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Optimization of the Analysis of Flavor Volatile Compounds by Liquid–Liquid Microextraction (LLME). Application to the Aroma Analysis of Melons, Peaches, Grapes, Strawberries, and Tomatoes

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A fast method based on liquid−liquid microextraction (LLME) has been developed for the analysis of volatile compounds in fruit and vegetable juices. The method was tested in an aqueous solution containing 49 common flavor compounds typically found in fruit aroma. Influence on extraction yield of the salts used, their levels, and the time of extraction was investigated. The efficiency of *n*-propyl gallate to inhibit the formation of secondary compounds from lipids during the crushing of fruit tissues was also tested. The proposed method was then applied to several authentic samples such as melons, peaches, grapes, strawberries, and tomatoes. The advantages and limitations of LLME are discussed.

KEYWORDS: **Flavor; volatile compounds; fruits; liquid−liquid microextraction**

INTRODUCTION

Liquid−liquid extraction (LLE) is certainly one of the most frequently reported methods in the literature for the isolation of volatile compounds in fruits and vegetables. Nevertheless, because LLE requires large amounts of high-purity solvents, this technique is relatively tedious and time-consuming and causes serious environmental problems. Furthermore, extracts often have to be concentrated hundreds of times, and loss of analytes and artifact formation, due to elevated temperatures, during the concentration step are generally observed. Numerous efforts have been made to improve the LLE methods, in particular with the introduction of liquid−liquid microextraction (LLME) techniques. LLME is a single-step extraction with a very high liquid sample/solvent ratio and a saturation of the aqueous phase with inorganic salts. Microextractions avoid the concentration process and reduce solvent consumption and time of analysis. In the 1980s, Jennings (*1*) and Kok et al. (*2*) had already proposed analytical methods based on this idea, but their results were semiquantitative. Up to now, LLME has been mainly used for pesticide residue determinations in water (*3*−*6*) or for volatiles analysis in grape (*7*) and wine (*8*−*13*). Except for the method developed by Cacho et al. (*7*), including steam stream distillation and microextraction, no study using microextraction has appeared in the literature on the determination of aroma components in fruits and vegetables. The aim of this work was to develope a fast and quantitative method for the determination of the main volatile compounds in fruits and vegetables. Melons, peaches, grapes, strawberries, and tomatoes have been analyzed using the proposed method.

MATERIALS AND METHODS

Solvent and Chemicals. Analytical grade dichloromethane (Pestanal, ≥99.8%), ammonium sulfate $(NH_4)_2SO_4$ (puriss. p.a., ≥99%), ethanol absolute (Spectranal, ≥99.8%), and *n*-alkane standards (C_8−C_{40}) were from Riedel-de Haën, and *n*-propyl gallate (≥98%) was from Fluka. A stock solution containing 49 reference compounds was prepared in ethanol (**Table 1**). Reference compounds were obtained from various suppliers given in parentheses: no. 1−11, 13−15, 18−27, 32−37, 40−42, 44, 48, and 49 (Aldrich); no. 16, 17, 30, and 38 (Fluka); no. 12, 28, 29, 31, 39, 43, and 45−47 (Interchim) (see **Table 1** for compound numbers). All reference compound purities were >95%. A flavor test solution was prepared by taking 25 μL of the stock solution and diluting it to 500.0 mL with deionized water, giving ∼1 ppm of each flavor compound (except for Furaneol, ∼3 ppm). A flavor standard solution in dichloromethane was also prepared by taking 50 μL of the stock solution and diluting it to 10.0 mL.

Juice Sample Preparation. Amounts of 250 g of sample (fruit or vegetable), 250 mL of *n*-propyl gallate (10 mM), and 25 μL of 2-octanol (3.32 μg/mL) (internal standard) were homogenized in a Waring blender for 2 min. The mixture was centrifuged (13000*g*, 5 min, 4 °C), and the supernatant was filtered on a stainless steel sieve (16 mesh).

Optimized LLME. Forty milliliters of the flavor test solution (or sample juice previously obtained) and 12.8 g of $(NH_4)_2SO_4$ (32%; w/v) were agitated until complete salt dissolution and ultracentrifuged (21000*g*, 5 min, 4 °C). The supernatant was then filtered through a Whatman paper filter (grade 113v) into a 50 mL screw-capped conical centrifuge tube (34 × 98 mm glass borosilicate) containing a magnetic stir bar (15 × 6 mm). Two hundred and fifty microliters of dichloromethane was added, and the mixture was extracted for 60 min under magnetic stirring at 4 °C. After removal of the magnetic stir bar, the tube was sonicated for 1 min in a Branson Ultrasonic Cleaner 5510 and centrifuged (1000*g*, 5 min, 4 °C). The dichloromethane extract was then recovered with a 50 μL syringe, transferred to a 100 μL vial, and immediately injected in GC-MS and GC-FID.

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Table 1. Recoveries of the Reference Compounds in the Flavor Test Solution by LLE and LLME Sampling

						recovery[a] (%)			
no.	RT (min)	RI[b]	mg/mL[c]	log *P*[d]	compound	LLE	LLME	*F*[e]	*p*[f]
1	7.0	1009	17.76	1.77	isobutyl acetate	66 (5)	66 (2)	0.1	
2	7.7	1028	17.89	1.85	ethyl butyrate	71 (5)	70 (2)	0.2	
3	8.3	1043	17.58	2.26	ethyl 2-methylbutyrate	65 (5)	61 (2)	3.1	
4	9.0	1062	18.13	1.82	butyl acetate	71 (5)	73 (2)	0.4	
5	9.2	1069	17.02	1.80	hexanal	67 (4)	72 (3)	6.4	
6	9.7	1084	17.70	4.35	β-pinene	12 (6)	2 (6)	442.9	***
7	10.8	1112	17.92	2.26	isoamyl acetate	70 (6)	68 (2)	1.0	
8	12.1	1141	17.45	4.62	α-phellandrene	15 (7)	4 (2)	223.7	***
9	12.7	1155	17.00	4.75	α-terpinene	18 (13)	5 (2)	81.8	***
10	13.5	1175	17.21	4.83	limonene	21 (5)	5 (2)	532.1	***
11	14.5	1198	16.75	1.58	(*E*)-2-hexenal	89 (6)	96 (3)	3.9	
12	15.5	1220	17.76	2.83	ethyl hexanoate	67 (6)	53 (3)	35.7	**
13	17.1	1258	17.74	2.83	hexyl acetate	69 (5)	53 (3)	41.9	**
14	19.0	1298	18.28	2.61	(*Z*)-3-hexenyl acetate	77 (6)	75 (3)	0.5	
15	19.8	1317	16.85	2.06	6-methyl-5-hepten-2-one	79 (6)	89 (3)	11.7	*
16	20.5	1340	18.69	2.03	hexanol	80 (5)	48 (5)	137.3	***
17	21.6	1366	17.49	1.61	(*Z*)-3-hexen-1-ol	78 (5)	27 (5)	409.4	***
18	22.3	1378	19.93	2.94	2-isobutylthiazole	80 (5)	89 (3)	9.4	*
19	22.6	1390	16.09	1.61	(*E*)-2-hexen-1-ol	81 (5)	37 (6)	243.3	***
20	23.6	1409	19.53	2.90	2-octanol	84 (6)	90 (3)	3.3	
21	24.0	1418	21.81	0.95	ethyl 2-(methylthio)acetate	79 (5)	96 (3)	33.3	**
22	25.9	1464	22.00	0.95	2-(methylthio)ethyl acetate	79 (6)	97 (4)	28.8	**
23	26.7	1485	21.81	1.71	benzaldehyde	75 (5)	82 (3)	5.9	
24	27.2	1497	21.96	–0.05	2-(methylthio)ethanol	38 (4)	3 (4)	1518.1	***
25	28.6	1532	21.89	2.97	linalool	84 (6)	94 (5)	6.4	
26	29.6	1561	20.36	0.62	mesifurane	81 (6)	65 (6)	18.7	*
27	30.6	1581	18.63	3.44	β-cyclocitral	74 (7)	63 (6)	6.8	
28	31.1	1597	21.21	1.44	3-(methylthio)propyl acetate	81 (7)	98 (6)	12.3	*
29	33.4	1661	19.08	0.60	γ-hexalactone	79 (9)	75 (5)	0.6	
30	33.7	1669	16.46	3.33	α-terpineol	81 (8)	81 (5)	0.1	
31	34.1	1686	20.21	0.44	3-(methyltio)propanol	54 (6)	4 (3)	793.5	***
32	34.6	1691	21.15	2.08	benzyl acetate	79 (7)	100 (6)	19.3	**
33	36.0	1727	23.67	2.60	methyl salicylate	76 (8)	89 (6)	7.2	
34	36.5	1746	16.02	3.56	citronellol	81 (9)	79 (9)	0.1	
35	37.5	1775	16.97	3.47	nerol	94 (11)	94 (1)	0.1	
36	39.2	1824	16.92	3.47	geraniol	98 (12)	91 (4)	1.2	
37	39.5	1837	20.08	1.08	benzyl alcohol	75 (7)	8 (9)	470.2	***
38	40.6	1871	19.36	1.57	2-phenylethanol	81 (9)	30 (10)	124.4	***
39	41.7	1897	17.73	4.29	β-ionone	70 (17)	26 (2)	41.5	**
40	44.5	1994	56.98	0.82	Furaneol	22 (15)	1 (6)	120.2	***
41	47.5	2077	17.82	2.57	γ-decalactone	88 (20)	91 (2)	0.1	
42	47.9	2091	19.67	2.73	eugenol	83 (15)	93 (0)	2.1	
43	48.6	2114	17.98	2.57	δ-decalactone	78 (7)	93 (2)	19.2	**
44	49.3	2144	20.57	2.26	methyl anthranilate	87 (14)	86 (3)	0.1	
45	50.0	2176	16.73	3.06	γ-undecalactone	86 (21)	58 (4)	7.4	
46	50.9	2220	15.78	3.06	δ-undecalactone	77 (9)	72 (3)	1.9	
47	52.2	2284	16.63	3.55	γ-dodecalactone	71 (8)	24 (6)	215.8	***
48	53.1	2330	16.30	3.55	δ-dodecalactone	82 (9)	50 (5)	47.2	**
49	55.2	2447	19.15	1.55	vanillin	92 (9)	32 (3)	154.9	***

[a] Mean of three replicates with standard deviation in parentheses. [b] Linear retention index based on a series of *n*-hydrocarbons. [c] Concentration in the stock solution. [d] Octanol/water partition coefficient estimated using Syracuse Research Corp., LogKow v. 1.6 (see Meylan, W. M.; Howard, P. H. *J. Pharm. Sci.* **1995**, *84*, 83–92). [e] $F_{(0.05;\ 1;\ 4)} = 7.7$; $F_{(0.01;\ 1;\ 4)} = 21.2$; $F_{(0.001;\ 1;\ 4)} = 74.1$. [f] *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Conventional LLE. One hundred and fifty milliliters of the flavor test solution (or sample juice previously obtained) was extracted three times with 50 mL (3 × 15 min) of dichloromethane under magnetic stirring at 4 °C. The solvent was then removed using a small Vigreux column at 50 °C and concentrated to ∼500 μL. The extract was then immediately injected in GC-MS and GC-FID.

Extraction Recoveries. The flavor test solution was extracted in triplicate using the optimized LLME and the conventional LLE methods. For both, the final volume of dichloromethane extract was precisely recovered and noted. To 50 μL of each recovered extract was added 50 μL of 4-nonanol (50 ppm), and the mixtures were homogenized and injected in GC-FID. The recoveries were calculated as a percent, based on the ratio of the peak areas of the reference compounds extracted to the peak area of 4-nonanol, using calibration graphs for each standard constructed using solutions at known concentrations.

GC-FID Conditions. A Varian 3800 gas chromatograph equipped with a SolGel-Wax (SGE) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The flow of hydrogen 5.7 carrier gas was 1 mL/min. The oven temperature was kept at 35 °C for 5 min, then programmed to 150 °C at 3 °C/min and to 250 °C at 5 °C/min, and kept at 250 °C for 10 min. Injections (2 μL) were performed at 220 °C in splitless mode (3 min) using a CombiPAL autosampler (CTC Analytics). The FID detector was kept at 250 °C. The levels of the volatile compounds in the authentic sample juices were expressed as 2-octanol equivalents (assuming all of the response factors were 1). The concentrations are to be considered as relative data because recovery after extraction and calibration factors related to the standard were not determined.

GC-MS Conditions. A Varian 3800 gas chromatograph was used with the same SolGel-Wax capillary column as above. Injections (1 μL) were performed at 220 °C in splitless mode (3 min) using a CombiPAL autosampler (CTC Analytics). The flow of helium 6.0 carrier gas was 1 mL/min. The oven temperature program was as above. A Saturn ion-trap mass spectrometer was used. Mass spectra were

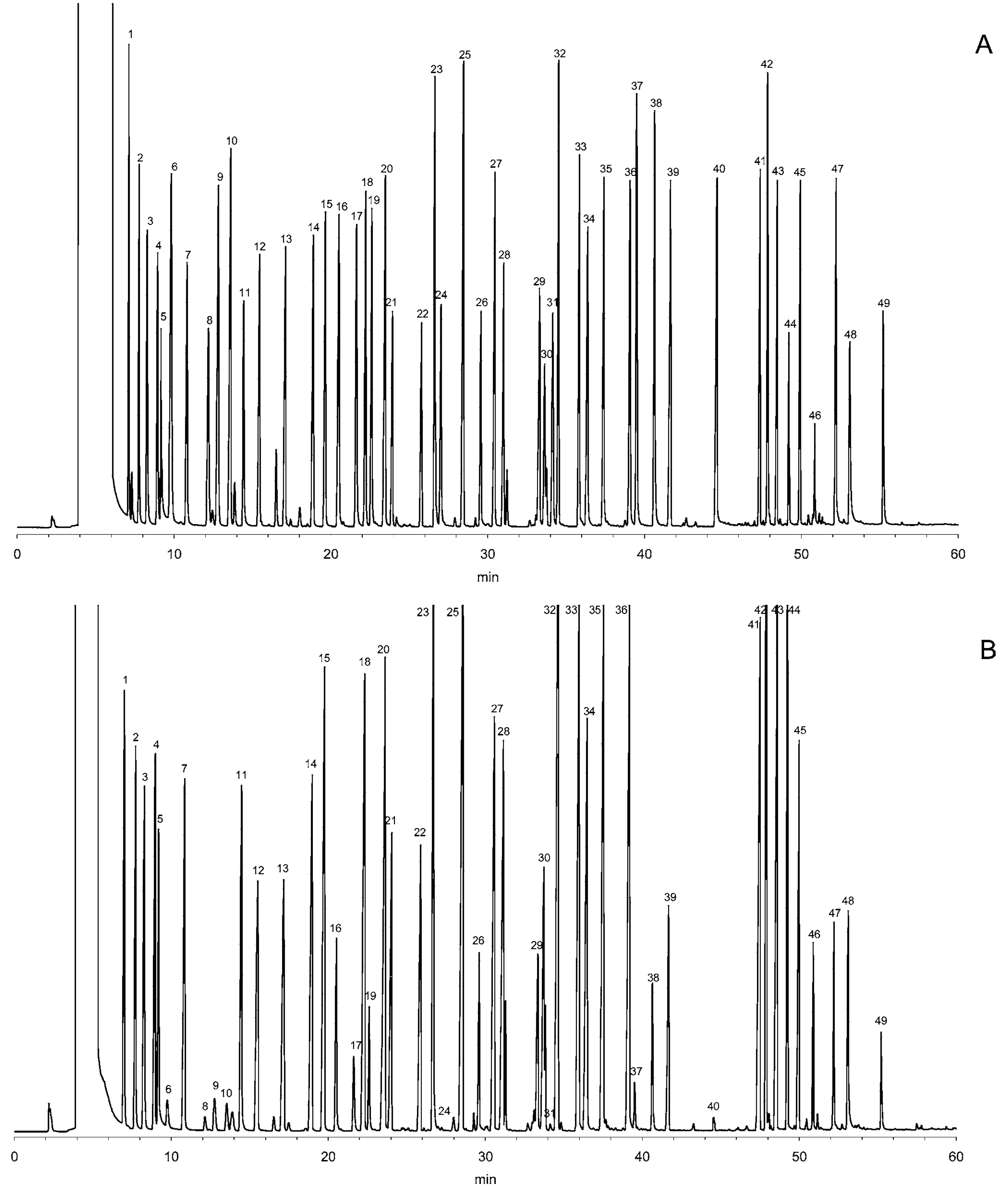

Figure 1. GC-FID chromatograms of the flavor test solution by direct injection (**A**) and by LLME sampling (**B**) (for component listing, see **Table 1**).

recorded in electron impact (EI) ionization mode. The ion trap, the manifold, and the transfer line temperatures were set, respectively, at 150, 45, and 250 °C. Mass spectra were scanned in the range m/z 30−350 amu at 1 s intervals. Identifications were carried out by comparison of linear retention indices and EI mass spectra with data from authentic compounds.

RESULTS AND DISCUSSION

LLME Performance. LLME was tested for common flavor compounds typically found in fruit aroma. **Figure 1** shows the GC-FID separation of the 49 volatile compounds from the flavor mixture (**Table 1**) both by direct injection and using the optimized microextraction procedure. The octanol/water partition coefficient (log P) and the linear retention index (RI) of the flavor compounds are shown in **Table 1**. The performance of the optimized LLME was compared to LLE by calculating the recoveries of the 49 flavor standards by both methods. A one-way analysis of variance was performed to determine the significant differences between LLME and LLE. The F test and

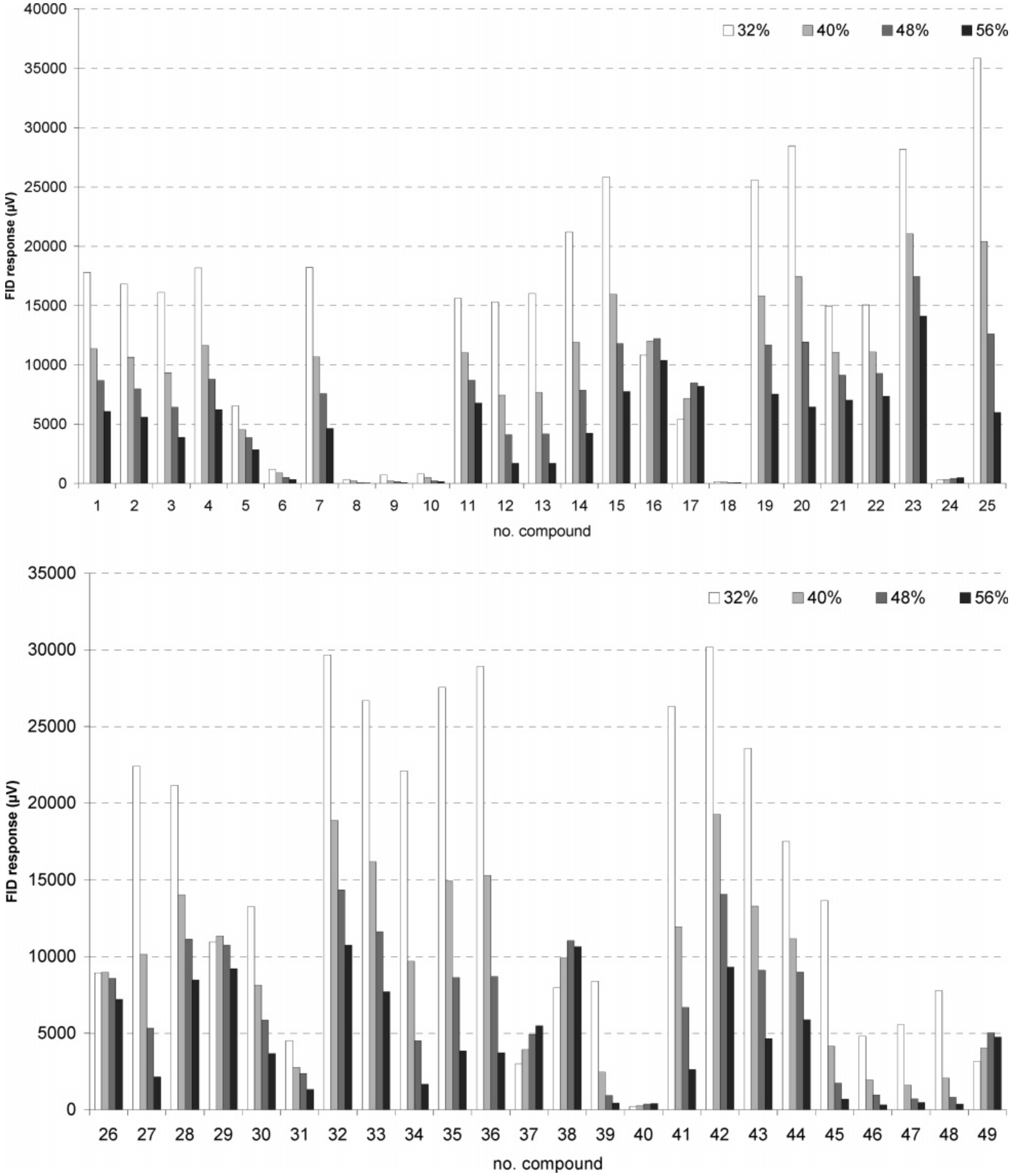

Figure 2. Effects of different $(NH_4)_2SO_4$ concentrations on the relative levels of reference compounds extracted by LLME (for component listing, see **Table 1**).

the *p* value of the ANOVA are also given in **Table 1**. As seen in **Figure 1** and **Table 1**, under the experimental conditions employed, most of the compounds are well extracted by LLME, particularly esters, thioesters, aldehydes, nitro compounds, terpenols, and lactones (except γ-dodecalactone). Generally, for these compounds, most of recoveries were found to be similar between the two methods, except for thioesters, benzyl acetate, δ-decalactone, 2-isobutylthiazole, and 6-methyl-5-hepten-2-one, for which recoveries by LLME were found to be significantly higher. In contrast, those observed for γ-hexalactone, mesifurane, hexyl acetate, ethyl hexanoate, and δ-dodecalactone were found to be significantly lower by the LLME method. As seen in **Table 1**, the main significant differences observed between the two methods were observed for C_6 alcohols, β-ionone, and three shikimic-derivatives (vanillin, 2-phenylethanol, and benzyl alcohol) and more particularly for terpenes, thioalcohols, and Furaneol, which are, respectively, fairly and badly extracted by the LLME method. As observed in **Table 1**, except for ethyl 2-(methylthio)acetate, 2-(methylthio)ethyl acetate, mesifurane, and γ-hexalactone, the efficiency of the LLME method is very poor for compounds with a log *P* lower or higher than 1, and it would seem that the most polar, and/or apolar, compounds are rather badly extracted by this method. Nevertheless, it is noteworthy that terpenes and Furaneol are also fairly extracted

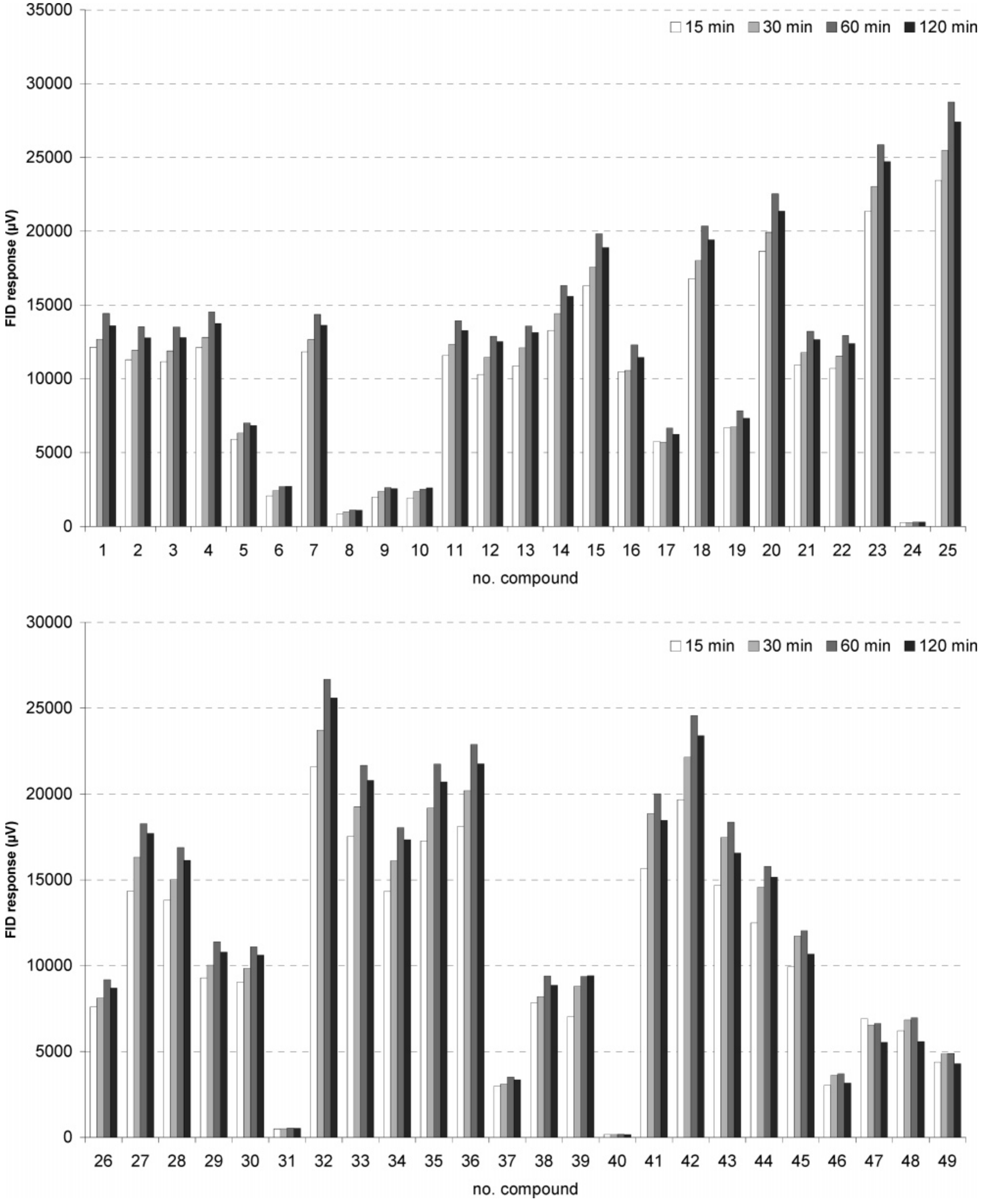

Figure 3. Effects of different times of extraction on the relative levels of reference compounds extracted by LLME (for component listing, see **Table 1**).

by the conventional LLE. **Table 1** shows the relative standard deviations (%RSD) for the recovery of flavor compounds by LLME. Most of the compounds, even those badly extracted, had a very good %RSD. The average relative standard deviation of 4% was judged to be satisfactory compared to that observed for LLE (8%). As observed in **Figure 1**, a detection limit of the order of 1−50 ppb was estimated for linalool, benzyl acetate, eugenol, geraniol, benzaldehyde, methyl salicylate, nerol, 2-octanol, γ-decalactone, δ-decalactone, 6-methyl-5-hepten-2-one, 2-isobutyl thiazole, methyl anthranilate, citronellol, β-cyclocitral, 3-(methylthio)propyl acetate, (*Z*)-3-hexenyl acetate, isoamyl acetate, butyl acetate, (*E*)-2-hexenal, ethyl 2-(methylthio)acetate, isobutyl acetate, ethyl 2-methylbutyrate, 2-(methylthio)ethyl acetate, hexyl acetate, and ethyl butyrate. Ethyl hexanoate, γ-undecalactone, α-terpineol, hexanal, β-ionone, γ-hexalactone, hexanol, δ-dodecalactone, mesifurane, 2-phenylethanol, γ-dodecalactone, (*E*)-2-hexen-1-ol, δ-undecalactone, (*Z*)-3-hexen-1-ol, vanillin, benzyl alcohol, α-terpinene, β-pinene, limonene,

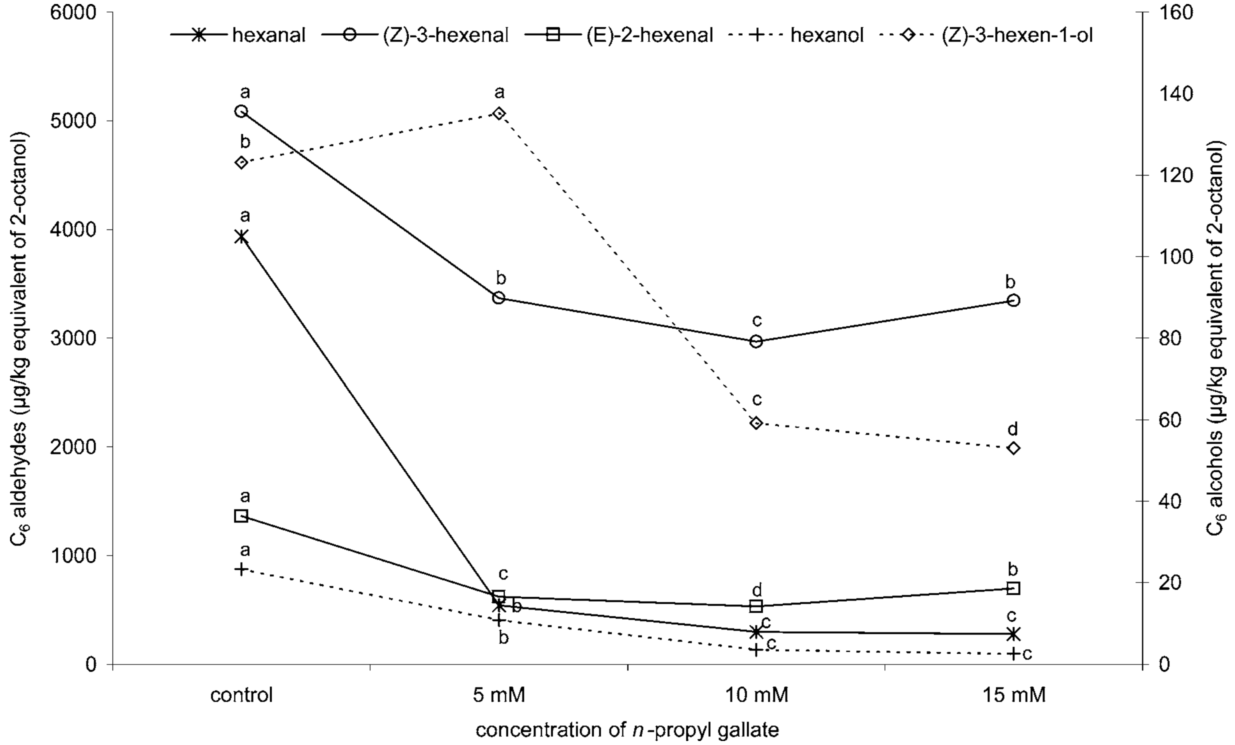

Figure 4. Effects of *n*-propyl gallate during homogenization on the levels of C_6 compounds in tomato (for a given compound, the levels with different letters are significantly different at $p < 0.05$).

and α-phellandrene could be detected at 0.05−1 ppm. A detection limit of 1 ppm was estimated for 2-(methylthio)-ethanol, 3-(methylthio)propanol, and Furaneol.

Extraction Optimization. In this study, different preliminary experiments were carried out using different salts (ammonium sulfate, sodium chloride, and calcium chloride). They have shown that ammonium sulfate was the most appropriate for achieving a good salting out effect. Use of sodium and/or calcium chloride (20−80%; w/v) generally resulted in a lower organic phase recovery and a high emulsion formation tendency. **Figure 2** shows the results of the extraction with different amounts of $(NH_4)_2SO_4$ (12.8−22.4 g of salt for 40 mL of sample, i.e., 32−56%; w/v). Except for hexanol (16), (Z)-3-hexen-1-ol (17), (*E*)-2-hexen-1-ol (19), 2-(methylthio)ethanol (24), benzyl alcohol (37), 2-phenylethanol (38), Furaneol (40), and vanillin (49), the FID response drastically decreased with increased amounts of salt, in particular for ethyl hexanoate (12), hexyl acetate (13), linalool (25), β-cyclocitral (27), nerol (35), geraniol (36), citronellol (34); β-ionone (39); γ-decalactone (41), γ- and δ-undecalactone (45, 46), and γ- and δ-dodecalactone (47, 48). Best results were observed with 32% ammonium sulfate. The time of extraction has also a great influence on the extraction efficiency. **Figure 3** shows the results of the extraction with different times of extraction (15−60 min). Best results were observed with 60 min.

Application to Real Samples. Because six-carbon (C_6-) volatiles—including the aldehydes hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenal, as well as their corresponding alcohols—in fruits and vegetables are known to be mainly produced from lipids during the crushing of plant tissues, the efficiency of *n*-propyl gallate (nPG), a well-known LOX inhibitor due to its electron-donating ability (*14*−*16*), was tested to reduce the formation of these secondary compounds during processing. **Figure 4** shows the levels of C_6 aldehydes and alcohols in a tomato sample in which enzyme deactivation was performed at different levels of nPG during homogenization. As seen in **Figure 4**, except for (*Z*)-3-hexen-1-ol at 5 mM of nPG, the C_6 compounds showed a similar pattern. Their levels, and in particular those of C_6 aldehydes, decreased drastically with increased concentrations of nPG and reached their lowest amounts for 10 mM LOX inhibitor. These results were consistent with those previously reported by Todd et al. (*17*) and Cass et al. (*18*).

The optimized method using a level of 10 mM of *n*-propyl gallate, 32% ammonium sulfate, and an extraction time of 60 min was then applied to the analysis of the volatile compounds of a red table grape (cv. Muscat de Hambourg), a Charentais cantaloupe melon (cv. Figaro), a strawberry (cv. Mara des Bois), a white peach (cv. Lauriered), and a grape tomato (cv. Cheers). **Figures 5**−**9** show the chromatograms of the different samples obtained by both conventional LLE and LLME. The precision of the LLME method was estimated by performing three replicate extractions. The corresponding standard deviation (SD) was calculated and expressed as a percentage (%RSD). Data are reported in **Tables 2**−**6**.

Charentais Melon. As seen in **Figure 5**, most of the flavor compounds extracted by conventional dichloromethane extraction were also extracted by LLME, albeit with somewhat different relative recoveries. The sensitivity of LLME in this example was generally comparable, even higher, than that of the conventional method for most compounds, particularly for esters (methyl 2-methylbutanoate, 2-methylpropyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, butyl acetate, 2-methylbutyl acetate, ethyl hexanoate, and hexyl acetate) and thioesters [methyl 2-(methylthio)acetate, ethyl 2-(methylthio)acetate, 2-(methylthio)ethyl acetate, and 3-(methylthio)propyl acetate], which are generally recognized as the most important volatile constituents of Charentais melon aroma (*19*−*23*). In contrast, alcohols (2-methylpropanol, butanol, isoamyl alcohol, 2-phenylethanol), acetoin (3-hydroxy-2-butanone), and thioalcohols [3-(methylthio)propanol and 2-(methylthio)ethanol] were, respectively, fairly and badly extracted. As observed in **Table 2**, except for (*Z*)-3-hexenyl acetate, benzaldehyde, and 3-phenyl-

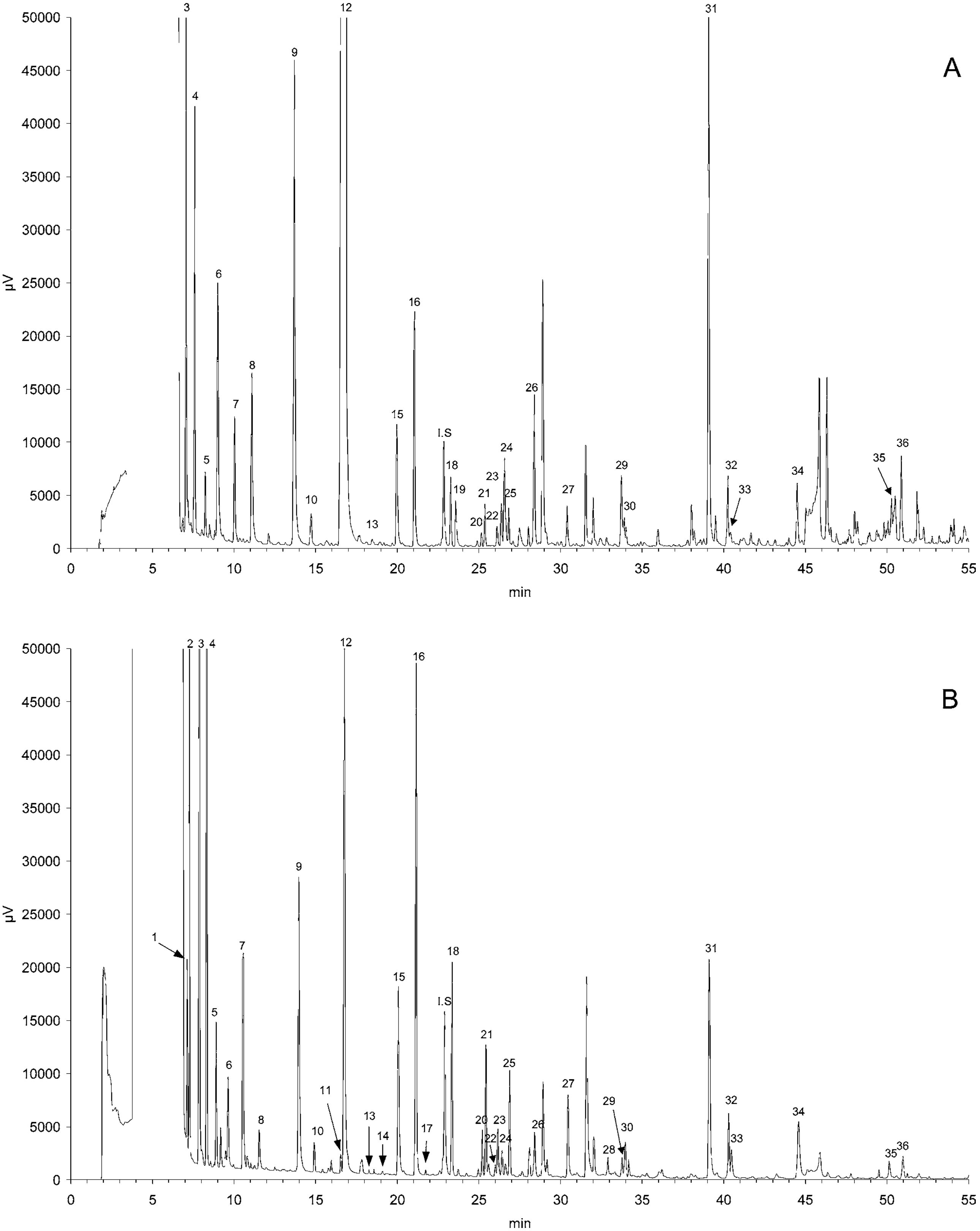

Figure 5. GC-FID chromatograms of a Charentais cantaloupe melon (cv. Figaro) by (**A**) conventional dichloromethane extraction and (**B**) LLME [1, methyl 2-methylbutanoate; 2, 2-methylpropyl acetate; 3, ethyl butanoate; 4, ethyl 2-methylbutanoate; 5, butyl acetate; 6, 2-methylpropanol; 7, 2-methylbutyl acetate; 8, butanol; 9, 2-methylbutanol; 10, ethyl hexanoate; 11, hexyl acetate; 12, 3-hydroxy-2-butanone (acetoin); 13, (*Z*)-3-hexenyl acetate; 14, 6-methyl-5-hepten-2-one; 15, hexanol; 16, (Z)-3-hexen-1-ol; 17, methyl 2-(methylthio)acetate; I.S., 2-octanol; 18, ethyl 2-(methylthio)acetate; 19, acetic acid; 20, 2-(methylthio)ethyl acetate; 21, 2,3-butanediol diacetate (isomer 1); 22, benzaldehyde; 23, ethyl 3-hydroxybutanoate; 24, 2-(methylthio)-ethanol; 25, 2,3-butanediol diacetate (isomer 2); 26, ethyl 3-(methylthio)propanoate; 27, 3-(methylthio)propyl acetate; 28, γ-hexalactone; 29, 3-(methylthio)-propanol; 30, benzyl acetate; 31, benzyl alcohol; 32, 2-phenylethanol; 33, γ-octalactone; 34, 3-phenyl-1-propanol; 35, 3-phenyl-2-propen-1-ol; 36, dihydroactinidiolide].

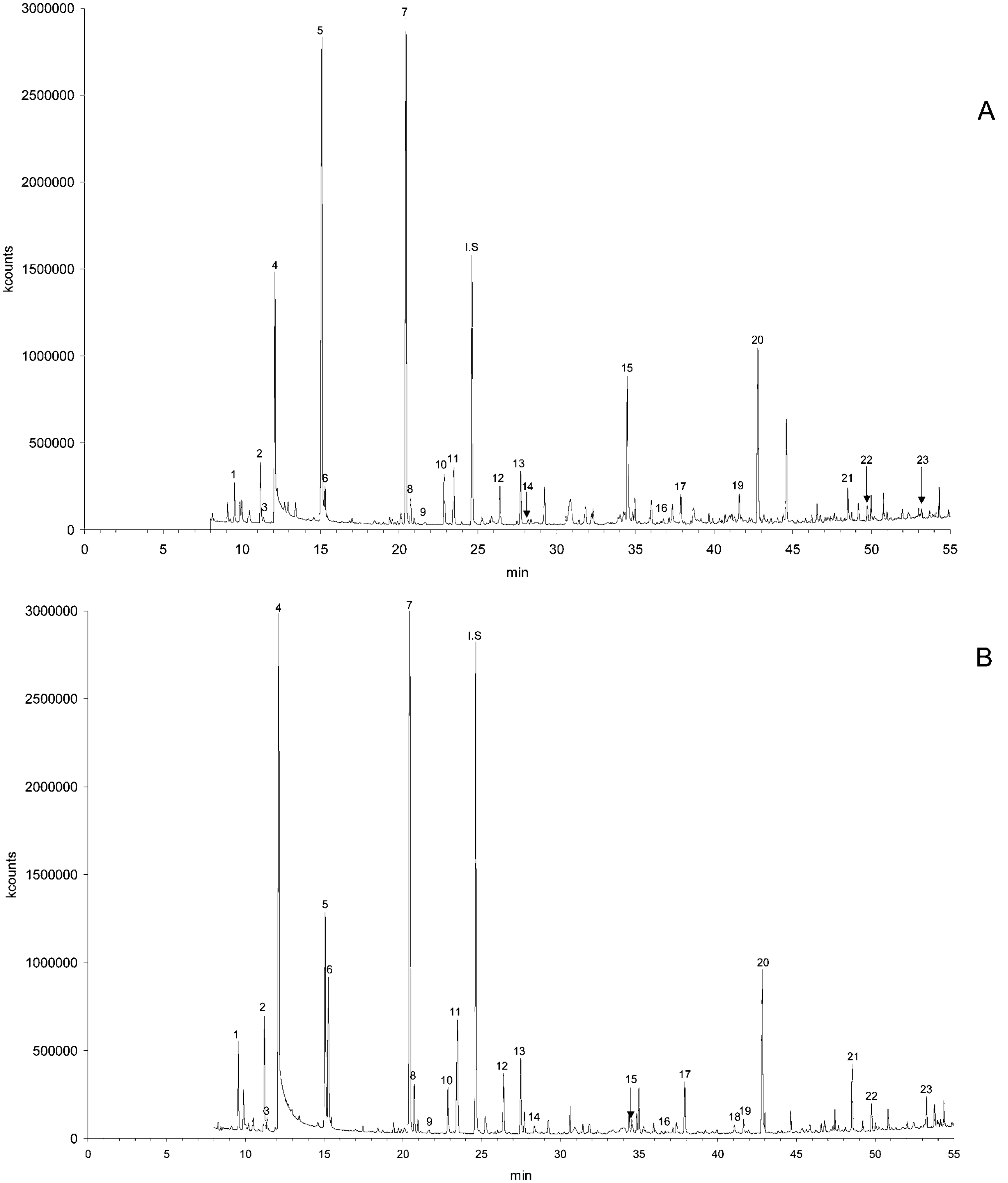

Figure 6. GC-MS chromatograms of a grape tomato (cv. Cheers) by (**A**) conventional dichloromethane extraction and (**B**) LLME [1, hexanal; 2, isobutyl cyanide; 3, (*E*)-2-pentenal; 4, (*Z*)-3-hexenal; 5, isoamyl alcohol; 6, (*E*)-2-hexenal; 7, 1-nitro-3-methylbutane; 8, 6-methyl-5-hepten-2-one; 9, hexanol; 10, (*Z*)-3-hexen-1-ol; 11, 2-isobutylthiazole; I.S., 2-octanol; 12, 6-methyl-5-hepten-2-ol; 13, 2-ethyl-1-hexanol; 14, benzaldehyde; 15, isovaleric acid; 16, geranial; 17, methyl salcylate; 18, guaiacol; 19, benzyl alcohol; 20, 2-phenylethanol; 21, 1-nitro-2-phenylethane; 22, eugenol; 23, dihydroactinidiolide].

2-propen-1-ol, the relative standard deviation values were below 10%. With an average relative standard deviation of 7.2%, the precision of the proposed method was judged to be satisfactory compared with other isolation procedures.

Grape Tomato. As seen in **Figure 6**, C_6 compounds [hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, and (*Z*)-3-hexen-1-ol], nitro compounds (isobutyl cyanide, 1-nitro-3-methylbutane, 2-isobutylthiazole, and 1-nitro-2-phenylethane), methyl salicylate, and 2-phenylethanol, compounds previously identified as important aroma components in tomato flavor (*24*, *25*), were generally better extracted by LLME. As previously observed, polar compounds such as isovaleric acid and isoamyl alcohol were poorly extracted compared to the classical LLE. As indicated in **Table 3**, the %RSD values ranged between 1 and 8.6%. With an average relative standard deviation of 5.2%, the precision of the method in this example was judged to be very good.

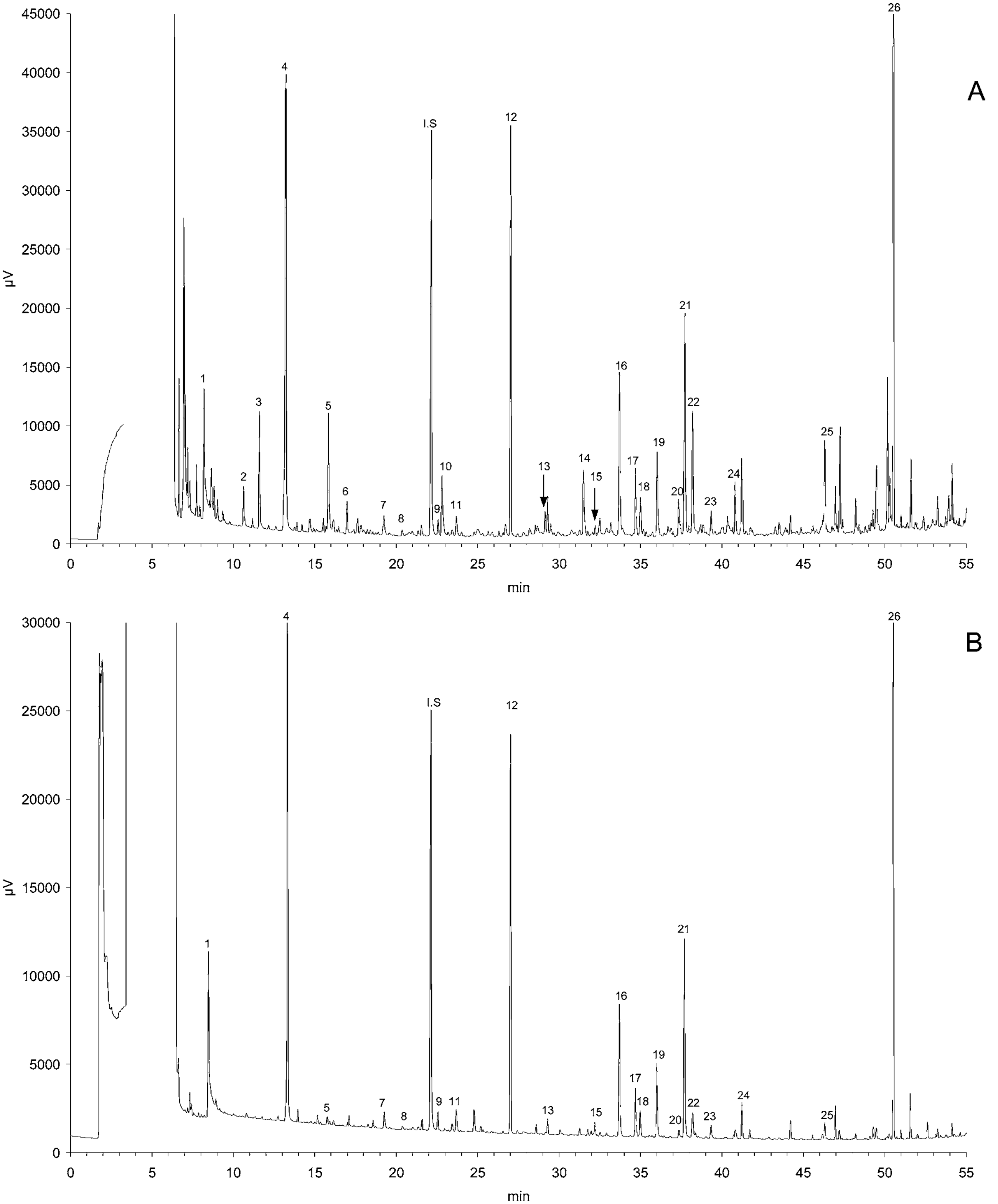

Figure 7. GC-FID chromatograms of a red table grape (cv. Muscat de Hambourg) by (**A**) conventional dichloromethane extraction and (**B**) LLME [1, hexanal; 2, butanol; 3, 3-penten-2-ol; 4, (*E*)-2-hexenal; 5, 3-hydroxy-2-butanone; 6, 4-methyl-2-pentanol; 7, hexanol; 8, (*Z*)-3-hexen-1-ol; I.S., 2-octanol; 9, (*E*)-furan linalool oxide; 10, acetic acid; 11, (*Z*)-furan linalool oxide; 12, linalool; 13, 3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol); 14, 1-methyl-2-pyrrolidinone; 15, α-terpineol; 16, (*E*)-pyran linalool oxide; 17, (*Z*)-pyran linalool oxide; 18, citronellol; 19, nerol; 20, hexanoic acid; 21, geraniol; 22, benzyl alcohol; 23, 2-phenylethanol; 24, 3,7-dimethyl-1,7-octadiene-3,7-diol (diol 37); 25, 2,6-dimethyl-1,7-octadiene-3,6-diol (diol 36); 26, geranic acid].

Red Table Grape. The importance of the terpenoid fraction in the characteristic “floral” aroma of Muscat grapes is now widely accepted, linalool, geraniol, nerol, citronellol, α-terpineol, and linalool oxides being, qualitatively and quantitatively, the main volatile compounds (*26*). As shown in **Figure 7**, excepted for butanol, 3-penten-2-ol, acetoin (3-hydroxy-2-butanone), 1-methyl-2-pyrrolidinone, and benzyl alcohol, the sensitivity of the LLME is comparable to that of the conventional method, in particular for C_6 aldehydes [hexanal and (*E*)-2-hexenal], monoterpenols (linalool, geraniol, nerol, citronellol, and α-ter-

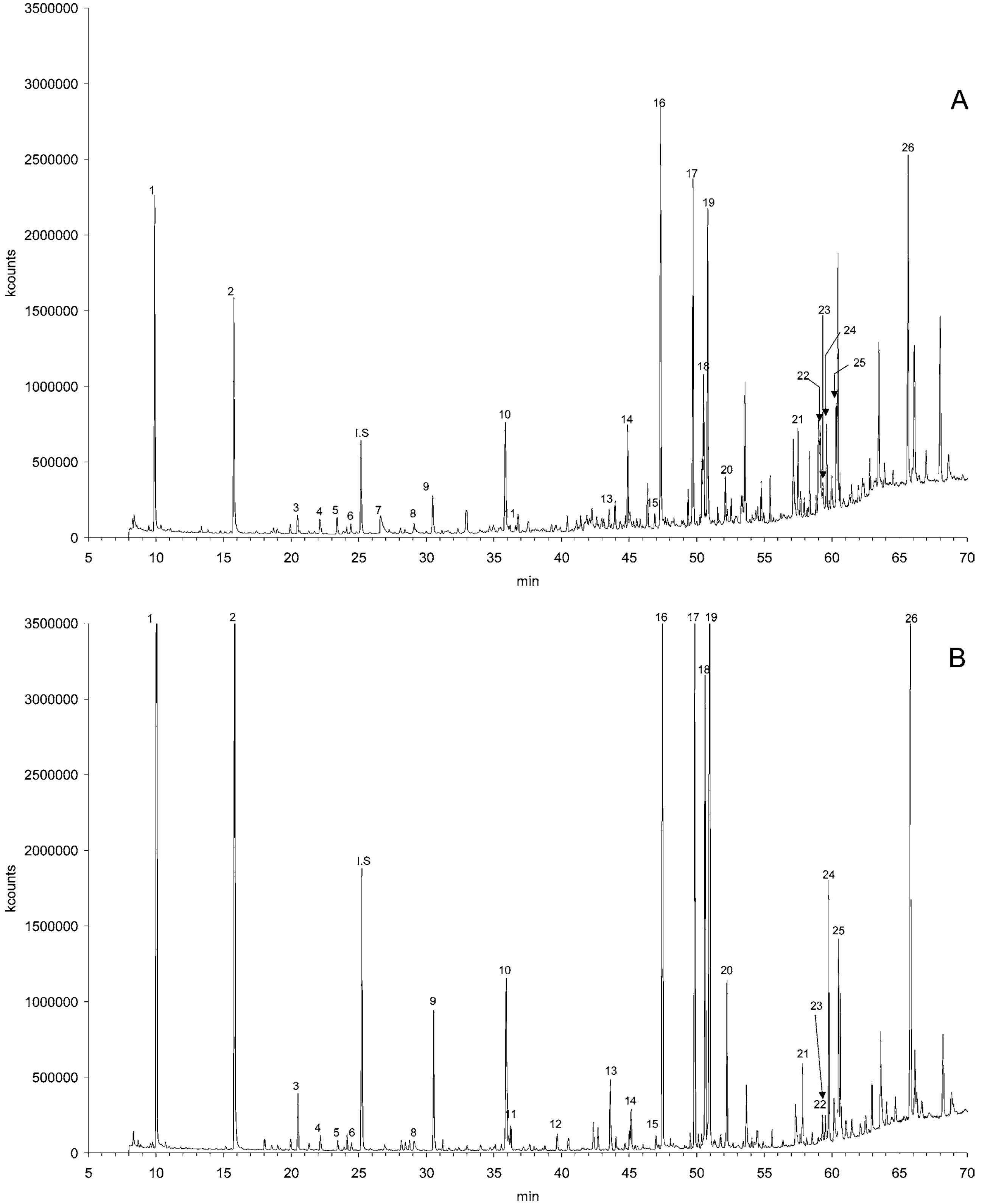

Figure 8. GC-MS chromatograms of a white peach (Lauriered) by (**A**) conventional dichloromethane extraction and (**B**) LLME [1, hexanal; 2, (*E*)-2-hexenal; 3, (*Z*)-3-hexenyl acetate; 4, hexanol; 5: (*Z*)-3-hexen-1-ol; 6, (*E*)-2-hexen-1-ol; I.S., 2-octanol; 7, acetic acid; 8, benzaldehyde; 9, linalool; 10, γ-hexalactone; 11, α-terpineol; 12, γ-heptalactone; 13, γ-octalactone; 14, 3,7-dimethyl-1,5-octadiene-3,7-diol; 15, γ-nonalactone; 16, unknown C_{13} norisoprenoid; 17, γ-decalactone; 18, γ-jasmolactone and 6-pentyl-α-pyrone; 19, δ-decalactone; 20, (*Z*)-7-decen-5-olide; 21, 3-hydroxy-7,8-dihydro-β-ionone (isomer 1); 22, 3-hydroxy-7,8-dihydro-β-ionone (isomer 2); 23, 3-hydroxy-7,8-dihydro-β-ionol; 24, 3-hydroxy-β-ionone; 25, 3-hydroxy-5,6-epoxy-β-ionone; 26, dehydrovomifoliol].

pineol), linalool oxides [furan and pyran linalool oxides (*E*, *Z*)], and geranic acid. As mentioned in **Table 4**, the %RSD values, ranging between 0.6 and 11.2%, were acceptable, the average relative standard deviation being ~6%.

White Peach. Peach and nectarine volatiles have been intensively investigated, and ~100 compounds have been identified. Among them, lactones, particularly γ- and δ-decalactone, have been reported as character impact compounds in

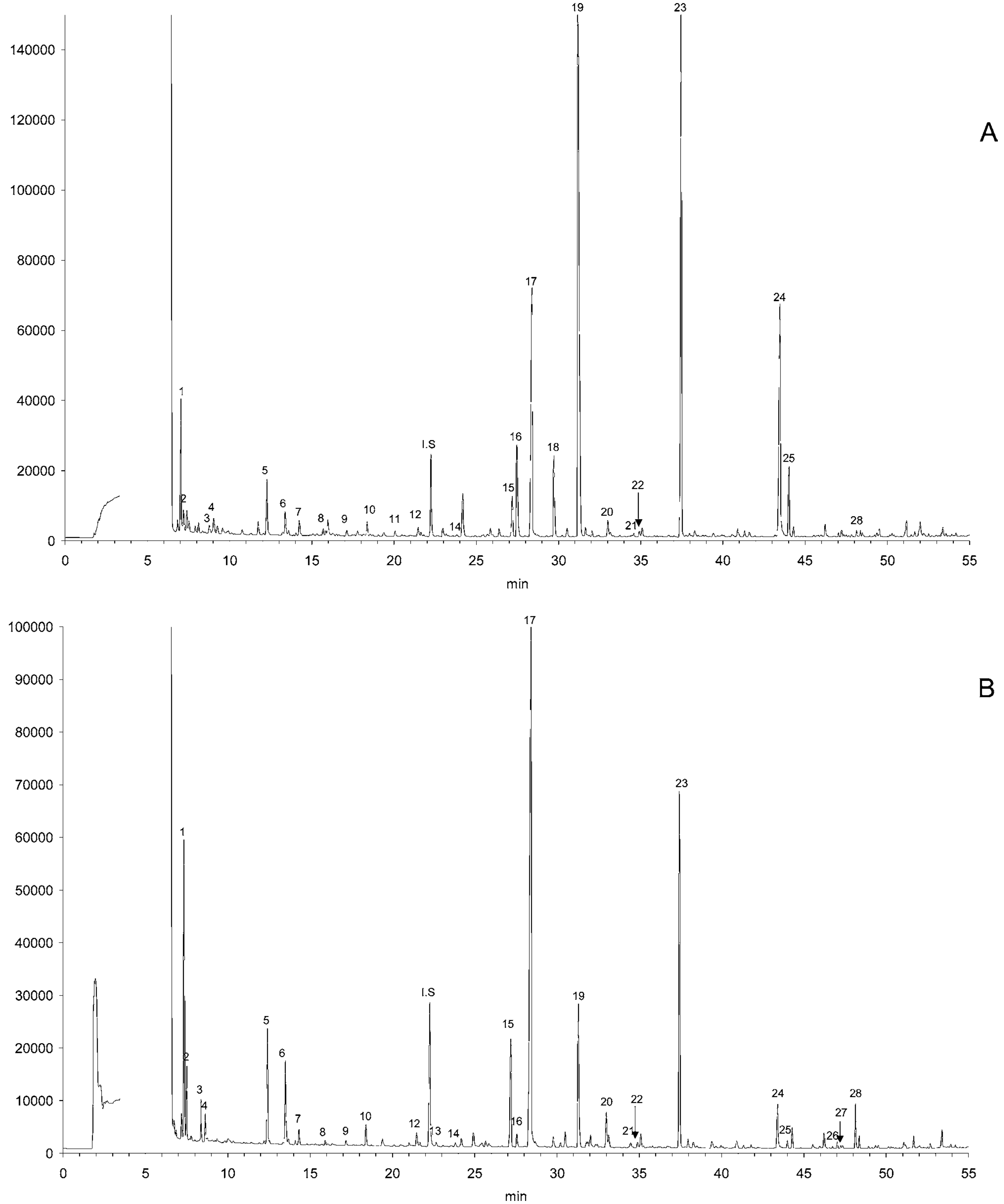

Figure 9. GC-FID chromatograms of a strawberry (cv. Mara des Bois) by (**A**) conventional dichloromethane extraction and (**B**) LLME [1, ethyl butanoate; 2, butyl acetate; 3, hexanal; 4, methyl hexanoate; 5, (*E*)-2-hexenal; 6, butyl acetate; 7, ethyl hexanoate; 8, hexyl acetate; 9, (*E*)-2-hexenyl acetate; 10, hexanol; 11, (*Z*)-3-hexen-1-ol; 12, (*E*)-2-hexen-1-ol; I.S., 2-octanol; 13, (*E*)-furan linalool oxide; 14, (*Z*)-furan linalool oxide; 15, linalool; 16, 2-methylpropanoic acid; 17, mesifurane; 18, butanoic acid; 19, 2-methylbutanoic acid; 20, benzyl acetate; 21, methyl salicylate; 22, methyl nicotinate; 23, hexanoic acid; 24, Furaneol; 25, nerolidol; 26, eugenol; 27, δ-decalactone; 28, methyl anthranilate].

peach flavor. The importance of other volatiles, such as C_6 aldehydes, terpenes, and C_{13} norisoprenoid compounds, has been also previously described (*27−31*). As seen in **Figure 8**, most of the compounds were generally better extracted by LLME, in particular C_6 aldehydes [hexanal and (*E*)-2-hexenal], linalool, lactones [γ- C_6, C_7, C_8, C_{10}, δ-C10, and (*Z*)-7-decen-5-olide], and C_{13} norisoprenoid compounds (3-hydroxy-β-ionone, 3-hydroxy-5,6-epoxy-β-ionone, and 4,5-dihydrovomifoliol). **Table 5** shows the precision of the method in this example. Except for an unknown C_{13} norisoprenoid compound and 3-hydroxy-

Table 2. Levels[a] of Volatile Compounds in Charentais Melon (Cv. Figaro)

no.	RI[b]	assignment[c]	compound	mean	SD	%RSD
1	1003	A	methyl 2-methylbutanoate	147.3	10.1	6.9
2	1009	A	2-methylpropyl acetate	579.3	43.6	7.5
3	1028	A	ethyl butanoate	1488.1	92.9	6.2
4	1043	A	ethyl 2-methylbutanoate	1040.2	86.7	8.3
5	1062	A	butyl acetate	211.5	13.8	6.5
6	1068	A	2-methylpropanol	160.6	11.6	7.2
7	1112	A	2-methylbutyl acetate	397.8	30.2	7.6
8	1118	A	butanol	82.5	5.6	6.8
9	1182	A	2-methylbutanol	641.5	33.4	5.2
10	1220	A	ethyl hexanoate	50.5	4.6	9.2
11	1258	A	hexyl acetate	24.4	2.4	9.8
12	1265	A	3-hydroxy-2-butanone (acetoin)	1330.2	101.5	7.6
13	1298	A	(*Z*)-3-hexenyl acetate	3.6	0.5	14.7
14	1317	A	6-methyl-5-hepten-2-one	5.6	0.3	5.8
15	1340	A	hexanol	415.0	20.2	4.9
16	1366	A	(*Z*)-3-hexen-1-ol	891.2	34.0	3.8
17	1386	A	methyl 2-(methylthio)acetate	4.4	0.2	3.7
18	1418	A	ethyl 2-(methylthio)acetate	331.2	15.8	4.8
20	1464	A	2-(methylthio)ethyl acetate	71.8	3.5	4.9
21	1483	C	2,3-butanediol diacetate (isomer 1)	198.2	10.6	5.3
22	1485	A	benzaldehyde	5.8	1.3	21.8
23	1497	C	ethyl 3-hydroxybutanoate	89.9	6.0	6.6
24	1502	A	2-(methylthio)ethanol	48.3	2.3	4.8
25	1521	C	2,3-butanediol diacetate (isomer 2)	164.4	9.4	5.7
26	1561	A	ethyl 3-(methylthio)propanoate	101.3	4.2	4.1
27	1597	A	3-(methylthio)propyl acetate	170.8	9.4	5.5
28	1661	A	γ-hexalactone	24.7	2.3	9.3
29	1686	A	3-(methylthio)propanol	35.9	2.4	6.7
30	1691	A	benzyl acetate	184.1	10.7	5.8
31	1837	A	benzyl alcohol	575.5	33.8	5.9
32	1871	A	2-phenylethanol	166.9	8.5	5.1
33	1878	A	γ-octalactone	57.8	5.0	8.7
34	1985	C	3-phenyl-1-propanol	195.8	15.0	7.7
35	2121	C	3-phenyl-2-propen-1-ol	42.7	4.3	10.2
36	2156	C	dihydroactinidiolide	90.3	7.9	8.8

[a] Values expressed in μg/kg equiv of 2-octanol. [b] Linear retention index based on a series of *n*-hydrocarbons. [c] A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; C, tentatively identified.

Table 3. Levels[a] of Volatile Compounds in Grape Tomato (Cv. Cheers)

no.	RI[b]	assignment[c]	compound	mean	SD	%RSD
1	1069	A	hexanal	45.9	2.5	5.4
2	1090	C	isobutyl cyanide	124.2	6.4	5.2
3	1096	C	(*E*)-2-pentenal	18.5	1.1	6.0
4	1111	A	(*Z*)-3-hexenal	887.1	33.6	3.8
5	1182	A	isoamyl alcohol	208.8	17.5	8.4
6	1198	A	(*E*)-2-hexenal	211.5	16.0	7.5
7	1308	B (*25*)	1-nitro-3-methylbutane	755.2	22.1	2.9
8	1317	A	6-methyl-5-hepten-2-one	33.7	1.2	3.6
9	1340	A	hexanol	2.4	0.1	4.0
10	1366	A	(*Z*)-3-hexen-1-ol	50.1	3.3	6.5
11	1378	A	2-isobutylthiazole	86.0	0.9	1.0
12	1461	C	6-methyl-5-hepten-2-ol	21.2	0.5	2.2
13	1488	C	2-ethyl-1-hexanol	44.1	0.7	1.5
14	1485	A	benzaldehyde	11.1	0.6	5.0
15	1581	A	isovaleric acid	7.4	0.6	7.9
16	1717	A	geranial	1.6	0.1	3.9
17	1727	A	methyl salicylate	29.9	1.3	4.4
18	1826	A	guaiacol	7.6	0.6	8.2
19	1837	A	benzyl alcohol	16.0	0.6	3.6
20	1871	A	2-phenylethanol	187.5	9.2	4.9
21	2032	B (*25*)	1-nitro-2-phenylethane	43.8	1.6	3.8
22	2091	A	eugenol	12.2	0.9	7.6
23	2156	C	dihydroactinidiolide	12.4	1.1	8.6

[a] Values expressed in μg/kg equiv of 2-octanol. [b] Linear retention index based on a series of *n*-hydrocarbons. [c] A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; B, identity tentatively assigned by comparing mass spectra with those obtained from the literature numbered and italicized in parentheses; C, tentatively identified.

Table 4. Levels[a] of Volatile Compounds in Red Table Grape (Cv. Muscat de Hambourg)

no.	RI[b]	assignment[c]	compound	mean	SD	%RSD
1	1069	A	hexanal	168.2	4.0	2.4
4	1198	A	(*E*)-2-hexenal	424.5	11.0	2.6
7	1340	A	hexanol	16.7	1.1	6.6
8	1366	A	(*Z*)-3-hexen-1-ol	3.3	0.4	11.2
9	1430	A	(*E*)-furan linalool oxide	16.4	0.8	4.6
11	1460	A	(*Z*)-furan linalool oxide	20.6	1.1	5.1
12	1532	A	linalool	262.9	9.6	3.7
13	1602	C	hotrienol	10.4	1.0	9.5
15	1668	A	α-terpineol	9.3	0.7	7.0
16	1716	A	(*E*)-pyran linalool oxide	115.0	10.0	8.7
17	1741	A	(*Z*)-pyran linalool oxide	43.7	3.8	8.7
18	1746	A	citronellol	22.1	0.6	2.7
18	1775	A	nerol	58.4	0.4	0.6
21	1824	A	geraniol	160.8	3.5	2.2
22	1837	A	benzyl alcohol	28.0	2.7	9.6
23	1871	A	2-phenylethanol	13.7	1.3	9.2
24	1910	A	3,7-dimethyl-1,7-octadiene-3,7-diol	28.0	2.2	7.9
25	2042	A	2,6-dimethyl-1,7-octadiene-3,6-diol	11.0	1.0	9.4
26	2151	A	geranic acid	426.9	27.8	6.5

[a] Values expressed in μg/kg equiv of 2-octanol. [b] Linear retention index based on a series of *n*-hydrocarbons. [c] A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; C, tentatively identified.

7,8-dihydro-β-ionone (isomer 2), the %RSD values, ranging between 0.6 and 12.8%, were judged to be satisfactory, the average relative standard deviation being ∼7%.

Strawberry. Because of its typical aroma, the volatile compounds of strawberry have been extensively studied, and >360 volatiles have been reported (*37*−*43*). Due to their very low odor threshold and their large amounts in several cultivars, Furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] (DHF) and mesifurane [2,5-dimethyl-4-methoxy-3(2*H*)-furanone] (DMF) are considered to be the two major flavor contributors. Previous studies have shown that linalool and esters, in particular, ethyl 2-methylbutanoate, methyl and ethyl butanoates, methyl and ethyl hexanoates, and hexyl and (*E*)-hex-2-enyl acetates, also significantly contribute to the strawberry aroma. Methyl anthranilate, which is characterized by an intense spicy-aromatic and flowery note, could be, according to Ulrich et al. (*44*), responsible for the typical character of the wild strawberry aroma. Finally, acids are generally described to have no impact on strawberry flavor, except for 2-methylbutanoic acid due to its relatively low odor threshold (180 ppb). As seen in **Figure 9**, the sensitivity of LLME is generally comparable to, or even higher than, that of the conventional LLE method for esters (ethyl butanoate, ethyl 2-methylbutanoate, butyl acetate, and methyl hexanoate), C_6 dldehydes [hexanal and (*E*)-2-hexenal], linalool, and mesifurane [2,5-dimethyl-4-methoxy-3(2*H*)-furanone]. In contrast, acids (2-methylpropanoic, butanoic, 2-methylbutanoic, and hexanoic acids) and Furaneol [2,5-dimethyl-

Table 5. Levels[a] of Volatile Compounds in White Peach (Cv. Lauriered)

no.	RI[b]	assign-ment[c]	compound	mean	SD	%RSD
1	1069	A	hexanal	1263.8	48.0	3.8
2	1198	A	(*E*)-2-hexenal	1031.5	27.4	2.7
3	1298	A	(*Z*)-3-hexenyl acetate	43.3	5.5	12.8
4	1340	A	hexanol	18.2	0.4	2.4
5	1366	A	(*Z*)-3-hexenol	11.7	0.8	6.9
6	1390	A	(*E*)-2-hexenol	9.9	0.7	7.5
8	1485	A	benzaldehyde	24.7	1.1	4.4
9	1532	A	linalool	159.0	6.1	3.8
10	1661	A	γ-hexalactone	452.1	9.0	2.0
11	1669	A	α-terpineol	15.4	0.5	3.4
12	1777	C	γ-heptalactone	23.2	0.9	3.9
13	1878	A	γ-octalactone	100.2	4.6	4.5
14	1910	C	3,7-dimethyl-1,5-octadiene-3,7-diol	49.4	2.1	4.2
15	1973	C	γ-nonalactone	14.1	1.2	8.6
16	1985		unknown C_{13} norisoprenoid[d]	667.0	174.7	26.2
17	2075	A	γ-decalactone	1004.3	100.8	10.0
18	2081	B (*32*)	γ-jasmolactone + 6-pentyl-α-pyrone	636.1	36.9	5.8
19	2114	A	δ-decalactone	1186.6	118.9	10.0
20	2121	B (*27*)	(*Z*)-7-decen-5-olide	206.7	14.3	6.9
21	2269	B (*33*)	3-hydroxy-7,8-dihydro-β-ionone (isomer 1)	111.5	1.3	1.2
22	2310	B (*33*)	3-hydroxy-7,8-dihydro-β-ionone (isomer 2)	13.6	4.6	33.9
23	2315	B (*34*)	3-hydroxy-7,8-dihydro-β-ionol	17.3	1.1	6.5
24	2317	B (*35*)	3-hydroxy-β-ionone	524.1	3.0	0.6
25	2339	B (*33*)	3-hydroxy-5,6-epoxy-β-ionone	397.9	9.5	2.4
26	2472	B (*36*)	4,5-dihydrovomifoliol	1828.0	33.7	1.8

[a] Values expressed in μg/kg equiv of 2-octanol. [b] Linear retention index based on a series of n-hydrocarbons. [c] A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; B, identity tentatively assigned by comparing mass spectra with those obtained from the literature numbered and italicized in parentheses; C, tentatively identified. [d] Major mass spectral fragments [*m*/*e* (%)]: 108 (100), 43 (70), 206 (69), 93 (67), 39 (62), 150 (44), 77 (30), 41 (28), 121 (27), 136 (26).

Table 6. Levels[a] of Volatile Compounds in Strawberry (Cv. Mara des Bois)

no.	RI[b]	assign-ment[c]	compound	mean	SD	%RSD
1	1028	A	ethyl butanoate	515.2	14.6	2.8
2	1043	A	ethyl 2-methylbutanoate	66.8	3.0	4.5
3	1062	A	butyl acetate	42.6	0.4	1.0
4	1069	A	hexanal	27.3	1.9	6.8
5	1159	A	methyl hexanoate	207.7	19.1	9.2
6	1198	A	(*E*)-2-hexenal	165.0	16.2	9.8
7	1120	A	ethyl hexanoate	33.1	1.6	4.9
8	1258	A	hexyl acetate	10.9	0.7	6.8
9	1319	A	(*E*)-2-hexenyl acetate	42.2	4.1	9.8
10	1340	A	hexanol	21.8	2.4	11.1
11	1366	A	(*Z*)-3-hexen-1-ol	5.0	0.8	15.8
12	1390	A	(*E*)-2-hexen-2-ol	34.1	2.8	8.1
13	1430	A	(*E*)-furan linalool oxide	5.8	0.6	10.5
14	1461	A	(*Z*)-furan linalool oxide	9.9	0.3	3.4
15	1532	A	linalool	251.5	12.9	5.1
16	1561	A	2-methylpropanoic acid	34.4	4.6	13.5
17	1565	A	mesifurane	1848.8	156.9	8.5
18	1624	A	butanoic acid	29.8	3.7	12.3
19	1665	A	2-methylbutanoic acid	376.8	51.5	13.7
20	1691	A	benzyl acetate	81.6	2.5	3.1
21	1743	A	methyl salicylate	15.9	0.6	3.9
22	1761	C	methyl nicotinate	15.1	2.1	13.6
23	1826	A	hexanoic acid	764.4	39.8	5.2
24	1994	A	Furaneol	97.2	15.5	15.9
25	2006	C	nerolidol	17.9	4.3	24.1
26	2091	A	eugenol	3.2	0.0	0.3
27	2114	A	δ-decalactone	4.5	0.3	6.2
28	2144	A	methyl anthranilate	58.8	1.5	2.5

[a] Values expressed in μg/kg equiv of 2-octanol. [b] Linear retention index based on a series of *n*-hydrocarbons. [c] A, identity confirmed by comparing mass spectra and retention time with those of authentic standards; C, tentatively identified.

4-hydroxy-3(2*H*)-furanone] are poorly extracted by LLME. Due to its water-soluble nature and its thermal instability, Furaneol (DHF) has always proved to be difficult to isolate by other isolation procedures (dynamic headspace, simultaneous distillation−extraction, or solid phase microextraction), and in most studies this compound is rarely quantified (*45*−*53*). Probably that the most reliable and accurate method for the quantitative analysis of DHF, and its methyl ether, is the stable isotope dilution analysis described by Sen et al. (*54*). Except for 2-methylbutanoic acid, which could contribute to strawberry aroma, the low affinity of LLME for fatty acids could be advantageous, for example, by reducing possible coelution of these compounds with other trace flavor constituents. As observed in **Table 6**, the %RSD values ranged between 0.3 and 24.1%. With an average relative standard deviation of 8.3%, the precision of the method in this example was judged to be acceptable.

Conclusion. Traditional LLE of volatile compounds is a widely used technique. However, this method involves large amounts of solvents, laborious and time-consuming extraction phases, and a possible thermal degradation of volatiles during the concentration step. In comparison, LLME provides many advantages. LLME is fast and simple and uses microquantities of solvent. Because this method requires no concentration step, LLME is less time-consuming than LLE and minimizes the risk of a thermal degradation of volatile compounds and the possible artifacts formation. This method also produces cleaner chromatograms compared with conventional LLE and prevents faster column degradation and rapid contamination of the MS source. Compared to conventional solvent extraction, except for some compounds, the sensitivity of LLME is generally comparable to, or even higher than, that for most volatile compounds. Finally, this method is generally as precise as classical extractions, or even better, and LLME is an interesting alternative for the analysis of volatile compounds in fruit and vegetable juices.

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