JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005,** *53,* 7837−7843 **7837**

Assessment of the Volatile Composition of Juices of Apricot, Peach, and Pear According to Two Pectolytic Treatments

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The behavior of volatile compounds according to two enzymatic treatments applied during the manufacture of fruit juice is described. More than 80 compounds were detected of a wide range of chemical families (alcohols, aldehydes, ketones, terpenoids, esters, norisoprenoids, ...). Theaspirane and α-isophoron were tentatively identified for the first time in apricot and peach fruits. The enzymes used, for extraction or clarification of fruit juices, modified the polysaccharides separated by molecular weight and the content of soluble polysaccharides. This could indicate differences in the fruit juice matrix, which could be related to observed changes in the volatile profile. In apricot, the enzymes enhanced the juice in terpenes and norisoprenoids as varietal compounds. In peach and pear, the enzymes used did not favor the amount of lactones and decadienoate esters, the character impact compounds, respectively.

KEYWORDS: Volatile compounds; polysaccharides by M_w; pectolytic enzymes; SPME; GPC

INTRODUCTION

Apricot, peach, and pear are some of the most important noncitric fruits in Mediterranean countries. Due to their nutritive value and aroma, these fruits are widely consumed as fresh fruit, canned fruit, and processed juice (*1*). Carbohydrates, organic acids, and phenolic compounds are the major constituents of fruit, and these compounds are useful for monitoring the quality of juices (*2*). According to Azondanlou et al. (*3*) the sum of sugars, organic acids, and volatile compounds (*4*, *5*) as well as the color, shape, and texture determine the sensory properties of fruits and vegetables. The formation of aroma compounds in fruit is a dynamic process during which the concentration of volatiles changes both qualitatively and quantitatively. Some studies about the influence of the degree of maturation in volatile composition are reported (*6*−*12*). After the harvest of the fruit, the technological manufacture could affect the flavor (*1*, *13*−*17*). In a previous study by Riu-Aumatell et al. (*4*) a wide variability of volatile compounds was detected in commercial fruit juices and nectars of apricot, peach, and pear, but only a few compounds were detected in all of the samples of the same fruit. This could indicate some variability due to the technology used in its production.

On the other hand, the nature of the food matrix affects the concentration of the odorants in the headspace, probably because there are selective interactions between some components of the fruit juice matrix and some odorants, thus affecting their volatility. Therefore, the physicochemical properties of the volatile compounds in combination with the chemical nature and structure of the food matrix can modify the concentration of the volatile compounds and its perception (*18*).

Pectolytic enzymes were used in the fruit juice manufacture to enhance the juice extraction and also to clarify the juice (*19*). These enzymes will modify the polysaccharides composition and probably will affect the behavior of the volatile compounds. No published studies exist about the effect of the pectolytic enzymes over the volatile composition of fruit juices except some studies on grape juice (*20*, *21*). The aim of this work was to assess whether the use of pectolytic enzyme preparation, in addition to facilitating juice processes, could change the volatile composition of apricot, peach, and pear juices. The polysaccharides composition by molecular weight was considered to be due to their capacity to modify the volatile profile. For each fruit (apricot, peach, and pear) three kinds of samples, controls, samples elaborated with an extraction enzyme, and samples treated with clarification enzyme, were compared.

MATERIALS AND METHODS

Samples. Twenty kilograms each of apricot, peach, and pear were purchased at commercial maturity from commercial establishments in Barcelona (Spain) and stored at 5 °C. Two types of each fruit were studied: two different lots of the cultivar Galta Roja for apricot and two varieties for peach (Maria Bianca and Royal Glory) and pear (Ercolini and Conference). The different lots of apricot were purchased on different days. Each 20 kg of fruit was divided in three groups to obtain three types of samples according to the enzymatic treatment applied. At the same time, the three treatments (control; clarification enzyme; extraction enzyme) were applied three times to avoid the variability of manufacture.

Preparation of Juice and Enzymatic Treatment. Prior to juicing, the fruits were washed with water and the apricot and peach stones were

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10.1021/jf051397z CCC: $30.25
Published on Web 09/10/2005

removed. Citric acid and sodium fluoride were added to the fruit (at 3 and 1 g/L, respectively) to preserve the samples. Three types of each fruit were obtained: controls without enzymatic treatment, samples treated with endozyme Pectofruit polyfruit (Spindal group AEB, Gretz-Amainvilliers, France) to increase the juice yield (extraction enzyme), and samples clarified with the endozyme Pectofruit pear (Spindal group AEB) to clarifiy the juice (clarification enzyme). The samples were coarsely cut and then crushed with a liquidizer (7000*g*, 10 s; Oster Designer, Delray Beach, FL), filtered with a nylon mesh, and centrifuged (2500*g*, 10 min, 10 °C) to provide the chemical analysis. The clear juice was bottled and stored at −18 °C. Extraction enzyme (5 g/100 kg) was added before sample crushing, whereas clarifying enzyme (5 g/100 L) was added after sample crushing. Both enzymes were added at room temperature. At the same time, clear juice volume was measured to calculate the juice output as milliliters of fruit juice per 100 g of fruit.

Chemicals. Hexane, ethyl acetate, ethanol, hexanal, 3-methylbutyl acetate, 1,2-dimethylbenzene, limonene, (*E*)-2-hexenal, 1-hexanol, ethyl octanoate, acetic acid, 2-furancarboxyaldehyde, benzaldehyde, (*E*)-2-nonenal, ethyl nonanoate, ethyl decanoate, heptanoic acid, hexanoic acid, ethyl dodecanoate, and 2-phenylethanol were purchased from Sigma-Aldrich (St. Louis, MO). Ethyl hexanoate, 6-methyl-5-hepten-2-one, linalool, 1-octanol, 3-nonanol, geraniol, and 2-methylhexanoic acid were purchased from Fluka (St. Louis, MO), whereas 4-methyl-2-pentanol, methyl acetate, 3-methylbutanal, ethyl propanoate, propyl acetate, 1-butanol, ethyl butanoate, ethyl 3-methylbutanoate, butyl acetate, 3-pentenol, hexyl acetate, (*Z*)-3-hexenol, fenchone, (*E*)-2-hexenol, 2-octanol, octyl acetate, isobornyl acetate, butanoic acid, 1-decanol, α-terpineol, (*Z,E*)-α-farnesene, (*E,E*)-α-farnesene, 2-decanol, 2-phenylethyl acetate, nerol, β-ionone, cinnamaldehyde, γ-nonalactone, octanoic acid, γ-decalactone, and decanoic acid were purchased from TCI (Chuo-Ku, Tokyo, Japan).

Analytical Methods. pH, soluble solid content (determined by refractometry), and titratable acidity were determined following the Codex recommendations (*22*). Maturation index was calculated as the ratio between soluble solids (°Brix) and titratable acidity (grams of citric acid per liter of juice). Glucose, fructose, and sucrose were assessed through the enzymatic method (*23*).

Polysaccharides were extracted from fruit juice following the methods of Segarra et al. (*24*). Total and acid polysaccharide contents were determined by using a spectrophotometric method (*24*). Polysaccharides of different molecular masses were separated by gel permeation chromatography (GPC) and quantified as described by López-Barajas et al. (*25*). Their molecular mass was identified with a calibration curve obtained with six polyacrylic acids (Sigma-Aldrich, St. Louis, MO) of M_r values between 240 and 2 kDa.

Volatile compounds were extracted with a headspace solid-phase microextraction (HS-SPME) method. The SPME fiber used was 2 cm of 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane coating bonded to a fused silica core (DVB-CAR-PDMS) (Supelco, Bellefonte, PA). This fiber was used previously in a wide range of beverages such as wine (*26*, *27*), orange juice (*28*), and apricot and tomato juices (*3*). The HS-SPME and the chromatographic conditions were the same as used by Riu-Aumatell et al. (*4*) for the volatile compounds determination in commercial fruit juices and nectars. A 5 mL juice sample was put in a 10 mL vial and then extracted at 40 °C for 30 min with magnetic stirring (700*g*). Volatiles were semiquantified using a 6890N network gas chromatograph equipped with a 5973 network mass selective detector (MS) (Agilent Technologies, Palo Alto, CA). The capillary column was a Supelcowax 10 with PEG 20M stationary phase (CW) (30 mm × 0.25 mm × 0.25 μm) (Bellefonte, PA). Helium was used as a carrier gas. The injector and detector temperatures were 250 and 280 °C, respectively. The temperature program was from 60 °C (held for 5 min) to 240 °C (held for 10 min) at 3 °C/min using splitless injection mode. Electron impact mass spectra were recorded at a voltage of 70 eV ionization energy in the 15−250 u mass range at 2 scans/s.

Volatile compounds were identified by comparing them with two spectral libraries (NIST/EPA/MSDC 49K Mass Spectral Database, Hewlett-Packard Co., Palo Alto, CA; and Registry of Mass Spectral Data with Structures, Wiley 6.1, New York), as well as with relative retention times when chemical standards were available. We also used the retention index standards (Sigma, St. Louis, MO) of C8 and C32 aliphatic hydrocarbons to calculate the Kovats index (KI) on CW and HP-5MS columns (Agilent Technologies, Palo Alto, CA) of the same dimensions as the analytical one. Quantification was carried out according to the internal standard method. The volatile compounds identified were quantified by considering the relative response factor to be 1 and were expressed as milligrams per liter equivalents (×100) of IS solution of 4-methyl-2-pentanol or 2-methylhexanoic acid (500 mg/L) prepared in methanol (SDS, Peypin, France). Compounds with a Kovats index from 812 to 1524 (CW phase) were quantified with 4-methyl-2-pentanol, whereas compounds with a Kovats index from 1530 to 2258 (CW phase) were quantified with 2-methylhexanoic acid.

Statistical Procedures. Discriminant analysis and multifactor ANOVA were performed with general and polysaccharide parameters to test the differences obtained between the two enzymatic treatments on the samples of fruit juices. To group volatile compounds that were affected by enzymes in each fruit studied, principal component analysis (PCA) was also applied. These statistical analyses were carried out by using the program Statgraphics Plus 5.1 (*29*).

RESULTS AND DISCUSSION

Table 1 provides the general parameter means including soluble polysaccharide composition and apricot, peach, and pear handmade juice polysaccharides separated by molecular weight according to the enzymatic treatment applied. The two varieties or two lots of each fruit ($n = 2$) and treatment triplicates ($n = 3$) were used to obtain the mean value ($n = 6$). When the multifactor ANOVA was performed (**Table 1**), it could be observed that the general parameters were significantly different according to the fruit considered. Peach was characterized by the highest levels of sucrose (*6*), apricot juice by glucose values, and pear juice by the highest content of fructose. Low pH and low total acidity were characteristic for peach and pear, respectively. When the enzymatic factor was considered, the two treatments decreased significantly the pH and caused an increase of 13% in sample acidity. Probably, the enzymes liberate several organic acids such as galacturonic acid due to the methylesterase activity present in enzymatic preparations and, thus, increase the acidity of the juice. As expected, the yield obtained (milliliters of juice per 100 g of fruit) with the extraction enzyme was higher than controls in all fruits studied, whereas the clarification enzyme in apricot and pear caused a yield decrease.

The behavior of polysaccharide values depended on the differences between varieties or between lots (data not shown). However, the enzymatic treatment influences the composition in total polysaccharides and polysaccharides of medium and low molecular weight (**Table 1**). The extraction enzyme increases the value of total soluble polysaccharides (41% on the average), especially for peach and pear fruits. The values of small polysaccharide fractions (M_r 1.7−1.3 and <1000) increased with the two enzymatic treatments, probably because they break down the medium polysaccharides (M_r 100−85 and 45−30), which at the same time decreased. Comparing the effect of both enzymes, the smallest polysaccharides (M_r <1000 Da) were higher in the juices treated with extraction enzyme. This may be explained by the higher extraction capacity of soluble polysaccharides of fruit and/or by the higher extraction time, which favors the contact between fruits and their juices.

Moreover, the discriminant analysis was carried out with the parameters of **Table 1** in order to group the samples according to the enzymatic treatment (**Figure 1**). The discriminant function 1 justified 76% of the variability, and it was defined for polysaccharides of M_r 45−30 and 1.7−1.3 kDa with discriminant function coefficients of 1.244 and −0.708, respectively. Function 2 justified 18% of the variability, and it was defined

Table 1. Mean ($n = 6$) of General Parameters and Carbohydrate Composition of Fruit Juices and Significance (p) Obtained by Multifactor ANOVA

	apricot			peach			pear					
		enzyme treatment			enzyme treatment			enzyme treatment				
	control	extraction	clarification	control	extraction	clarification	control	extraction	clarification	A[a]	B[b]	AB[c]
pH	4.40	4.09	4.23	3.81	3.70	3.72	4.28	4.10	4.18	0.0001	0.0001	ns[d]
°Brix (g/L)	12.73	13.87	13.01	12.05	9.08	8.78	11.49	12.94	10.85	0.0001	ns	0.0197
total acidity (g of citric acid/L)	4.32	4.45	4.91	4.66	5.25	5.68	2.21	2.66	2.71	0.0001	0.0270	ns
maturation index[e]	3.03	3.22	2.77	2.58	1.85	1.56	5.23	4.97	4.03	0.0001	0.0040	ns
yield (mL of juice/100 g fruit)	73	78	55	63	82	67	70	71	63	ns	0.0047	ns
glucose (g/L)	0.41	0.34	0.36	0.12	0.14	0.21	0.14	0.15	0.18	0.0001	ns	ns
fructose (g/L)	0.31	0.25	0.27	0.16	0.16	0.23	0.54	0.59	0.65	0.0001	ns	ns
sucrose (g/L)	0.21	0.12	0.16	0.50	0.56	0.41	0.02	0.03	0.02	0.0001	ns	ns
TPS[f] (g/L)	8.53	8.15	5.65	5.45	8.19	4.79	3.67	7.11	4.49	ns	0.0153	ns
APS[g] (g/L)	8.31	6.17	4.32	2.66	6.78	3.01	3.32	4.43	3.48	0.0187	ns	ns
M_r 540–440 kDa	0.02	0.04	0.03		0.04		0.09	0.12	0.03	0.0046	ns	ns
M_r 320–215 kDa				0.05	0.17	0.09	0.29		0.02	ns	ns	0.0070
M_r 100–85 kDa	2.39	0.46	0.17	1.65	0.60	0.09	0.27	0.51	0.09	ns	0.0020	ns
M_r 45–30 kDa	2.31	0.96		1.16	0.92	0.04	1.25	0.53	0.06	0.0269	0.0001	0.0292
M_r 17–13 kDa	1.22	0.38	0.43	0.78	0.63	0.63	0.41	0.67	0.57	ns	ns	ns
M_r 1.7–1.3 kDa	1.00	0.86	0.50	0.27	1.23	0.47	0.38	1.05	0.52	ns	0.0329	ns
M_r < 1000 Da	2.29	5.45	4.52	1.54	4.59	3.48	0.97	4.24	3.21	ns	0.0001	ns

[a] A, fruit factor. [b] B, enzymatic treatment factor. [c] AB, interaction between fruit and enzymatic treatment factors. [d] Not significant result. [e] Maturity index was calculated as the ratio between soluble solid (°Brix) and total acidity (g of citric acid/L of juice). [f] Total polysaccharides. [g] Acid polysaccharides.

for polysaccharides of low molecular weight (<1000 Da) and glucose content with discriminant functions coefficients of −0.759 and 0.763, respectively (data not shown). The separation of the juices elaborated with three different methods was >75% according to the classification made by cross-validation. Ninety-four percent of the juices enzymatically clarified were correctly classified (the only sample wrongly placed was assigned in the other enzyme-treated group), and 77% of the juices treated with extraction enzyme were correctly placed (three samples were wrongly placed; one of them in the control group and the other two in the enzymatically clarified group).

Characterization of Volatile Composition. The volatile profile obtained by GC-MS is shown in **Table 2**. Seventy-two compounds in apricot samples, 80 compounds in peach, and 96 compounds in pear juice were detected. In **Table 2** can be observed the list of volatile compounds sorted through the retention time obtained with CW phase, the KI obtained by HP-5 phase, the identification method (A, comparison with retention time based on the use of reference standards; B, mass spectrum), and the median value ($n = 18$) of each fruit: two varieties or two lots (2) for three replicates of treatment (3) and triplicates

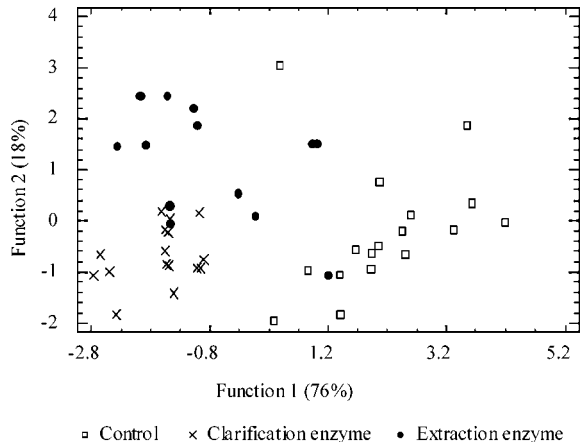

Figure 1. Discriminant analysis of general parameters of apricot, peach, and pear according to the enzymatic treatment used.

determination (3). The significance levels (p) obtained by analysis of variance among the three types of samples according to the enzymatic treatment are also shown in **Table 2** (enzyme factor). The volatile compounds identified only by mass spectrum were tentatively identified.

A wide range of chemical families is present in **Table 2**: aldehydes, ketones, alcohols, esters, lactones, terpenoids and aromatic compounds, hydrocarbons, furans, C_{13}-norisoprenoids, and carboxylic acids. By comparison of the results in the current study with those of Riu-Aumatell (*4*), aldehydes and alcohols of six carbons were better detected, probably because the triple-phase fiber has more polar characteristics and the extraction capacity of these compounds was higher than that of PDMS fiber used in the previous study. The presence of C6 compounds was probably due to lipooxygenase activity, which was initialized by the disruption of the fruit tissues when the juice was extracted (*10*).

Some compounds are of special significance because of their importance for fruit sample aroma. The most abundant volatile compounds determined in apricot juices in the current study were benzaldehyde, some esters, norisoprenoids, and terpenoid compounds (**Table 2**). The characteristic contents of benzaldehyde, linalool, and esters were described previously by Guichard (*30*, *31*) and Takeoka et al. (*32*) for apricot.

Esters, alcohols, and terpenoids were the main peach compounds (**Table 2**). Also, the lactones previously described in the literature (*1*, *5*−*7*, *9*, *13*) as responsible of peach flavor were clearly determined. In peach samples, benzaldehyde, methyl and ethyl acetate, and some lactones were the quantitatively main compounds (**Table 2**). Benzaldehyde, described as typical of stone fruits, was responsible for the almond nutty and stone fruit aroma in the peach pulp (*7*, *33*) and probably arises from the cyanogenic glycoside amygdalin, a typical constituent of *Prunus* spp. (*13*, *32*). Some norisoprenoids tentatively identified as α-isophoron (*m*/*z* 39, 82, 138) and theaspirane B (*m*/*z* 82, 96, 138) were detected for the first time in apricot and peach samples (**Table 2**). Norisoprenoids were compounds from the degradation of carotenoids, which have significant aroma impact in other fruits such as grape, apple, lychee, and mango (*28*).

Table 2. Volatile Compounds Identified by HS-SPME and GC-MS by Fruit, Mean, and Significance Value (*p*)

		KI CW phase[a]	KI HP5 phase[b]	ID[c]	apricot ($n = 18$)	enzyme factor[g]	peach ($n = 18$)	enzyme factor	pear ($n = 18$)	enzyme factor
1	hexane	812	600	A, B	1.94[f]		1.66		1.76	
2	ethanal[d]	837	nd[e]	B	4.66		1.59		1.96	
3	methyl acetate	902	nd	A, B	4.36		3.12		0.01	
4	ethyl acetate	977	602	A, B	29.00	0.0000	23.89		2.38	
5	2-butanone[d]	1000	nd	B	5.00	0.0006	6.36		0.89	
6	3-methylbutanal	1005	629	A, B					0.66	
7	ethanol	1010	668	A,B	48.24	0.0000	15.11		7.31	
8	ethyl propanoate	1021	nd	A,B			1.23			
9	propyl acetate	1030	nd	A,B			1.21		0.01	
10	pentanal[d]	1034	732	B	3.77		2.16		2.30	
11	2-methylpropanol[d]	1043	797	B	3.58		0.72		1.81	
12	methyl 2-methylbutanoate[d]	1052	776	B					1.24	0.0194
13	ethyl butanoate	1069	804	A, B	1.26		0.48	0.0475	0.22	
14	methylbenzene[d]	1073	674	B	2.06		1.54		1.31	
15	ethyl 2-methylbutanoate[d]	1083	849	B					1.07	
16	ethyl 3-methylbutanoate	1096	nd	A,B			0.54			
17	2-hexen-4-one[d]	1099	685	B					0.61	
18	butyl acetate	1100	710	A,B			0.57		1.36	
19	hexanal	1105	700	A, B	4.94		1.46	0.0328	14.01	
20	1-butanol	1116	nd	A, B			0.29			
21	3-methylbutyl acetate	1139	911	A,B			0.58		0.54	
22	1,2-dimethylbenzene	1159	753	A, B	0.26		0.15		0.41	
23	3-pentenol	1177	809	A, B					0.25	
24	pentyl acetate[d]	1193	nd	B			1.07		0.01	
25	isocineole[d]	1199	1008	B	0.01		1.27			
26	1-heptanal[d]	1206	872	B					0.89	
27	pentyl propanoate[d]	1208	nd	B			0.42	0.0175		
28	limonene	1213	1021	A, B	1.26		0.92	0.0187	0.81	0.0054
29	eucalyptol[d]	1222	1023	B	1.07		0.38			
30	butyl butanoate[d]	1232	nd	B					0.04	
31	(*E*)-2-hexenal	1234	854	A, B	1.28		0.31	0.0007	0.62	
32	ethyl hexanoate	1245	994	A, B	0.55		0.27	0.0170	0.01	
33	pseudocumene[d]	1252	961	B	1.10		1.76		0.86	
34	pentanol[d]	1261	764	B	0.19		0.22		0.36	
35	3-octanone[d]	1266	896	B					0.04	
36	*p*-cymene[d]	1275	1017	B	0.24		0.14			
37	hexyl acetate	1282	1008	A, B	0.10		0.24		0.54	
38	(*E*)-β-ocimene[d]	1286	1031	B	0.30					
39	3-hydroxy-2-butanone[d]	1290	nd	B	1.15	0.0043	0.26	0.0189	0.10	
40	1-octanal[d]	1299	1006	B					0.09	
41	(*E*)-isolimonene[d]	1306	nd	B	0.16					
42	3-octenone[d]	1310	979	B					0.43	
43	3-hexenyl acetate[d]	1321	1009	B			0.10		0.10	
44	(*Z*)-2-octenal[d]	1329	1049	B					1.21	0.0244
45	2-hexenyl acetate[d]	1338	nd	B			0.06			
46	6-methyl-5-hepten-2-one	1340	980	A, B	0.36	0.0194	0.16		2.52	0.0267
47	1-hexanol	1356	858	A, B	0.64		1.07		4.44	
48	ethyl 4-hexanoate[d]	1365	nd	B					0.12	
49	(*Z*)-3-hexenol	1382	nd	A,B			0.06		0.33	
50	fenchone	1385	nd	A,B			0.09			
51	ethyl 2-methyloctanoate[d]	1388	1156	B	0.34		0.21		0.33	
52	1-nonanal[d]	1394	1098	B			0.20		0.26	0.0296
53	citronellal[d]	1398	1145	B					0.52	0.0043
54	(*E*)-2-hexenol	1403	nd	A, B	0.26	0.0036	0.56	0.0001	0.06	
55	butyl hexanoate[d]	1412	nd	B					0.02	
56	hexyl butanoate[d]	1414	nd	B					0.03	
57	2-octanol	1420	1004	A,B			0.01		0.03	0.0334
58	(*E*)-2-octenal[d]	1425	1060	B					1.24	
59	ethyl octanoate	1431	1191	A, B	0.62	0.0337	0.48	0.0126	0.33	
60	megastigme-7(*E*)-9,13-triene[d]	1439	1350	B	0.45					
61	acetic acid	1443	606	A, B	3.52		0.86		0.78	
62	3-octenol[d]	1447	972	B	0.68		0.25		1.24	
63	2-furancarboxyaldehyde	1454	817	A, B	0.39				0.26	
64	nerol oxide[d]	1460	1147	B			0.01		0.31	0.0406
65	1,2,3,4-tetrahydro-1,5,7-trimethylnaphthalene[d]	1462	1242	B	0.57		0.01			
66	octyl acetate	1469	1149	A, B			0.20		0.03	
67	benzaldehyde	1504	953	A, B	75.31		35.80	0.0001	0.30	
68	theaspirane B[d]	1523	1286	B	5.59	0.0010	0.29			
69	(*E*)-2-nonenal	1524	1162	A, B	1.86	0.0040			0.90	
70	ethyl nonanoate	1530	1288	A, B	5.98		1.18		2.62	
71	linalool	1544	1093	A,B	44.69	0.0004	2.19	0.0025	1.67	
72	1-octanol	1553	1064	A, B	1.37		1.02	0.0331	2.39	

Table 2. (Continued)

		KI CW phase[a]	KI HP5 phase[b]	ID[c]	apricot ($n = 18$)	enzyme factor[g]	peach ($n = 18$)	enzyme factor	pear ($n = 18$)	enzyme factor
73	5-methylfurfural[d]	1562	nd	B	0.32					
74	megastigme-4,6(*E*),8(*E*)-triene[d]	1568	1329	B	4.67	0.0007				
75	isobornyl acetate	1568	1275	A,B			1.31		0.76	
76	α-fenchyl alcohol[d]	1572	1276	B			2.35		0.01	
77	α-isophoron[d]	1576	1051	B	0.21		0.36			
78	2,6-nonadienal[d]	1577	1155	B					1.52	
79	4-terpineol[d]	1591	nd	B			1.13			
80	megastigme-4,6(*E*),8(*Z*)-triene[d]	1598	1317	B	4.18	0.0021				
81	β-ciclocytral[d]	1603	1210	B	3.17		0.87		0.81	
82	megastigme-4,6(*Z*),8(*Z*)-triene[d]	1606	1251	B	20.50	0.0000				
83	hexyl hexanoate[d]	1608	nd	B					2.54	
84	2-decenol[d]	1612	nd	B					4.51	
85	butanoic acid	1620	820	A,B			0.70	0.0038	0.49	0.0063
86	menthol[d]	1632	1171	B	1.63	0.0455	1.65	0.0005	1.23	
87	ethyl decanoate	1635	1386	A,B	5.25		3.15			
88	(*E*)-2-decenal[d]	1638	nd	B					7.20	
89	unknown 1646 *m*/*z* 73, 147, 207	1646	nd	B	3.10	0.0173	1.29	0.0071		
90	ethyl benzonoate[d]	1651	1162	B	12.07	0.0012				
91	1,2,3,4-tetrahydro-1,5,8-trimethylnaphthalene[d]	1655	1200	B	6.15	0.0001				
92	2-decanol	1657	nd	A,B	1.14					
93	3-nonanol	1658	nd	A,B			1.02		2.76	
94	heptanoic acid	1662	nd	A,B			0.42	0.0008		
95	unknown 1671 *m*/*z* 74, 121, 161	1671	nd	B					0.01	
96	γ-hexalactone[d]	1683	1047	B	0.60		4.61	0.0394		
97	α-terpineol	1689	1181	A, B	3.73	0.0013	0.79	0.0138	0.46	0.0434
98	(*Z*)-2,(*Z*)-4-decadienal[d]	1696	nd	B					2.16	0.0007
99	α-muurolene[d]	1711	nd	B					0.01	
100	naphthalene[d]	1719	1170	B	1.20	0.0063				
101	1,2-dihydro-1,1,6-trimethylnaphthalene[d]	1724	1340	B	4.26	0.0050	0.55			
102	(*Z*,*E*)-α-farnesene	1725	nd	A, B					1.25	
103	δ-cadinene[d]	1744	1548	B					0.42	
104	(*E*,*E*)-α-farnesene	1747	nd	A, B					7.78	0.0059
105	2,4-decadienal[d]	1762	1208	B					1.19	
106	1-decanol	1763	1269	A,B			0.22		0.26	
107	2-phenylethyl acetate	1777	1260	A, B			0.01		0.29	
108	γ-heptalactone[d]	1789	1248	B			0.01			
109	methyl (*E*)-2,(*Z*)-4-decadienoate[d]	1794	1386	B					1.13	0.0461
110	nerol	1797	1251	A, B	0.96	0.0000				
111	(*E*)-2,(*E*)-4-decadienal[d]	1809	1305	B					0.47	
112	β-damascenone[d]	1810	1386	B			0.58	0.0373	0.80	
113	ethyl (*E*)-2,(*E*)-4-decadienoate[d]	1842	1445	B					0.01	
114	hexanoic acid	1843	1019	A, B	1.09	0.0298	0.47	0.0321		
115	ethyl (*E*)-2,(*Z*)-4-decadienoate[d]	1845	1457	B					7.13	0.0123
116	geraniol	1848	1246	A, B	2.70	0.0227				
117	ethyl dodecanoate	1848	1598	A, B			0.58		0.01	
118	geranyl acetone[d]	1853	1441	B	2.94	0.0000	1.12	0.0264	2.93	
119	ethyl (*Z*)-2,(*E*)-4-decadienoate[d]	1859	nd	B					0.01	
120	2-phenylethanol	1902	1118	A, B	0.39		0.25			
121	α-calacorene[d]	1904	1525	B					0.77	
122	γ-octalactone[d]	1906	1259	B			0.33	0.0004		
123	ethyl (*Z*)-2,(*E*)-6-dodecadienoate[d]	1917	nd	B					0.01	
124	β-ionone	1931	1472	A, B	4.26	0.0014	1.41	0.0367	1.32	
125	dihydro-β-ionone[d]	1952	1405	B	5.94	0.0006				
126	dihydro-β-ionol[d]	1966	nd	B	1.66	0.0306				
127	4,5-dimethylfurfural[d]	1978	nd	B	3.79	0.0071				
128	1,8-dimethylnaphthalene[d]	1989	1404	B					0.52	
129	methyleugenol[d]	2016	nd	B					0.30	
130	cinnamaldehyde	2039	1258	A, B	1.91	0.0002	1.63	0.0052	2.76	
131	γ-nonalactone	2055	1366	A, B	0.26		0.33			
132	octanoic acid	2068	1279	A, B					0.53	
133	ethyl cinnamate[d]	2129	1451	B	0.94	0.0212				
134	γ-decalactone	2142	1454	A, B	0.78	0.0046	3.03		0.01	
135	eugenol[d]	2164	1346	B			0.41			
136	decanoic acid	2175	1357	A, B	0.52				0.69	
137	unknown 2258 *m*/*z* 73, 101, 144	2258	1133	B	19.10	0.0243	4.63		0.01	
138	δ-dodecalactone[d]	2467	1677	B	0.98					

[a] Carbowax phase. [b] HP5 phase. [c] Identification (A, comparison of retention time with reference standards; B, mass spectrometry). [d] Tentatively identified. [e] Not detected. [f] mg/L equivalents of internal standard. [g] Significance value obtained by enzyme factor (p).

These compounds can be formed as a result of in vivo degradation or thermal degradation generated during the processing of foods containing carotenoids (*28*).

In pear fruit the volatile composition was mainly composed by oxygenated compounds (such as aldehydes, alcohols, and ketones) and esters, as was described previously in the literature

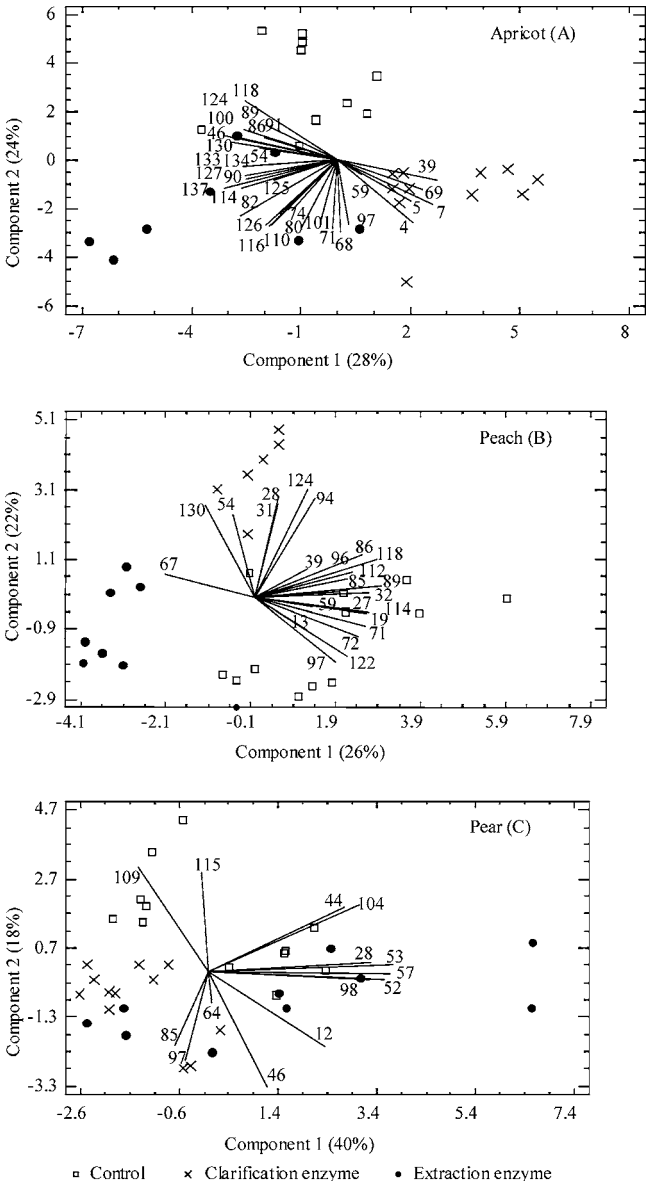

Figure 2. PCA of volatile compounds (significant by one-way ANOVA) according to the enzymatic treatment realized in apricot (**A**), peach (**B**), and pear (**C**).

(*14*−*16*, *34*, *35*). In the current study (**Table 2**) hexanal, cinnamaldehyde, methyl and ethyl decadienoates, and farnesenes were clearly detected. Ethyl esters of 2,4-decadienoic acids were the impact character compounds responsible for pleasant pear-like odor (*14*, *16*); also, α-farnesenes were found as a relatively major components (*11*).

Effect of Enzymatic Treatment on Volatile Composition. The differences observed with the enzymatic treatment affect only quantitatively the volatile compounds. **Figure 2** shows the PCA performed with the volatile compounds that were statistically significantly affected by the enzyme treatment (**Table 2**). The multifactor ANOVA results indicate that the effect of enzymatic treatment depends on the fruit variety or lot (data not shown).

In **Figure 2A** can be observed apricot juices PCA. Samples were grouped according to the enzyme used in elaboration. Component 1 separates the samples obtained with the two enzymes used in this study, whereas component 2 separates control samples and the juices obtained with enzymes. Control samples were the poorest in volatile compounds, mainly terpenoids and norisoprenoids with pleasant odor: megastigme-4,6(*E*)-8(*E*)-triene, megastigme-4,6(*E*)-8(*Z*)-triene, megastigme-4,6(*Z*)-8(*Z*)-triene, 1,2-dihydro-1,1,6-trimethylnaphthalene, 1,2,3,4-tetrahydro-1,5,8-trimethylnaphthalene, α-terpineol, nerol, theaspirane B, geraniol, dihydro-β-ionol, and linalool (with an increase of 50% mainly in the samples treated with extraction enzyme) (**Figure 2A**; **Table 2**). This could be due to an enzyme effect over soluble polysaccharide composition. According to the literature, pectins could affect the flavor release in different ways: the viscosity caused by pectins (*36*) and the binding of the aroma compounds with the food matrix (*37*) could cause a decrease in flavor release. The value of total and acid polysaccharides and the polysaccharide fractions of M_r 100−85, 45−30, 17−13, and 1.7−1.3 kDa were higher in apricot control juices (**Table 1**), which could cause a retention of volatile compounds and thus a decrease of these compounds in the headspace. Another possibility for the minor content of varietal compounds in control samples could be the glycosidic activity in enzyme preparations. This activity could hydrolyze glycosidic precursors, releasing free terpenes and norisoprenoids of its aglycons (**Table 1**; M_r <1000), so varietal substances could be enhanced. Juices treated with clarification enzyme could present higher amounts of ethyl acetate, 2-butanone, ethanol, 3-hydroxy-2-butanone, and (*E*)-2-nonenal than extraction enzyme samples.

The PCA performed with peach (**Figure 2B**; **Table 2**) showed that several volatile compounds were separated according to the enzymatic treatment. Samples treated with extraction enzyme had significantly lesser amounts of hexanal, pentyl propanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, 1-octanol, linalool, α-terpineol, menthol, hexanoic acid, geranyl acetone, and lactones γ-hexalactone and γ-octalactone. Lactones γ-octalactone (detected in only the Royal Glory variety) and γ-hexalactond, described in the literature as responsible for peach flavor (*1*, *5*−*7*, *9*, *13*), decreased 63 and 43%, respectively, in extraction samples. Only benzaldehyde seems to increase with the extraction enzyme, whereas the contents of limonene, heptanoic acid, and β-ionone were increased with the clarification enzyme. In **Table 1** can be observed that in the extraction enzyme treated samples, the yield value, total and acid polysaccharides, and polysaccharides of M_r 540−440 and 320−215 kDa were higher. However, in this case the treatment with extraction enzyme did not increase the varietal volatile compounds.

For pear juices (**Figure 2C**; **Table 2**) control samples have a volatile composition different from that of the enzyme-treated samples. Clarification enzyme juices were poorest in (*Z*)-2,(*Z*)-4-decadienal, limonene, citronellal, 2-octanol, 1-nonanal, (*Z*)-2-octenal, and (*E*,*E*)-α-farnesene. Control samples of variety Conference had higher amounts of methyl (*E*)-2,(*Z*)-4-decadienoate, and ethyl (*E*)-2,(*Z*)-4-decadienoate, the impact character compounds in pear. The clarification enzyme used in the current study for pear has not been the most appropriate one due to its negative effects on the amount of decadienoate esters.

These preliminary results indicate that various volatile compounds could be affected by the applied procedure even if more specific studies are required to increase this knowledge. In conclusion, the data obtained show that the use of enzymes during the elaboration of fruit juice affects the volatile composition with different behaviors on the studied fruits. In the case of apricot, the enzymes used enhanced the flavor in pleasant odors such as terpenes and norisoprenoids. Peaches treated with extraction enzyme were the poorest in volatile compounds, probably because the high polysaccharide content in these samples could cause a higher retention of volatile compounds.

Finally, in pear fruit the enzymatic treatment used did not favor the content of decadienoate esters, the impact character compound of pears.

LITERATURE CITED

(1) Derail, C.; Hofmann, T.; Schieberle, P. Differences in key odorants of handmade juice of yellow-flesh peaches (*Prunus persica* L.) induced by the workup procedure. *J. Agric. Food Chem.* **1999**, *47*, 4742−4745.

(2) Versari, A.; Castellari, M.; Parpinello, G. P.; Riponi, C.; Galassi, S. Characterisation of peach juices obtained from cultivars Redhaven, Suncrest and Maria Marta grown in Italy. *Food Chem.* **2002**, *76*, 181−185.

(3) Azondanlou, R.; Darbellay, C.; Luisier, J.-L.; Villettaz, J.-C.; Amadò, R. Development of a model for quality assessment of tomatoes and apricots. *Lebensm.-Wiss. -Technol.* **2003**, *36*, 223−233.

(4) Riu-Aumatell, M.; Castellari, M.; López-Tamames, E.; Galassi, S.; Buxaderas, S. Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GC/MS. *Food Chem.* **2004**, *87*, 627−637.

(5) Visai, C.; Vanoli, M. Volatile compound production during growth and ripening of peaches and nectarines. *Sci. Hortic.* **1997**, *70*, 15−24.

(6) Chapman, G. W.; Horvat, R. J. Changes in nonvolatile acids, sugars, pectin and sugar composition of pectin during peach (Cv. Monroe) maturation. *J. Agric. Food Chem.* **1990**, *38*, 383−387.

(7) Robertson, J. A.; Meredith, F. I.; Horvat, R. J.; Senter, S. D. Effect of cold storage and maturity on the physical and chemical characteristics and volatile constituents of peaches (Cv. Cresthaven). *J. Agric. Food Chem.* **1990**, *38*, 620−624.

(8) Chapman, G. W.; Horvat, R. J.; Forbus, W. R. Physical and chemical changes during the maturation of peaches (Cv. Majestic). *J. Agric. Food Chem.* **1991**, *39*, 867−870.

(9) Aubert, C.; Gunata, Z.; Ambid, C.; Baumes, R. Changes in physicochemical characteristics and volatile constituents of yellow- and white-fleshed nectarines during maturation and artificial ripening. *J. Agric. Food Chem.* **2003**, *51*, 3083−3091.

(10) Gomez, E.; Ledbetter, C. A. Development of volatile compounds during fruit maturation: characterization of apricot and plum × apricot hybrids. *J. Sci. Food Agric.* **1997**, *74*, 541−546.

(11) Shiota, H. Changes in the volatile composition of La France pear during maturation. *J. Sci. Food. Agric.* **1990**, *52*, 421−429.

(12) Lalel, H. J. D.; Singh, Z.; Tan, S. C. Aroma volatiles production during fruit ripening of "Kensington" Pride mango. *Postharvest Biol. Technol.* **2003**, *27*, 323−336.

(13) Sumitani, H.; Suekane, S.; Nakatani, A.; Tatsuka, K. Changes in composition of volatile compounds in high-pressure treated peach. *J. Agric. Food Chem.* **1994**, *42*, 785−790.

(14) Kralj-Cigic, I.; Zupancic-Kralj, L. Changes in odour of Bartlett pear brandy influenced by sunlight irradiation. *Chemosphere* **1999**, *38*, 1299−1303.

(15) Lara, I.; Miró, R. M.; Fuentes, T.; Sayez, G.; Graell, J.; López, M. L. Biosynthesis of volatile aroma compounds in pear fruit stored under long-term controlled-atmosphere conditions. *Postharvest Biol. Technol.* **2003**, *29*, 29−39.

(16) Chervin, C.; Speirs, J.; Loveys, B.; Patterson, B. D. Influence of low oxygen storage on aroma compounds of whole pears and crushed pear flesh. *Postharvest Biol. Technol.* **2000**, *19*, 279−285.

(17) Lambert, Y.; Demazeau, G.; Lagerteau, A.; Bouvier, J.-M. Changes in aromatic composition of strawberry after high-pressure treatment. *Food Chem.* **1999**, *67*, 7−16.

(18) Bezman, Y.; Mayer, F.; Takeoka, G. R.; Buttery, R. G.; Ben-Oliel, G.; Rabinowitch, H. D.; Naim, M. Differential effects of tomato (*Lycopersicon esculentum* Mill) matrix on the volatility of important aroma compounds. *J. Agric. Food Chem.* **2003**, *51*, 722−726.

(19) Council Directive 2001/112/EC of December 20, 2001, relating to fruit juices and certain similar products intended for human consumption.

(20) Lao, C.; López-Tamames, E.; Buxaderas, S.; De la Torre-Boronat, M. C. Grape pectic enzyme treatment effect on white musts and wines composition. *J. Food Sci.* **1996**, *61*, 553−556.

(21) Lao, C.; López-Tamames, E.; Lamuela-Raventós, R. M.; Buxaderas, S.; De la Torre-Boronat, M. C. Pectic enzyme treatment effects on quality of white grape musts and wines. *J. Food Sci.* **1997**, *62*, 1142−1149.

(22) General standard for fruit juices preserved exclusively by physical means not covered by individual standards. Codex Stan 164-1989, Roma, Italy, 1989.

(23) Boehringer-Mannheim, GmbH, Biochemica, Germany, 1992.

(24) Segarra, I.; Lao, C.; López-Tamames, E.; de la Torre-Boronat, M. C. Spectrophotometric method for the analysis of polysaccharide levels in winemaking products. *Am. J. Enol. Vitic.* **1995**, *46*, 564−570.

(25) López-Barajas, M.; López-Tamames, E.; Buxaderas, S. Improved size-exclusion high performance liquid chromatographic method for the simple analysis of grape juice and wine polysaccharides. *J. Chromatogr. A* **1998**, *823*, 339−347.

(26) Torrens, J.; Riu-Aumatell, M.; López-Tamames, E.; Buxaderas, S. Volatile compounds of red and white wines by headspace solid-phase microextraction using different fibers. *J. Chromatogr. Sci.* **2004**, *42*, 310−316.

(27) Tat, L.; Comuzzo, P.; Stolfo, I.; Battistutta, F. Optimization of wine headspace analysis by solid-phase microextraction capillary gas chromatography with mass spectrometric and flame ionization detection. *Food Chem.* **2005**, *93*, 361−369.

(28) Mahattanatawee, K.; Rouseff, R.; Valim, F. M.; Naim, M. Identification and aroma impact of norisoprenoids in orange juice. *J. Agric. Food Chem.* **2005**, *53*, 393−397.

(29) *Statgraphics 5.1* (1994−2000); Statistical Graphics Corp.: Rockville, MD.

(30) Guichard, E. Quantification of some volatile aromatic compounds of apricot by adding standards. *J. Food Sci.* **1988**, *53*, 1902−1904.

(31) Guichard, E.; Souty, M. Comparison of the relative quantities of aroma compounds found in fresh arpicot (*Prunus armeniaca*) from six different varieties. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 301−307.

(32) Takeoka, G. R.; Flath, R. A.; Mon, T. R.; Teranishi, R.; Guentert, M. Volatile constituents of apricot (*Prunus armeniaca*). *J. Agric. Food Chem.* **1990**, *38*, 471−477.

(33) Narain, N.; Hsieh, T. C.-Y.; Johnson, C. E. Dynamic headspace concentration and gas chromatography of volatile flavor components in peach. *J. Food Sci.* **1990**, *55*, 1303−1307.

(34) Suwanagul, A.; Richardson, D. G. Pear fruit volatiles characterized by SPME and capillary GLC/mass spectroscopy. *Acta Hortic.* **1998**, *475*, 599−603.

(35) Suwanagul, A.; Richardson, D. G. Identification of headspace volatile compounds from different pear (*Pyrus communis* L.) varieties. *Acta Hortic.* **1998**, *475*, 605−623.

(36) Roberts, D. D.; Elmore, S.; Langely, K. R.; Bakker, J. Effect of sucrose, guar gum and carboxymethylcellulose on the release of volatile flavor compounds under dynamic conditions. *J. Agric. Food Chem.* **1996**, *44*, 1321−1326.

(37) Hansson, A.; Andersson, J.; Leufvén, A. The effect of sugars and pectin on flavor release from a soft drink-related model system. *Food Chem.* **2001**, *72*, 363−368.

Received for review June 13, 2005. Revised manuscript received July 26, 2005. Accepted July 31, 2005. This study was made possible thanks to financial assistance from the Generalitat de Catalunya (Spain), Project 2001SGR-00131; from the Ministerio de Educación y Cultura (MEC) (Spain), Project HI1999-0121; and from the Comisión Interministerial de Ciencia y Tecnología (CICYT) (Spain), Project VIN01-051.

JF051397Z