Optimized Likens–Nickerson Methodology for Quantifying Honey Flavors

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Dichloromethane extraction under an inert atmosphere followed by simultaneous steam distillation–dichloromethane extraction appears to be a useful method for honey flavor quantification. The organoleptic features of extracts obtained in this way closely match those of the honey samples. Recovery factors obtained for a large number of chemicals highlight the critical impact of parameters such as oxygen level, extraction time, and cold finger temperature. While recovery is excellent for around 70 tested chemicals when these optimized conditions are used, recovery factors must be taken into account for accurate quantification of hydrophilic compounds.

INTRODUCTION

To isolate volatile components from a complex matrix such as honey and to obtain very representative extracts remain major challenges to flavor chemists. Over the past 30 years, most studies in this field have been restricted to qualitative determinations. In the specific field of honey, accurate quantification now appears to be essential to evaluating flavor changes linked to new processing methods or long storage (microbiological or chemical degradation). Such knowledge would further be helpful in ascertaining a honey's floral origin without the ambiguity inherent in organoleptic tests. In this context, high concentrations of hydroxy ketones have already been reported as characteristic of *Eucalyptus* spp. and *Banksia* spp. honeys (Graddon et al., 1979). Citrus honeys (e.g. orange and lemon) are known to contain methyl anthranilate, a compound that other honeys seem to contain at concentrations of less than 0.5 ppm (Serra, 1988; White, 1975). A recent study (Bouseta et al., 1992) aimed at identifying the headspace composition of 84 unifloral honeys also revealed a range of compounds characteristic of the floral source (aldehydes in lavender honey; acetone in fir honey; diketones, sulfur compounds, and alkanes in eucalyptus honey). Further studies on less volatile flavor compounds are needed, however, to differentiate other kinds of honeys.

Scant quantitative data have been published in this area, probably due to the lack of accurate extraction methods. In 1973, Tschogowadse et al. attempted to isolate terpenoids in honey by steam distillation. One year later, Tsuneya et al. (1974) isolated 8-*p*-menthene-1,2-diol from an ether extract from 116 kg of linden (*Tilia* spp.) honey. Simple solvent extraction followed by concentration either under nitrogen or in a rotary evaporator was used by many workers in the following years (Berahia et al., 1993; Bonaga et al., 1986; Graddon et al., 1979; Steeg and Montag, 1987, 1988; Wootton et al., 1978). More recently, Tan et al. (1988, 1989a,b,
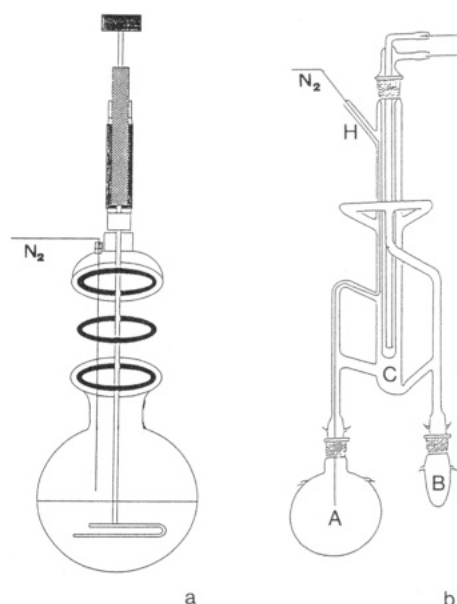
Figure 1. (a) Pre-extraction apparatus; (b) microextractor for simultaneous steam distillation–solvent extraction.

1990) proposed continuous liquid/liquid extraction with diethyl ether for the extraction of polar phenolic and acidic substances. This method was applied by Wilkins et al. (1993) to determine linalool derivatives and other heavy components in New Zealand honeys. Ferber and Nursten (1977) evaluated numerous flavor extraction protocols before selecting vacuum distillation at 65 °C as the method of choice. Bicchi et al. (1983) were the first to emphasize the importance of pre-extracting flavor compounds from sugars prior to heating. They proposed a two-step protocol including preliminary acetone extraction followed by simultaneous Likens–Nickerson steam distillation and solvent extraction (Likens and Nickerson, 1964; Nickerson and Likens, 1966).

In the present work, we have optimized this two-step method. Very good recovery factors are measured for most chemicals when very strict conditions are maintained. This makes it possible to plan a real quantification. Possibilities and limitations of the method are

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Table 1. Dichloromethane Extract Mean Concentrations (Parts per Billion) for Triplicates, Standard Deviations (SD), and Recovery Factors (R, Mean × 100/Initial Concentration, Percent) Obtained by Increasing Steam Distillation Time (Step 2)

steam distillation time (min)	benzaldehyde, initial concn 1219 ppb			camphene, initial concn 980 ppb			β-pinene, initial concn 1003 ppb			terpineol, initial concn 1450 ppb			verbenone, initial concn 1152 ppb			bornyl acetate, initial concn 1344 ppb			trans-caryophyllene, initial concn 1180 ppb		
	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)
15	682	12	56	475	14	49	512	11	51	891	37	61	582	58	51	804	33	60	719	32	61
30	1028	51	84	609	36	62	667	28	66	1435	122	99	1080	95	94	1308	121	97	1154	93	98
45	1042	10	86	750	37	77	800	33	80	1424	31	99	1125	18	98	1296	20	97	1159	10	98
60	946	13	78	670	14	68	657	36	66	1358	67	94	1105	81	96	1259	85	94	1108	53	94
90	972	26	80	599	20	61	652	23	65	1385	54	96	1105	54	96	1270	54	95	1130	53	96
120	866	18	71	542	20	55	555	48	55	1359	89	94	1171	122	102	1233	82	92	1045	51	89

Table 2. Dichloromethane Extract Mean Concentrations (Parts per Billion) for Triplicates, Standard Deviations (SD), and Recovery Factors (R, Mean × 100/Initial Concentration, Percent) Obtained by Decreasing Cold Finger Temperature (Step 2)

| cold finger temp (°C)	benzaldehyde, initial concn 1219 ppb			camphene, initial concn 980 ppb			β-pinene, initial concn 1003 ppb			terpineol, initial concn 1450 ppb			verbenone, initial concn 1152 ppb			bornyl acetate, initial concn 1344 ppb			trans-caryophyllene, initial concn 1180 ppb		
	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)
20	927	65	76	316	52	33	376	59	38	1305	98	90	1037	54	90	1196	51	89	1015	17	86
10	962	35	79	290	124	39	421	109	42	1305	28	90	1048	20	91	1156	71	86	1003	62	85
0	897	7	74	577	29	59	622	28	62	1276	28	88	1025	62	89	1169	23	87	1050	23	89
−5	885	16	73	575	46	59	632	43	63	1392	72	96	1163	56	101	1317	69	98	1192	57	101
−10	1048	10	86	755	37	77	802	33	80	1435	31	99	1129	18	98	1304	20	97	1156	10	98
−15	951	11	78	735	33	75	742	35	74	1247	134	86	1048	115	91	1183	125	88	1027	116	87
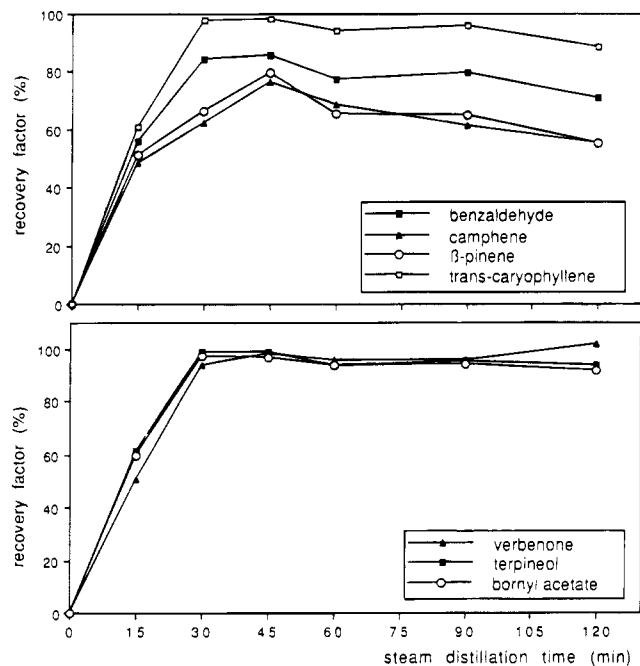
Figure 2. Mean recovery factors (percent) from triplicates obtained by increasing steam distillation time (step 2).
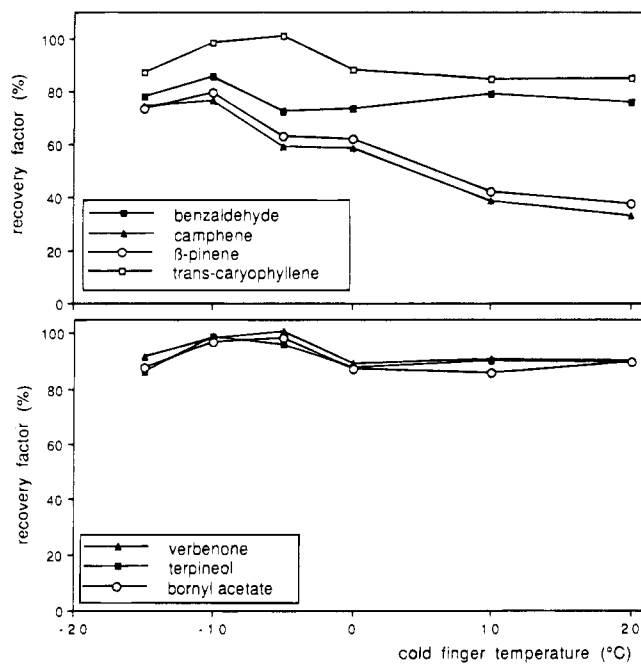

Figure 3. Mean recovery factors (percent) from triplicates obtained by decreasing cold finger temperature (step 2).

described. This technique has yielded organoleptically highly representative extracts for 220 unifloral honeys (data to be published).

MATERIALS AND METHODS

Honey Sample. A commercial Canadian honey was used for the extraction optimization. Pollen analyses revealed the presence of 97% clover pollen grains.

Honey Flavor Extraction. Solvent extraction (step 1) was first performed to remove the flavor compounds from the sugar matrix, which could induce artifacts by nonenzymatic browning reactions. After the vessel was purged with high-purity nitrogen, 100 g of honey and 200 mL of bidistilled dichloromethane were poured into the extraction apparatus shown in Figure 1a. The mixture was stirred for 60 min at 140 rpm under a 2 mL/min nitrogen stream to avoid oxidation reactions.

Table 3. Dichloromethane Extract Mean Concentrations (Parts per Billion) for Quadruplicates (Except 0 °C, in Triplicates), Standard Deviations (SD), and Recovery Factors (R, Mean × 100/Initial Concentration, Percent) Obtained by Increasing Nitrogen Flow Rate (Step 2)

N flow rate (mL/min)	benzaldehyde, initial concn 896 ppb			camphene, initial concn 926 ppb			β-pinene, initial concn 599 ppb			terpineol, initial concn 600 ppb			verbenone, initial concn 683 ppb			bornyl acetate, initial concn 500 ppb			trans-caryophyllene, initial concn 438 ppb		
	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)	concn (ppb)	SD (ppb)	R (%)
0	771	10	86	713	43	77	479	24	80	588	16	98	669	13	98	480	9	96	429	5	98
2	878	17	98	898	38	97	581	29	97	594	13	99	689	15	101	505	15	101	429	20	98
5	896	29	100	898	44	97	575	35	96	594	17	99	683	19	100	500	14	100	420	21	96
10	824	17	92	852	18	92	551	16	92	564	4	94	649	3	95	475	3	95	398	10	91
60	699	74	78	167	46	18	108	29	18	522	16	87	601	18	88	300	42	60	210	20	48

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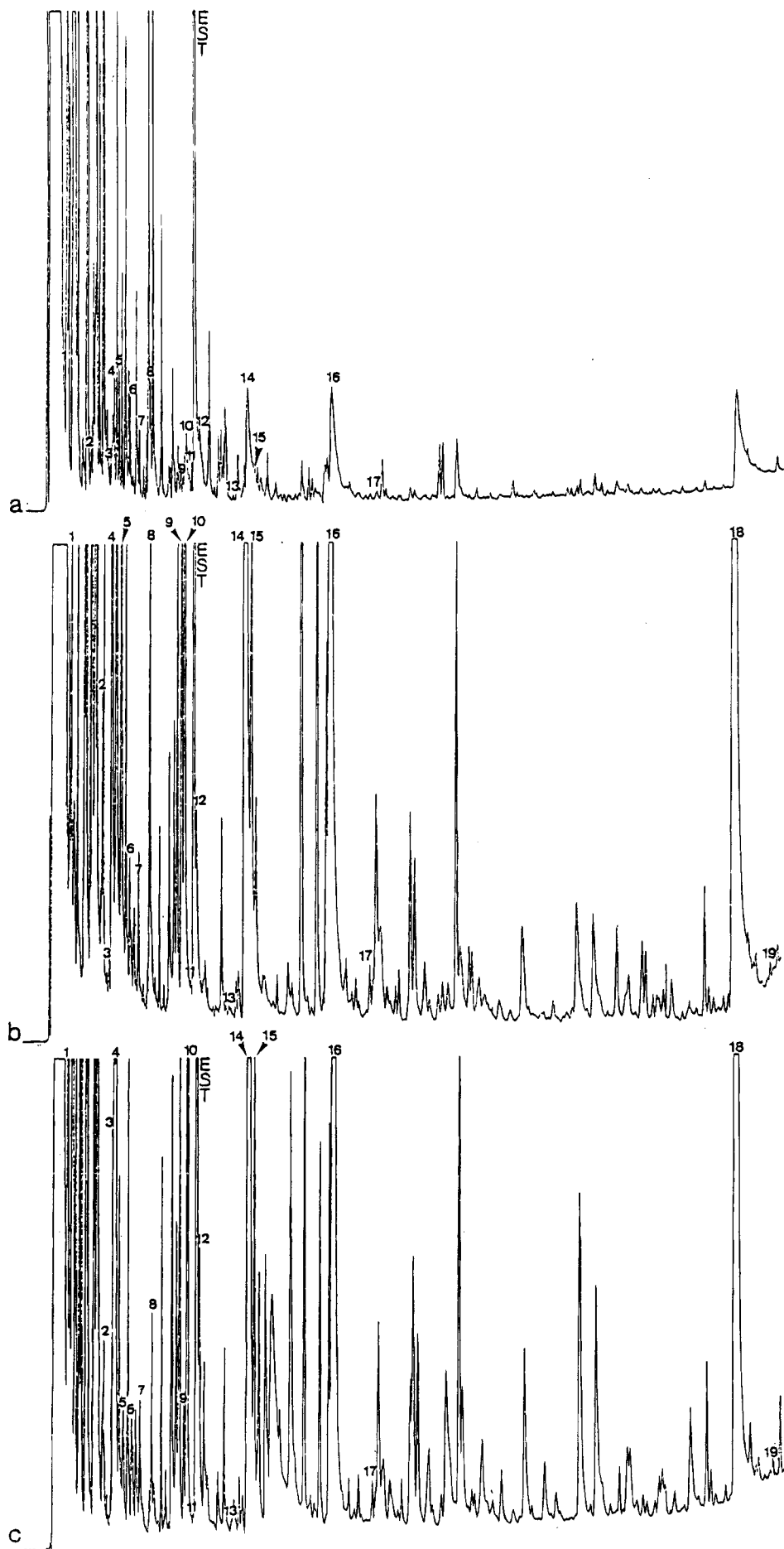
Figure 4. Chromatograms of honey extract with pentane (a), acetone (b), and dichloromethane (c).

Table 4. Honey Sample Extraction with Three Different Solvents (Step 1)[a]

compound	PN	RT (min)	identified by	pentane		acetone		dichloromethane	
				av (ppb)	CV (%)	av (ppb)	C V (%)	av (ppb)	C V (%)
methylfuran	1	5.6	G C	3	35	385	8	202	6
caproaldehyde	2	8.9	GC–MS	6	19	24	9	15	7
octane	3	9.5	GC–MS	2	99	1	19	20	11
furfural	4	9.7	GC–MS	5	18	656	2	467	8
furfuryl alcohol	5	10.1	GC–MS	28	22	370	24	51	5
1-hexanol	6	11.4	GC–MS	12	22	18	4	18	8
m-xylene	7	12.2	GC–MS	5	18	12	19	14	7
acetylfuran	8	13.2	GC–MS	35	18	247	28	35	10
5-methylfuraldehyde	9	16.0	GC–MS	8	24	157	8	22	5
benzaldehyde	10	16.3	GC–MS	9	18	254	4	230	10
α-pinene	11	16.9	GC–MS	8	21	1	12	5	8
phenol	12	17.4	GC–MS	3	32	51	18	51	20
β-pinene	13	19.6	GC–MS	8	19	19	10	22	7
benzyl alcohol	14	21.6	GC–MS	27	131	647	8	1109	13
phenylacetaldehyde	15	22.2	GC–MS	7	142	131	16	70	12
phenethyl alcohol	16	29.3	GC–MS	86	18	678	7	918	9
camphor	17	33.4	GC–MS	5	18	53	3	39	10
coumarin	18	66.5	CG–MS	262	10	2568	2	4451	5
trans-caryophyllene	19	68.7	GC–MS	11	19	3	6	3	10

[a] Peak numbering (PN) gives the order of elution through the column; RT, column retention time (min); GC, gas chromatographic retention data compared with those of authentic samples; MS, mass spectral data compared with those of library compounds and/or those of authentic samples. Average concentrations (ppb, calculated with a 100% recovery factor) and coefficients of variation (CV, standard deviation × 100/mean, %) obtained for three (in acetone and pentane) and five (dichloromethane) analyses of the same sample.

The dichloromethane extract was concentrated to 1 mL in a Kuderna-Danish flask maintained in a 45 °C water bath.

Steam distillation–solvent extraction (step 2) was carried out in a microextractor (Alltech 8910, Figure 1b) to remove flavor compounds from the coextracted heavy matrix; this yielded an extract suitable for on-column chromatographic injection. The previously obtained 1 mL extract was transferred to flask A (see Figure 1b) with five 200 µL aliquots of dichloromethane used for washing the vessel and 30 mL of ultrapure (Milli-Q water purification system, Millipore, Bedford, MA) deoxygenated water. Dichloromethane and ultrapure, deoxygenated water (1.5 mL each) were introduced into area C by arm H. A few clean grains of carborundum were successively introduced into flasks A and B. Prior to the procedure, the entire system was purged with nitrogen (2–3 mL/min) for 5 min. Flask A was then heated in a 140 °C oil bath. After 3 min, flask B was heated in a 90 °C water bath. The vapors were condensed in area C by means of a cold finger maintained at −10 °C by a cryostat. The entire steam distillation–solvent extraction procedure was carried out under a 2 mL/min nitrogen flow. The steam distillation was stopped after 45 min, and 2 mL of the dichloromethane extract was removed from flask B. The dichloromethane layer in area C was then collected in flask B by introducing 3 × 1 mL of dichloromethane through arm H; flask B was finally washed with 3 × 0.5 mL of dichloromethane. Fifty microliters of 1000 ppm chloroheptane was added to the combined extracts as an external standard. The extract was then concentrated to 0.25–0.5 mL in a Snyder Kuderna and a micro-Dufton column. One microliter was analyzed by GC and GC–MS.

Gas Chromatography Analytical Conditions. For gas chromatography, we used a Hewlett-Packard Model 5890 gas chromatograph equipped with a Hewlett-Packard Model 7673 automatic sampler, a cold on-column injector, a flame ionization detector, and a Shimadzu CR4A integrator. Analysis of the honey volatile compounds was carried out on a 50 m × 0.32 mm, wall-coated, open tubular (WCOT) CP-SIL5 CB capillary column (film thickness, 1.2 µm). The oven temperature was programmed to rise from 30 to 85 °C at 55 °C/min, then to 145 °C at 1 °C/min, and to 250 °C at 3 °C/min. The carrier gas was helium at a flow rate of 1.5 mL/min. The injector temperature was maintained at 3 °C above the oven temperature. The detector temperature was 275 °C. The minimum peak area for data acquisition was set at 500 µV·s.

Gas Chromatography–Mass Spectrometry Analytical Conditions. The column (see above) was directly connected to an HP 5988 quadrupole mass spectrometer. Electron

impact mass spectra were recorded at 70 eV. Spectral recording throughout elution was automatically performed with the HP59970C software. Peaks were identified by their enhancement after coinjection of authentic standard compounds and with the help of the NBS/EPA/NIH mass spectra library.

RESULTS AND DISCUSSION

Simultaneous Steam Distillation–Solvent Extraction (Step 2) Optimization. To obtain an accurate method for quantifying honey flavors, we optimized step 2 with respect to the distillation time, the cold finger temperature, and the oxygen level. This was done on a test mixture composed of the following suspected honey constituents: mono- and sesquiterpenes (camphene, β-pinene, and *trans*-caryophyllene), terpenic alcohols (terpineol), ketones (verbenone), esters (bornyl acetate), and aromatics (benzaldehyde). Recovery factors were checked with a 30 mL standard mixture of the above-listed compounds, diluted to concentrations close to 1 ppm in ultrapure, deoxygenated water. The pH of 5.7 at the beginning of the extraction was equal to the pH of a real honey extract [pH value obtained after extraction (step 1) of honey; see below].

We first determined the kinetic parameters of the steam distillation–solvent extraction step using all of the experimental conditions described under Materials and Methods apart from the nitrogen flow. Results on triplicates are listed in Table 1. Figure 2 clearly shows that the recovery factor reaches a maximum after 30 min. After 45 min and probably due to losses, the extraction efficiency slightly decreases for the most volatile compounds (benzaldehyde and monoterpenes; see Figure 2). With a 45 min extraction time, all of the recovery factors exceed 77%, with 97–99% for terpinol, verbenone, bornyl acetate, and *trans*-caryophyllene.

Next, we determined the optimal temperature of the cold finger. The data reported in Table 2 and Figure 3 emphasize how critical this parameter is. A temperature above −5 °C significantly decreases the extraction efficiency. The three most volatile chemicals, benzaldehyde, camphene, and β-pinene, even require a temperature of −10 °C. In the case of monoterpenes, less efficient condensations (boiling points under 165 °C) and

oxidation reactions are assumed to occur when the cold finger temperature exceeds 0 °C, leading to very low recovery (33–42%).

As suggested above, monoterpenes must be protected from oxidation. To determine the real impact of oxygen, steam distillation–solvent extraction was performed with and without a stream of nitrogen gas. Table 3 shows the favorable effect of a 2 mL/min nitrogen flow. However, as the nitrogen flow rate is increased, the recoveries of benzaldehyde, camphene, and β-pinene decrease from 98, 97, and 97% (at 2 mL/min), respectively, to 78, 18, and 18% (at 60 mL/min), respectively. As expected, higher nitrogen flow rates reduce the efficiency with which the more volatile compounds are condensed in the cold finger. Recovery ratios exceeding 97% were reproducibly obtained with a 2 mL/min nitrogen flow for all standard compounds. As will be demonstrated in the last section, however, this method is not recommended for temperature-sensitive molecules such as C_4–C_5 lactones.

In our preliminary tests, the nature of the vessel used to evaporate the solvent proved also to be of prime importance. Rotary evaporators and nitrogen purges, both frequently used for honey extracts, lead to the loss (up to 90%) of many volatiles. The system used here, i.e. the Kuderna-Danish flask (for concentration to 1 mL) and micro-Dufton column (for concentration to 0.25 mL), avoids the loss of any compound.

Solvent Extraction (Step 1) Optimization. The favorable effect of a nitrogen flow was also proven at this first step. In triplicate measurements, peak intensities varied significantly according to the size of the oxidized fraction unless nitrogen was used. Therefore, all extractions described below were carried out under an inert atmosphere.

Three different extracting solvents, pentane, acetone, and dichloromethane, were investigated on a Canadian honey. All of the experimental parameters were as described under Materials and Methods, including the steam distillation–solvent extraction step (step 2). Low yields of volatile extracts were obtained with pentane (Figure 4a), but qualitatively matching chromatograms (see Figure 4b,c) were obtained with the two other solvents. After acetone extraction, however, the extracts were richer in furan derivatives (Table 4, PN 1, 4, 5, 8, and 9), suggesting that nonenzymatic browning reactions may occur more easily. This observation can be related to the higher solubility of fructose and glucose in acetone than in dichloromethane. Moreover, concentration to 1 mL is time-consuming when acetone is used. The small amounts of furan derivatives detected in the dichloromethane extract were not due to an artifact but came from the honey sample itself, as demonstrated by another method dedicated to the analysis of more volatile compounds (Bouseta et al., 1992).

We further determined on the same honey sample the optimal dichloromethane extraction time in triplicates. The kinetic curves obtained differed considerably from one compound to another (see Figure 5). For all compounds, the amount extracted increased, as expected, with time up to about 60 min, due to slow solubilization of the flavor compounds. After this time, step 1 efficiency decreased for monoterpenes (depicted for β-pinene in Figure 5). A similar effect was observed a bit later for benzyl alcohol. On the other hand, no significant loss was noticed until 120 min for compounds
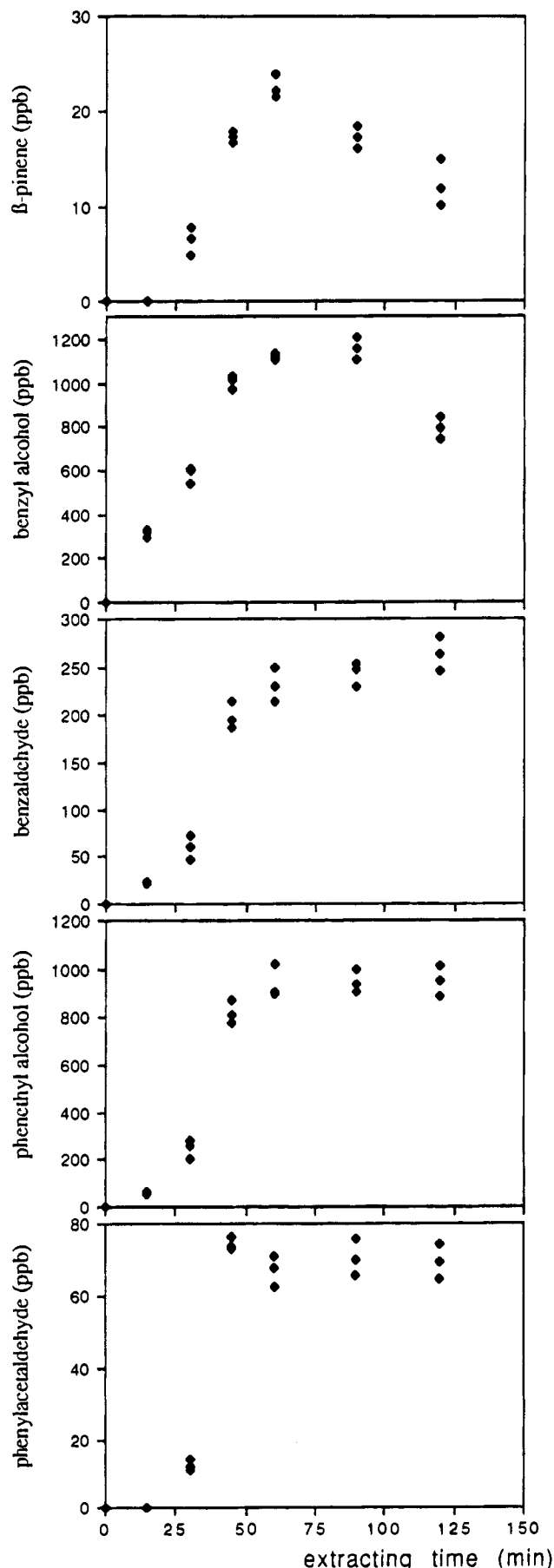
Figure 5. Honey volatile concentrations calculated with a 100% recovery factor for different extracting times in step 1 (experiences in triplicates).

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Table 5. Coefficients of Variation (CV, Standard Deviation × 100/Mean, Percent) and Recovery Factors (R, Mean × 100/Expected Concentration, Percent) Obtained for Five Analyses (Steps 1 and 2) of the Same Test Mixture (Concentrations around 100 ppb)

compound	RT (min)	CV (%)	R (%)	compound	RT (min)	CV (%)	R (%)
hydrocarbons				aldehydes/ketones			
octane	9.6	11	94	3,4-hexanedione	8.8	11	96
m-xylene	12.2	8	90	caproaldehyde	8.9	8	98
o-xylene	13.4	8	97	2-furaldehyde	9.8	5	87
nonane	14.1	9	83	trans-2-hexenal	10.7	9	89
α-pinene	16.6	8	89	2-heptanone	12.4	6	83
β-citronellene	16.7	3	101	heptanal	13.1	8	94
camphene	17.5	5	89	trans-2-heptenal	16.0	6	94
sabinene	19.0	9	91	5-methylfurfural	16.1	7	85
β-pinene	19.5	11	87	benzaldehyde	16.4	9	96
2-carene	21.3	10	92	2-octanone	18.7	7	78
α-phellandrene	21.3	5	94	octanal	19.7	8	105
3-carene	22.0	5	99	salicylaldehyde	22.6	11	76
p-cymene	22.6	8	79	trans-2-octenal	24.5	12	78
limonene	23.4	8	99	2-nonanone	27.7	9	98
γ-terpinene	26.0	9	92	l-fenchone	28.1	12	99
1,2,3,4-tetramethylbenzene	35.3	7	102	thujone	29.8	10	87
trans-caryophyllene	68.7	10	103	o-methylacetophenone	31.8	11	92
α-humulene	71.6	9	98	propiophenone	34.7	3	108
hexadecane	81.2	7	94	menthone	35.0	11	101
alcohols/phenols/ethers				2-decanone	38.9	10	99
3-methyl-3-buten-1-ol	7.3	4	95	verbenone	40.4	8	106
3-methyl-2-buten-1-ol	8.2	3	97	trans,trans-2,4-nonadienal	40.8	7	98
4-hydroxy-4-methyl-2-pentanone	10.2	28	34	p-anisaldehyde	44.0	4	84
furfuryl alcohol	10.6	29	28	carvone	44.4	11	91
n-hexanol	11.5	8	93	pulegone	44.5	8	101
cyclohexanol	12.4	3	103	perillaldehyde	48.3	9	103
phenol	17.5	25	43	2-undecanone	51.4	7	77
2-octanol	19.7	5	104	trans,trans-2,4-decadienal	53.5	9	73
benzyl alcohol	21.7	26	38	esters			
1,8-cineole	23.4	7	100	γ-butyrolactone	12.1	23	8
1-phenylethyl alcohol	24.3	14	72	γ-valerolactone	14.5	36	18
p-cresol	25.3	6	74	isoamyl butyrate	24.0	9	104
guaiacol	26.8	3	98	phenylethyl acetate	45.3	3	109
phenethyl alcohol	29.2	7	60	linalyl acetate	47.3	10	91
linalool	29.2	25	119	bornyl acetate	51.3	10	102
camphor	33.6	10	93	sulfur compound			
4-ethylphenol	35.0	14	62	dimethyl disulfide	7.8	8	86
borneol	36.8	12	102	furans			
menthol	37.6	9	102	2-methylfuran	5.5	22	72
terpinene-4-ol	38.1	10	96	2-acetylfuran	13.2	12	68
α-terpineol	39.4	10	105	nitrogen compounds			
4-allylanisole	39.7	8	100	indole	49.0	10	62
3-phenylpropan-1-ol	42.4	20	53	methyl anthranilate	55.8	18	70
β-citronellol	43.3	3	108				
trans-anethole	50.2	8	101				
thymol	50.7	8	105				
cinnamyl alcohol	51.0	15	28				
carvacrol	51.9	5	103				
eugenol	58.7	10	85				

such as benzaldehyde, phenylethyl alcohol, or phenyl-acetaldehyde. A 60 min solvent extraction time was selected.

Reproducibility of Standard Mixture Extraction (Steps 1 and 2). The reproducibility of the optimized method (see above), calculated for five consecutive analyses of a standard mixture, is given in Table 5. For most low-polarity compounds (hydrocarbons, aldehydes, ketones, acyclic esters, dimethyl disulfide, terpenic alcohols, etc.), variation coefficients below 12% and recovery factors above 70% (above 90% for 35 chemicals) are obtained. Poor recovery factors are calculated, however, for hydrophilic alcohols (low volatility) such as 4-hydroxy-4-methyl-2-pentanone, furfuryl alcohol, phenol, benzyl alcohol, phenethyl alcohol, 4-ethylphenol, 3-phenylpropan-1-ol, and cinnamyl alcohol. In such cases, recovery factors must be taken into account for accurate quantification. As shown in Table 5, the method is not recommended for C_4–C_5 lactones.

LITERATURE CITED

Berahia, T.; Cerrati, C.; Sabatier, S.; Amiot, M. J. Gas chromatography-mass spectrometry analysis of flavonoids in honey. Sci. Aliments **1993**, 13, 15–24.

Bicchi, C.; Belliardo, F.; Frattini, C. Identification of the volatile components of some piedmontese honeys. J. Apic. Res. **1983**, 22, 130–136.

Bonaga, G.; Giumanini, A. G. The volatile fraction of chestnut honey. J. Apic. Res. **1986**, 25, 113–120.

Bonaga, G.; Giumanini, A. G.; Gliozzi, G. Chemical composition of chestnut honey: analysis of the hydrocarbon fraction. J. Agric. Food Chem. **1986**, 34, 319–326.

Bouseta, A.; Collin, S.; Dufour, J. P. Characteristic aroma profiles of unifloral honeys obtained with a dynamic head-space GC-MS system. J. Apic. Res. **1992**, 31, 96–109.

Ferber, C. E. M.; Nursten, H. E. The aroma of beeswax. J. Sci. Food Agric. **1977**, 28, 511–518.

Graddon, A. D.; Morrisson, J. D.; Smith, J. F. Volatile constituents of some unifloral australian honeys. J. Agric. Food Chem. **1979**, 27, 832–837.

Honey Flavors

J. Agric. Food Chem., Vol. 43, No. 7, 1995 **1897**

Likens, S. T.; Nickerson, G. B. Determination of certain hop oil constituents in brewing products. *ASBC Proc.* **1964**, 5–13.

Nickerson, G. B.; Likens, S. T. Gas chromatographic evidence for the occurrence of hop oil components in beer. *J. Chromatogr.* **1966**, *21*, 1–5.

Serra, J. Determination of methyl anthranilate in citrus honey (citrus sp.) of eastern spain and its influence on the diastase activity of the honey. *Alimentaria* **1988**, *197*, 37–40.

Steeg, E.; Montag, A. Proof of aromatic carboxylic acids in honey. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 17–19.

Steeg, E.; Montag, A. Quantitative determination of aromatic carboxylic acids in honey. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 115–120.

Tan, S.-T., Holland, P. T.; Wilkins, A. L.; Molan, P. C. Extractives from New Zealand honeys. 1. White clover, manuka, and kanuka unifloral honeys. *J. Agric. Food Chem.* **1988**, *36*, 453–460.

Tan, S. T.; Wilkins, A. L.; Molan, P. C.; Holland, P. T.; Reid, M. A chemical approach to the determination of floral sources of New Zealand honeys. *J. Apic. Res.* **1989a**, *28*, 212–222.

Tan, S.-T.; Wilkins, A. L.; Holland, P. T.; McGhie, T. K. Extractives from New Zealand honeys. 2. Degraded carotenoids and other substances from heather honey. *J. Agric. Food Chem.* **1989b**, *37*, 1217–1221.

Tan, S. T.; Wilkins, A. L.; Holland, P. T.; McGhie, T. K. Extractives from New Zealand honeys. 3. Unifloral thyme and willow honey constituents. *J. Agric. Food Chem.* **1990**, *38*, 1833–1838.

Tschogowadse, S. K.; Koblianidse, G. L.; Dembizkij, A. D. Aromatic components of honeys. *Lebensmittelindustrie* **1973**, *20*, 225–228.

Tsuneya, T.; Shibai, T.; Yoshioka, A.; Shiga, M. The study of shina (linden, *Tilia japonica* Simk.) honey flavor. *Koryo* **1974**, *109*, 29–35.

White, J. Composition of honey. In *Honey, a Comprehensive Survey*; Crane, Ed.; Heinemann: London, 1975; pp 157–206.

Wilkins, A. L.; Lu, Y.; Tan, S. T. Extractives from New Zealand honeys. 4. Linalool derivatives and other components from nodding thistle (*Carduus nutans*) honey. *J. Agric. Food Chem.* **1993**, *41*, 873–878.

Wootton, M.; Edwards, R. A.; Faraji-Haremi, R. Effect of accelerated storage conditions on the chemical composition and properties of Australian honeys. 3. Changes in volatile components. *J. Apic. Res.* **1978**, *17*, 167–172.