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Aroma Study of Essences in Non-Alcoholic Soft Drinks. Isolation and Concentration of Aromas

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Introduction

Our appetite for eating or drinking is mainly influenced by the presence of flavouring substances. Natural aromas are isolated from vegetable or animal parts by physical processes. The essences isolated are generally constituted of hundreds of products.

Owing to the identification of these compounds, flavour experts have succeeded in composing synthetically various aroma entities.

In order to identify the composition of an aroma complex, the analyst has to apply the most efficient isolation, concentration and detection techniques. In direct analysis, the so-called «head space» technique gives the composition of the vapour phase which is in equilibrium with the aqueous suspension of the material maintained at a given temperature. This technique does not generally give satisfactory results for volatiles presenting a relatively high boiling point. Furthermore sorption effects of volatiles on solid particles of the food materials provoke also a distorted image of the proportions between the various flavour constituents. Various enrichments methods were used to overcome these facts (1—8). In this work, we use an indirect analysis in which flavour samples are processed by applying three successive steps: isolation, concentration, separation and identification by using the combination of gas chromatography with the mass spectrography.

The flavour substances of natural essences were isolated using four methods:

A. simple extraction
B. continuous liquid-liquid extraction
C. single and double stage vacuum distillation
D. steam distillation.

Our purpose was to find the most efficient isolation technique and to control any possible artefact formation.

After an aging period of one month the various essences, kept at 4°C in contact with air, are identified a second time in order to check the stability of the natural flavour.

Experimental

Essence samples

Fresh natural essence samples of different trade marks were analysed.

— No 1209: lemon essence
— No 1213: lemon essence
— No 1214: lemon essence
— No 1218: lemon essence
— No 1221: lemon essence
— No 1223: lemon essence
— No 1225: lemon essence
— No 1228: lemon essence
— No 1210: orange essence
— No 1211: orange essence
— No 1215: orange essence
— No 1220: orange essence
— No 1222: orange essence
— No 1224: orange essence
— No 1226: orange essence
— No 1227: orange essence
— No 1229: orange essence
— No 1212: pineapple essence
— No 1219: cola essence
— No 1230: mandarin essence

Although the trade marks of the essences are known by the authors, there is no information about the state origin of the essence samples.

Samples of citrus essences were kept at 4°C in contact with air.

Isolation techniques

Simple extraction

Mix 3 ml of pure essence with 100 ml of distilled water. Add the water solution dropwise to 50 ml of petroleum ether (Malinckrodt). Transfer the total volume to a separatory funnel, shake vigorously during 1 minute and allow the mixture to stand until the two layers are separated. Remove the water phase and dry the organic phase with anhydrous sodium sulphate. Reextract the removed water layer with 50 ml of petroleum ether. After separation of the two phases discard the water and dry the solvent with anhydrous sodium sulphate.

After filtration put the two organic extracts together in an erlenmeyer flask, add 1 ml of internal standard (0.5 mg/ml pyrene) and concentrate the volume at room temperature on a rotavapor until 1 ml.

Continuous liquid-liquid extraction (fig. 1)

Mix homogeneously 3 ml of pure essence with 200 ml of distilled water. Transfer the essence solution to the extractor. Put 50 ml of petroleum ether in an erlenmeyer flask which is attached to the extractor by means of a sidetube. Warm up the organic solvent until boiling. The arising vapour is condensed within a Liebig cooler and drops through a membrane into the water solution. Bubbling through the aqueous essence ether extracts the aroma components. Along an overflow the flavour substances return to the erlenmeyer flask together with the petroleum ether. The extraction time is two hours. Dry and concentrate the organic phase as described in simple extraction.

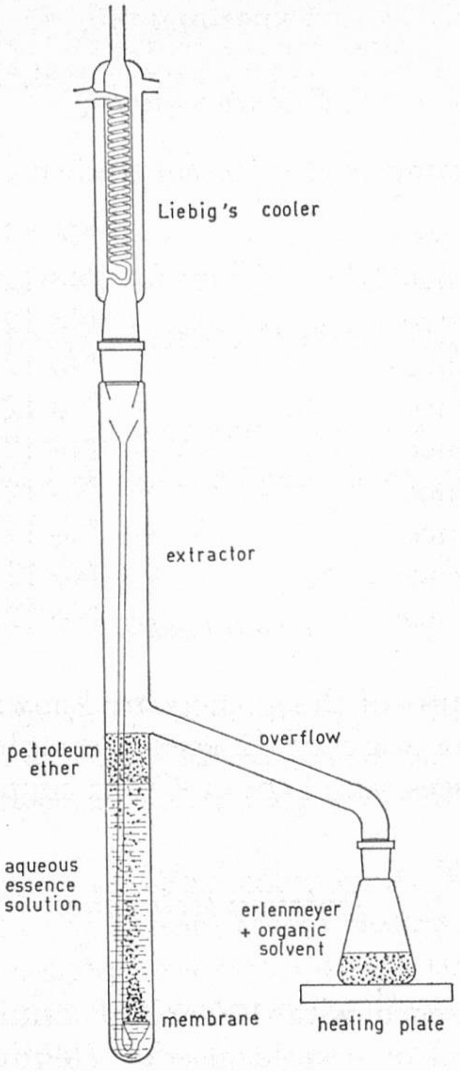


Fig. 1. Apparatus for the isolation of volatile compounds from essences by continuous liquid-liquid extraction

Vacuum distillation (fig. 2)

Mix homogeneously 3 ml natural essence with 100 ml of distilled water. Transfer the total volume to a 250 ml Claisen flask. At a pressure of 1 mm Hg, 90 ml of the water phase is distilled over at room temperature and collected in an ice-trap. The distillate is extracted, dried and concentrated as mentioned above.

In double stage vacuum distillation (ice-nitrogen) the volatile components are collected in the nitrogen trap during the first thirty minutes of the distillation. The less volatile substances and the water are condensed in the ice-trap. The extraction, the drying process and the concentration of the two distillates are carried out as described in the first isolation method.

Steam distillation (fig. 3)

Mix 3 ml of natural essence with 300 ml of distilled water. Transfer the aqueous essence into a three-necked 1 liter flask. Warm up the volume to 60°C and continuously admit steam into the aqueous essence. Collect 400 ml of steam distillate into 50 ml of petroleum ether. Extract, dry and concentrate the distillate as earlier described.

The recovered essences are analyzed with a Varian gaschromatograph 2720 equipped with a flame ionization detector.

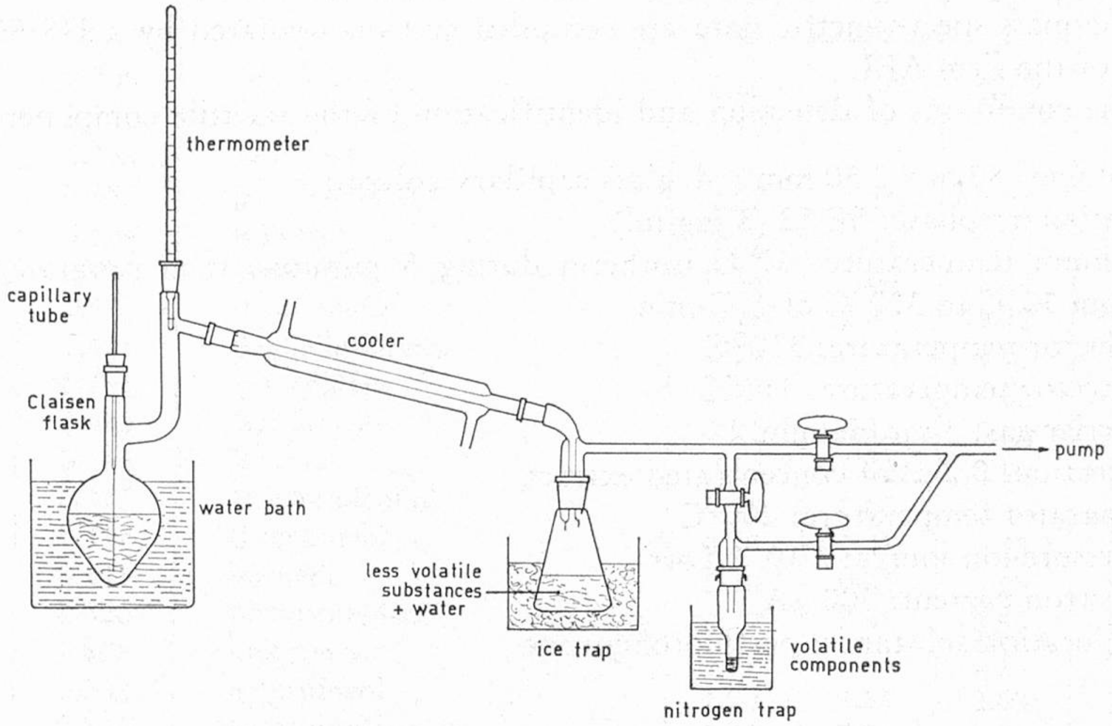


Fig. 2. Apparatus for the isolation of volatile compounds from essences by double stage vacuum distillation

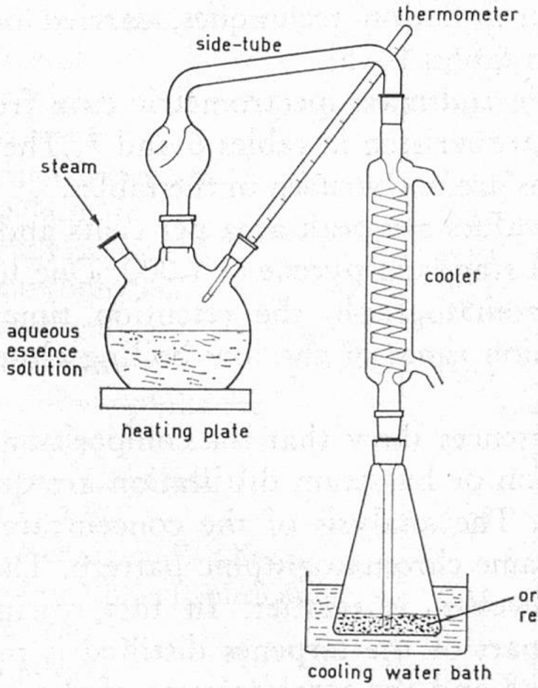


Fig. 3. Apparatus for the isolation of volatile compounds from essences by steam distillation

Retention times, areas and percentages of the registered peaks on the gaschromatograms are calculated by a Hewlett-Packard computer system 3352C.

The gaschromatographic peaks are identified by the combined system gaschromatography-mass spectrometry-computer.

The MS 30 double beam mass spectrometer of the firm AEI is linked with the gaschromatograph by means of a Williams membrane separator.

The mass spectrometric data are compiled and manipulated by a DS 50 computer of the firm AEI.

The conditions of detection and identification of the volatile components are:

- column: 50 m x 0.50 mm i. d. glass capillary column
- stationary phase: SE 52 (5 mg/ml)
- column temperature: 30°C isotherm during 5 minutes; then programmation from 30°C to 300°C at 4°C/min
- injector temperature: 310°C
- detector temperature: 310°C
- carrier gas: 3 ml He/min
- injection: 0,2 µl of concentrated extract
- separator temperature: 200°C
- pressure ion source: 2.10^{-6} Torr
- electron current: 300 µA
- calibration substance: perfluorokerosine

Results and discussion

The results of the four isolation techniques, carried out on different citrus essences, are summarized in tables 1—5.

The gaschromatographic and mass-spectrometric data from steam distillates of lemon and orange essence are written in tables 6 and 7. The identified compounds having small concentrations are not written in the tables.

The indicated percent values are peak area per cents and the relative retention times relate to the internal standard pyrene = 1.000. Due to the unstable starting temperature of the gaschromatograph, the retention times and the calculated, uncorrected relative retention times of the low boiling eluted compounds are not reproducible.

The results of lemon essences show that the composition of recovered essences isolated by simple extraction or by steam distillation are qualitatively and quantitatively almost identical. The analysis of the concentrate obtained by vacuum distillation illustrates the same chromatographic pattern. The concentration of the volatile monoterpenes, however, is smaller. In fact, owing to the insufficient cooling of the ice-trap, a part of the terpenes distilled is pumped away. Various components are not detected and the total amount of the monoterpenes observed is very low (± 14%). Besides, the oxygenated fraction as alcohols, aldehydes and esters, represent ± 80% of the total concentrate. Two factors could be responsible

Table 1. Gaschromatographic and mass-spectrometric data of sample 1209 (lemon essence)

Peak number	Relative Retention Times	Identification	Percentage			
			A	B	C	D
1.	0.306	α(+)-pinene	0.60		0.18	0.56
2.	0.321	camphene	0.10			0.09
3.	0.349	sabinene			0.10	
4.	0.351	β(+)-pinene	2.57	0.11	0.76	2.30
5.	0.368	myrcene	1.72	0.21	0.94	1.58
6.	0.390	eucalyptol + ?	0.88	0.66	0.24	0.86
7.	0.415	d-limonene	46.48	12.42	39.39	45.40
8.	0.421	β-phellandrene	0.16		0.11	0.15
9.	0.431	γ-terpinene	2.48	0.38	1.65	2.10
10.	0.453	terpinolene	0.91	0.22	0.64	0.76
11.	0.465	linalool	2.32	5.03	2.84	2.96
12.	0.473	fenchylalcohol	0.30	0.60	0.46	0.37
13.	0.497	β-terpineol	0.18	0.38	0.23	0.20
14.	0.513	borneol	0.20	0.41	0.25	0.23
15.	0.520	terpinene-4-ol	0.27	0.55	0.33	0.31
16.	0.526	unknown	0.21		0.17	0.20
17.	0.532	α-terpineol	1.93	4.64	2.80	2.51
18.	0.536	α-terpinylacetate	0.39	0.81	0.52	0.45
19.	0.542	decanal	0.50		0.47	0.40
20.	0.561	nerol	0.53	1.12	0.87	0.20
21.	0.570	neral	11.50	25.76	15.46	12.74
22.	0.574	linalylacetate	1.70	1.07	2.17	0.88
23.	0.579	geraniol	0.55	1.47	1.09	0.95
24.	0.590	geranial	16.69	36.35	22.54	18.30
25.	0.632	unknown	0.08		0.09	0.06
26.	0.634	citronellylacetate	0.11		0.14	0.08
27.	0.640	nerylacetate	0.36	0.19	0.49	0.32
28.	0.651	geranylacetate	0.84	0.44	1.17	0.74
29.	0.674	β-caryophyllene	0.17		0.23	0.08
30.	0.723	bisabolene			0.09	
31.	0.871	nootkatone	0.34	0.36	0.09	0.33
32.	0.916	di-iso-butylphthalate				0.06
33.	0.926	unknown				0.10
34.	0.936	di-butylphthalate				0.04
35.	1.000	PYRENE	0.27	0.81	0.03	0.26
36.	1.108	di-(2ethylhexyl)-adipate				0.29
37.	1.160	di-(2ethylhexyl)-phthalate	0.36	0.66		0.18
38.	1.187	unknown	0.18			
39.	1.215	unknown		0.31		

A: simple extraction
B: continuous liquid-liquid extraction
C: vacuum distillation
D: steam distillation

Table 2. Gaschromatographic and mass-spectrometric data of sample 1210 (orange essence)

Peak number	Relative Retention Times	Identification	Percentage			
			A	B	C	D
1.	0.303	α-thujene	0.08			0.12
2.	0.310	α(+)-pinene	0.71	0.32		1.14
3.	0.351	sabinene	0.54	0.30		0.50
4.	0.353	β(+)-pinene	0.29	0.14		0.43
5.	0.371	myrcene	2.68	1.22	0.53	3.60
6.	0.387	eucalyptol + octanal				0.25
7.	0.422	limonene	83.55	81.25	43.07	82.61
8.	0.434	γ-terpinene	1.87	0.90	0.58	2.27
9.	0.447	octanol	0.33	0.63	1.19	0.30
10.	0.455	terpinolene	0.36	0.71	1.69	0.32
11.	0.465	linalool	1.52	3.55	8.76	1.21
12.	0.520	terpinene-4-ol		0.15	0.55	
13.	0.530	α-terpineol	0.37	0.14	3.43	0.31
14.	0.541	decanal	0.84	0.79	1.32	0.83
15.	0.566	carvone	0.25	0.50	0.73	0.21
16.	0.576	neral	0.19	0.58	2.30	0.19
17.	0.608	geranial + piperitenone	0.47	1.22	3.41	0.38
18.	0.666	dodecanal	0.06	0.13	0.54	0.18
19.	0.714	valencene				0.14
20.	0.730	elemol			0.54	
21.	0.818	β-sinensal	0.15		1.37	0.16
22.	0.844	α-sinensal	0.10		0.64	0.10
23.	0.872	nootkatone	0.13			0.08
24.	0.915	di-iso-butylphthalate			0.62	0.07
25.	0.946	di-butylphthalate			0.52	0.09
26.	1.000	PYRENE	0.38	0.81	3.81	0.29
27.	1.108	di-(2ethylhexyl)-adipate				0.37
28.	1.160	di-(2ethylhexyl)-phthalate	0.62	0.94	3.93	0.25

Table 3. Gaschromatographic and mass-spectrometric data of sample 1212 (pineapple essence)

Peak number	Relative Retention Times	Identification	Percentage			
			A	B	C	D
1.	0.138	ethylbutyrate	3.24	7.25		2.38
2.	0.200	2methylbutylacetate	2.07	4.60		1.69
3.	0.240	unknown		0.27		
4.	0.259	α(+)-pinene	0.78	0.31	0.42	1.10

Peak number	Relative Retention Times	Identification	Percentage			
			A	B	C	D
5.	0.286	isobutylisobutyrate	1.62	2.84	0.58	1.36
6.	0.305	β(+)-pinene	0.89	0.44	0.51	0.88
7.	0.326	myrcene	3.04	1.32	2.85	3.85
8.	0.345	unknown	0.68	0.78	0.51	0.75
9.	0.390	limonene	63.06	43.14	72.88	64.16
10.	0.397	isoamylisobutyrate	0.22	0.33	0.21	0.21
11.	0.400	isoamylbutyrate	1.24	1.41	1.08	1.12
12.	0.421	allylcaproate	5.91	8.23	5.56	5.11
13.	0.435	linalool	1.12	2.41	1.14	0.95
14.	0.451	unknown		0.18		
15.	0.464	unknown		0.19		
16.	0.490	ethylbenzoate	0.64	1.43	0.66	0.58
17.	0.495	unknown	0.12	0.29	0.15	0.15
18.	0.499	terpinene-4-ol	2.08	2.02	2.15	1.90
19.	0.507	α-terpineol	0.23	0.54	0.26	0.22
20.	0.511	ethylcaprylate	0.13	0.17	0.14	0.12
21.	0.515	unknown		0.22		
22.	0.517	decanal	0.41	0.36	0.45	0.44
23.	0.527	unknown		0.35	0.16	0.12
24.	0.537	unknown		0.26		
25.	0.545	carvone	0.23	0.65	0.16	0.23
26.	0.568	neral	0.20	0.46	0.15	0.19
27.	0.575	isobornylacetate	0.15	0.14	0.21	0.15
28.	0.580	ethylpelargonate	0.97	0.54	1.24	1.15
29.	0.613	benzylbutanoate	1.32	2.23	1.40	1.29
30.	0.619	hydroxycarvomenthol?	0.19	0.29	0.23	0.18
31.	0.636	nerylacetate	0.11	0.12	0.12	0.12
32.	0.642	unknown		0.26		0.12
33.	0.661	unknown	2.00	1.23	2.20	2.15
34.	0.670	unknown		0.21	0.18	0.20
35.	0.702	valencene	0.21	0.25	0.18	0.10
36.	0.734	elemol	2.76	2.71	0.85	2.57
37.	0.736	unknown		0.81	0.23	
38.	0.748	γ-decalactone	0.98	1.80	0.17	0.96
39.	0.788	eudesmol	0.08	0.07		0.07
40.	0.789	unknown	0.07	0.06		0.07
41.	0.867	nootkatone		0.28		
42.	0.912	di-iso-butylphthalate				0.10
43.	0.933	di-butylphthalate				0.08
44.	1.000	PYRENE	0.19	0.23	0.11	0.10
45.	1.112	di-(2ethylhexyl)-adipate				0.35
46.	1.116	di-(2ethylhexyl)-phthalate	0.39	0.24	0.12	0.26

Table 4. Gaschromatographic and mass-spectrometric data of sample 1219 (cola essence)

Peak number	Relative Retention Times	Identification	Percentage				
			A	B	C	D_1	D_2
1.	0.258	α(+)-pinene				0.65	
2.	0.304	β(+)-pinene				0.69	
3.	0.321	myrcene				1.12	
4.	0.345	1,4-cineol				1.31	
5.	0.363	limonene	3.06			55.61	3.22
6.	0.387	γ-terpinene	4.38			1.38	2.50
7.	0.434	fenchylalcohol	4.95		1.60	0.93	2.52
8.	0.453	1-terpinenol	3.38		4.30	1.27	2.89
9.	0.460	β-terpineol				1.64	
10.	0.477	isoborneol	4.77		5.61	1.57	3.70
11.	0.486	terpinene-4-ol				0.40	
12.	0.498	α-terpineol			3.33	6.12	12.02
13.	0.502	α-terpinylacetate				2.50	3.70
14.	0.556	cinnamaldehyde (trans)	14.28		31.84	2.84	6.91
15.	0.559	cinnamaldehyde (cis)				2.13	
16.	0.651	β-caryophyllene				0.20	
17.	0.660	bergamotene				0.22	
18.	0.702	bisabolene				0.49	
19.	0.887	di-iso-butylphthalate				0.23	
20.	0.909	butyl-iso-butylphthalate	1.23			0.47	0.97
21.	0.931	di-butylphthalate	2.07			0.35	
22.	0.969	unknown	1.88	2.85			
23.	0.992	unknown	2.22		2.76		1.24
24.	1.000	PYRENE	46.03	64.75	41.24	7.64	18.29
25.	1.092	methyldihydroabietate				0.26	
26.	1.114	di-(2etylhexyl)-adipate		12.63	1.61	5.69	12.08
27.	1.168	di-(2ethylhexyl)-phthalate	3.05	4.06	1.90	1.94	20.68
28.	1.264	unknown	3.27	2.92			

A: simple extraction
B: continuous liquid-liquid extraction
C: vacuum distillation
D_1: steam distillation
D_2: results of the distillation of the essence after one month aging time

Table 5. Gaschromatographic and mass-spectrometric data from steam distillate of the mandarin essence 1230

Peak number	Relative Retention Times	Identification	Percentage
1.	0.241	α-thujene	1.23
2.	0.250	α(+)-pinene	3.33
3.	0.295	sabinene	0.26
4.	0.299	β(+)-pinene	3.07
5.	0.316	myrcene	2.75
6.	0.342	p-cymene	0.54
7.	0.370	d-limonene	59.17
8.	0.394	γ-terpinene	24.76
9.	0.414	terpinolene	1.47
10.	0.425	linalool	0.24
11.	0.488	terpinene-4-ol	0.17
12.	0.492	α-terpineol	0.32
13.	0.571	perillaldehyde	0.09
14.	0.647	methyl methylanthranilate	0.91
15.	0.655	α-farnesene (trans trans)	0.15
16.	0.708	butylhydroxytoluene (tert.)	0.12
17.	0.709	bisabolene	0.14
18.	0.836	α-sinensal	0.28
19.	1.000	PYRENE	0.53
20.	1.170	di-(2ethylhexyl)-phthalate	0.22

Table 6. Gaschromatographic and mass-spectrometric data from steam distillates of lemon essences

Peak no	Relative Retention Times	Identification	Percentage							
			1209	1213	1214	1218	1221	1223	1225	1228
1.	0.303	α-thujene				0.06				
2.	0.306	α(+)-pinene	0.56	0.48	0.42	1.14	2.09	1.26	0.70	0.40
3.	0.351	β(+)-pinene	2.30	2.00	1.13	2.18	10.40	3.42	1.10	2.28
4.	0.368	myrcene	1.58	1.12	0.68	3.46	3.28	2.71	0.89	1.26
5.	0.390	eucalyptol + octanal	0.86	0.46		0.68		2.06	0.60	0.56
6.	0.415	d-limonene	45.40	56.28	40.90	66.69	51.53	48.25	72.75	56.93
7.	0.421	β-phellandrene				0.17				
8.	0.431	γ-terpinene	2.10	1.60	0.75	5.24	5.16	7.58	0.90	5.39
9.	0.453	terpinolene	0.76	0.55	0.24	0.68	0.42	3.63	0.57	1.63
10.	0.465	linalool	2.96	2.20	2.01	0.94	1.45	0.88	1.91	0.68

Peak no	Relative Retention Times	Identification	Percentage							
			1209	1213	1214	1218	1221	1223	1225	1228
11.	0.473	fenchylalcohol	0.37	0.23		0.19		0.62	0.23	0.28
12.	0.492	1-terpinenol				0.17		0.91		0.41
13.	0.500	β-terpineol				0.22		0.68		0.33
14.	0.510	isoborneol				0.15		0.91		0.21
15.	0.520	terpinene-4-ol	0.31	0.13	0.34	0.92	2.44	1.40	0.23	0.65
16.	0.532	α-terpineol	2.51	1.87	2.92	2.35	2.62	6.67	1.25	4.48
17.	0.536	α-terpinylacetate	0.45	0.30		0.39	0.35	1.07		0.49
18.	0.542	decanal	0.40	0.24		0.32	0.75	0.34	0.24	
19.	0.561	nerol	0.20	0.64	0.71	0.31	0.64	0.27	0.87	0.61
20.	0.570	neral	12.74	8.20	14.26	0.96	2.60	3.09	4.37	5.95
20a.	0.574	linalylacetate	0.88	0.90	0.36		0.26		0.49	
21.	0.579	geraniol	0.95	0.78	0.42	0.58	0.71	0.78	1.52	1.47
22.	0.590	geranial	18.30	13.61	23.37	1.17	4.06	4.90	7.61	9.03
23.	0.593	unknown				0.32				
23a.	0.634	citronellylacetate	0.08				0.82	0.11		
24.	0.640	nerylacetate	0.32	0.32	1.17	0.49	0.71	0.68		0.58
25.	0.651	geranylacetate	0.74	0.69	0.89	0.46	1.59	1.04		0.66
26.	0.674	β-caryophyllene	0.08	0.13		0.23		0.27		0.35
27.	0.680	bergamotene				0.21		0.14		0.33
28.	0.711	butylhydroxyanisole (tert.)				0.14				
29.	0.725	bisabolene				0.41		0.22		0.76
29a.	0.766	butylhydroxytoluene (tert.)					0.56	0.42		
29b.	0.871	nootkatone	0.33	0.33						
30.	0.915	di-isobutyl-phthalate				0.05				
31.	0.926	isobutyl-butylphthalate				0.09				
32.	0.936	dibutylphthalate				0.08				
33.	0.991	heneicosane				0.26				
34.	1.000	PYRENE	0.26	1.11	3.03	1.53	0.92	0.93	0.94	0.99
35.	1.034	docosane				0.61				
36.	1.075	tricosane				0.88				
37.	1.093	methyl-dihydroabietate				0.21				
38.	1.114	tetracosane* + di-(2-ethylhexyl) adipate	0.29	0.38		1.54		0.25	0.66	0.55
39.	1.150	pentacosane				0.50				
40.	1.168	di-(2ethylhexyl)-phthalate	0.18	0.51		0.33		0.24	0.31	0.32
41.	1.186	hexacosane				0.26				
42.	1.221	heptacosane				0.12				

* only detected in sample 1218
The registered compounds with peak numbers a or b are not identified in sample 1218

Table 7. Gaschromatographic and mass-spectrometric data from steam distillates of orange essences

Peak number	Relative Retention Times	Identification	Percentage								
			1210	1211	1215	1220	1222	1224	1226	1227	1229
1.	0,303	α-thujene	0.12								
2.	0.310	a(+)-pinene	1.26	1.35	0.15	0.91	1.73	0.71	1.07	1.06	1.09
3.	0.351	sabinene	0.50								
4.	0.353	β(+)-pinene	0.43	1.02	0.23	0.54	3.42	0.15	0.67	0.38	
5.	0.371	myrcene	3.60	3.70	1.08	3.30	4.33	3.14	2.62	4.16	4.36
6.	0.387	eucalyptol + octanal	0.25				0.82	0.42		2.28	
7.	0.422	d-limonene	82.61	80.27	87.75	75.10	72.17	88.39	82.77	81.70	84.73
8.	0.434	γ-terpinene	2.27	4.51	0.77	2.43	1.89	0.11	2.37	0.11	0.22
9.	0.447	octanol	0.30								0.53
10.	0.455	terpinolene	0.32	0.40		0.40	0.22	0.11	0.17	0.21	0.17
11.	0.465	linalool	1.21	1.32	2.95	3.43	1.17	1.17	0.88	3.54	0.88
12.	0.474	fenchylalcohol		0.10						0.33	
13.	0.520	terpinene-4-ol		0.21	0.24	0.70	0.59	0.38	0.15	0.35	0.69
14.	0.530	α-terpineol	0.31	0.35	0.57	0.47	0.37	0.32	0.23	0.37	0.23
15.	0.541	decanal	0.83	1.17	0.93	0.73	0.81	1.03	0.85	1.69	0.36
16.	0.566	carvone	0.21	0.26		0.26				0.16	
17.	0.571	perillaldehyde				0.30					
18.	0.576	neral	0.19				0.25				
19.	0.588	geraniol	0.38								
20.	0.608	geranial + piperitenone	0.38	0.36						0.39	
21.	0.634	citronellylacetate			0.30		0.30				
22.	0.640	nerylacetate				0.17	0.22				
23.	0.651	geranylacetate				0.15	0.50				
24.	0.666	dodecanal	0.18				0.33			0.12	
25.	0.669	β-elemene					0.25				
26.	0.674	β-caryophyllene				0.19	0.43				

Peak number	Relative Retention Times	Identification	Percentage								
			1210	1211	1215	1220	1222	1224	1226	1227	1229
27.	0.682	bergamotene				0.22	0.29				
28.	0.698	butylhydroxyanisole (tert.)						0.18			
29.	0.700	dimethylstyrene							1.71		
30.	0.702	α+β humulene					0.22				
31.	0.703	unknown (m/e: 115,43)							1.03		
32.	0.703	citronellylpropionate			0.26						
33.	0.714	valencene	0.14	0.12		0.11	0.25	0.54			0.28
34.	0.718	calarene					0.28				
35.	0.719	dodecanol				0.40					
36.	0.723	bisabolene				0.35	0.33				
37.	0.732	butylhydroxytoluene (tert.)				0.49	0.18				
38.	0.738	δ-cadinene					0.14				
39.	0.818	β-sinensal	0.16	0.15					0.17		
40.	0.844	α-sinensal	0.10	0.15					0.13		
41.	0.872	nootkatone	0.08			0.10		0.13			
42.	0.915	di-iso-butylphthalate	0.07								
43.	0.946	di-butylphthalate	0.09								
44.	1.000	PYRENE	0.29								
45.	1.108	di-(2ethylhexyl)-adipate	0.37	0.40		1.56	1.29	0.62	0.84	0.32	0.54
46.	1.160	di-(2ethylhexyl)-phthalate	0.25	0.22		0.66	0.47	0.37	0.43	0.25	1.17

m/e 115,43: most intens fragment ions of not identified mass spectrum for this phenomenon: first, the monoterpenes are oxidized during the extraction process, second, the volatile essences remain adsorbed on the non-dissolved essence material.

The results proceeding from orange essence demonstrate a less-important difference. The qualitative and quantitative composition of the isolated concentrates A, B and D are practically identical. Vacuum distillation proves again that the monoterpenes are mostly evaporated.

An analogous phenomenon is observed in cola and pineapple essences. In pineapple essence, however, we found that the composition of the B concentrate present qualitative and quantitative differences with regard to the other concentrates. Furthermore it seems that the isolation of the esters is more efficient with continuous liquid-liquid extraction than with the other isolation techniques.

The efficiency of these isolation techniques is very well illustrated by the data listed in table 4. The low levels of many components in the liquid-liquid extraction concentrate is a characteristic feature. It means again that the flavours remain adsorbed on the non-dissolved material. Even the concentrates of the methods A and C show also low recoveries. The base peak has been identified as cinnamaldehyde. In the steam distillate this substance only represents a minor percentage of the total amount of the aroma isolated. As the principal component we observe limonene. Another remarkable fact is the detection of the monoterpene alcohols in the steam distillate of the cola essence only.

Finally the plasticizers, identified as adipates and phthalates, are only detected in the concentrates of the steam distillate. The low volatility of these products explains their absence in the extracts of the other isolation methods.

The results from the steam distillate of the mandarin essence 1230, summarized in table 5, show that the d-limonene is the principal component (60%). A second, very important compound detected is γ-terpinene (25%). Other significant monoterpenes identified are thujene, α-pinene, β-pinene, myrcene and terpinolene.

Peak 14 has been identified as the methylester of methylanthranilic acid. The mass spectrum corresponding with this product (fig. 4) is characterized by an intense molecular peak ion at m/e 165. Other intense fragment ions are registered

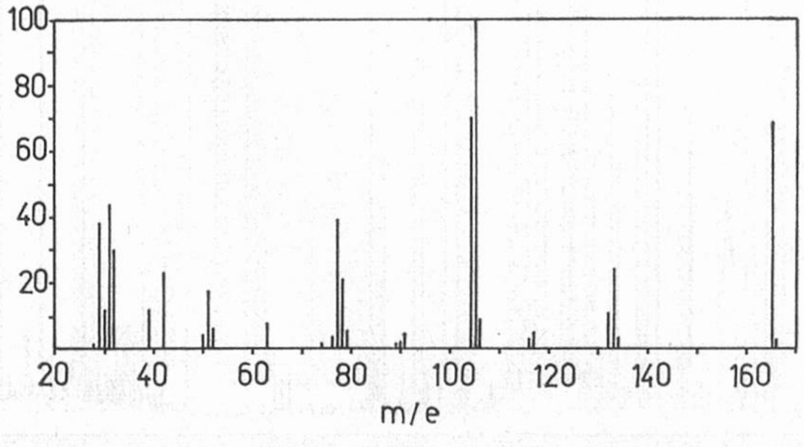


Fig. 4. Mass spectrum of methyl methylanthranilate

at masses m/e 105, 104, 132, 31, 77 and 29. The presence of the compound is vitally important for the value of the mandarin essence. If this product is not present, we can conclude that a less valuable tangerine essence is concerned.

Peak no 18 has been identified as α-sinensal. Although this aromatic substance is most frequently observed in the orange essence, we have to conclude that this compound can also be found within mandarin essence.

Figure 5a represents the chromatogram of the steam distillate of the natural lemon essence No 1218. The constitution of the aromagram consists of two groups: the volatile flavours eluted between 70°C and 150°C and the less volatile substances emerged from 180°C at 280°C.

On the chromatogram corresponding with the single stage vacuum distillate (fig. 5d) we do not observe the low boiling volatiles. Furthermore the detection of the substances isolated is less intense and the homologous series of hydrocarbons C21—C27 (peaks 33—42) and plasticizers are not observed. We have a very clear idea of the composition of the essence of sample 1218 if we investigate the aromagrams of the vacuum distillate with two traps (fig. 5b and c). With the exception of peaks 28 (identified as butylhydroxytoluene) (tert.) and 33—42 (identified as a homologous series of aliphatic hydrocarbonns and plasticizers) we can conclude that the detected peaks of the combined chromatograms 5b and 5c are identical to those observed on the chromatogram of the steam distillate.

The results summarized in colums D_1 and D_2 of table 4 show that the composition of flavouring substances varies according to their aging times. We notice that

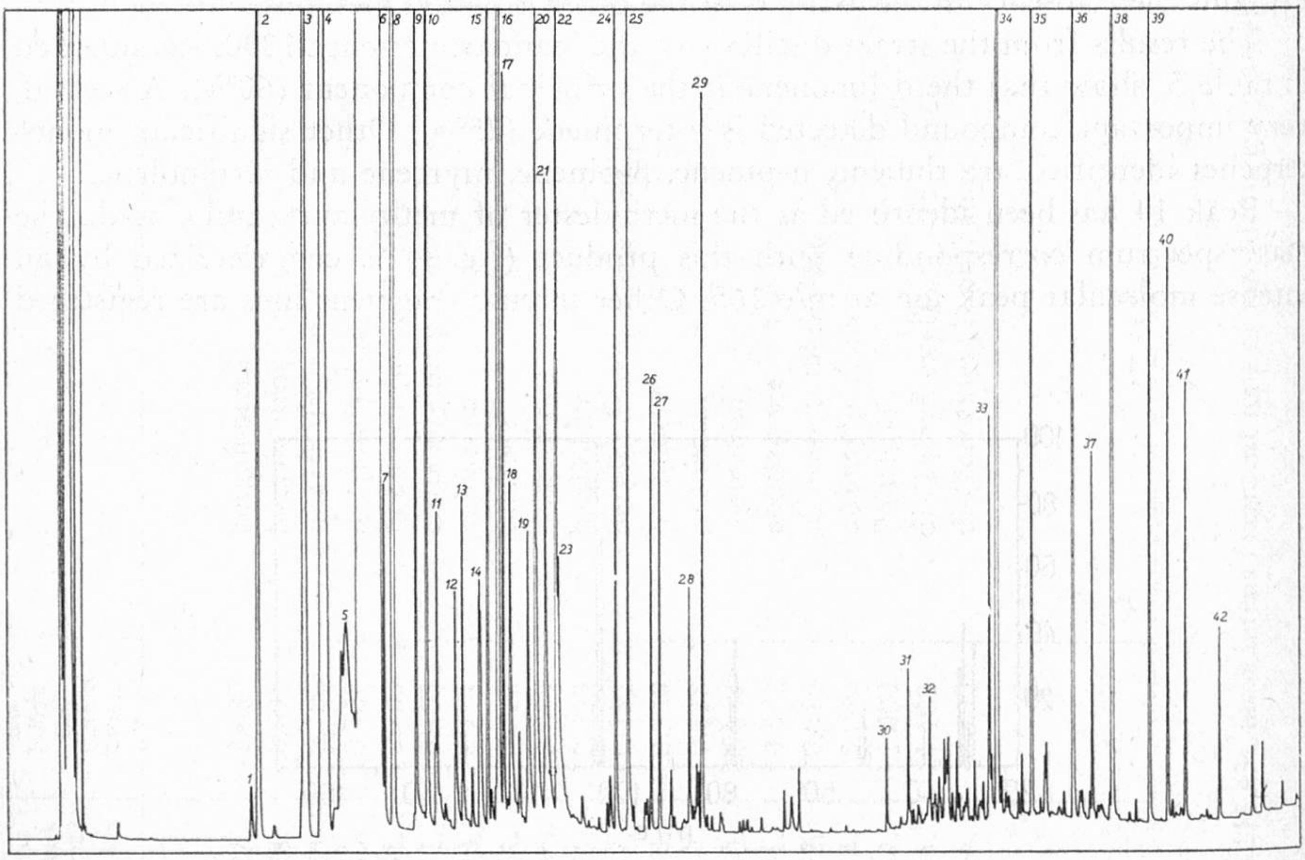


Fig. 5a. Chromatogram of a steam distillate of natural lemon essence

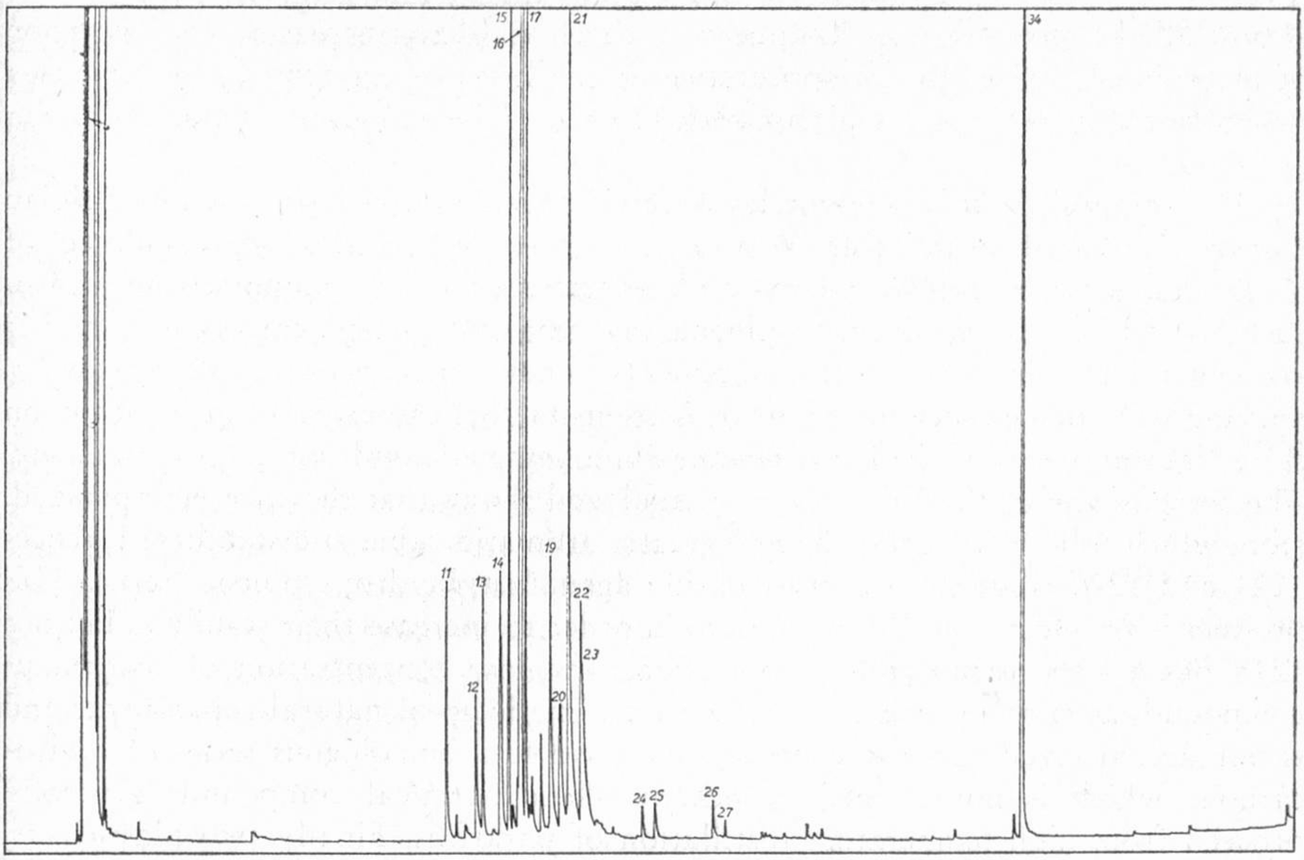


Fig. 5b. Chromatogram of a double stage vacuum distillate of natural lemon essence (nitrogen-trap)

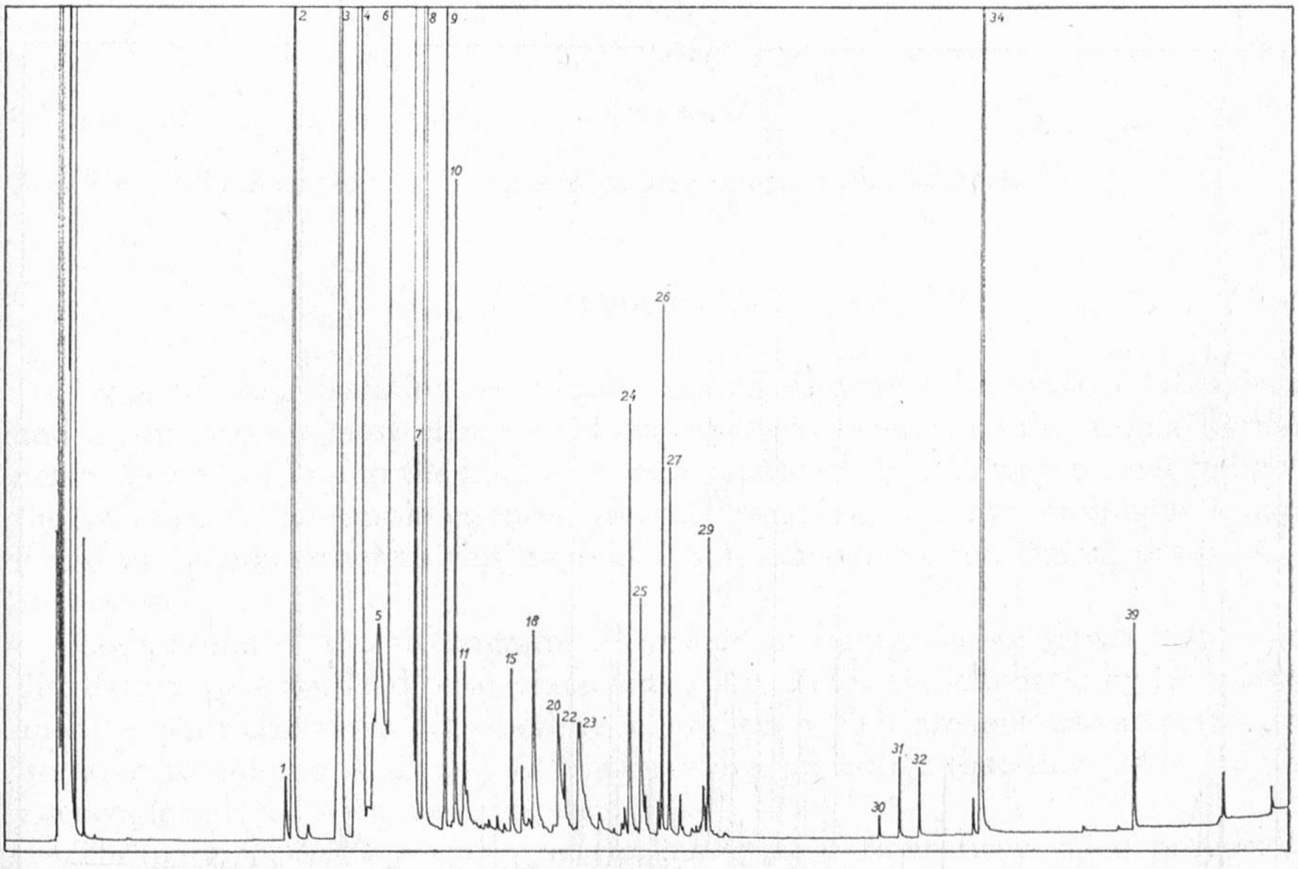


Fig. 5c. Chromatogram of a double stage vacuum distillate of natural lemon essence (ice-trap)

almost all the monoterpenes disappear or partly oxidize (increasing concentration of α-terpineol). After the disappearance of the low boiling aromas we remark a proportional increase of the plasticizers. However, their absolute quantity remains the same.

The composition of different lemon and orange essences, sold on the Belgian market, is described in tables 6 and 7. The lemon essences are composed of aldehydes, esters, alcohols, mono- and sesquiterpenes. The monoterpene hydrocarbons, d-limonene, α-pinene, β-pinene, myrcene and γ-terpinene occur in higher concentrations than the other components. They range between 50% to 85%, varying from one essence to the other. Various factors can exert an influence upon these figures: origin of the fruit, picking-time, seasonal conditions, aging time, etc. The composition of the lemon essences analyzed shows that they are deterpenized, from which follows that they have a greater aromatic value and stability. Essences 1221 and 1223 contain an anti-oxidation agent butylhydroxytoluene (tert.). This product is frequently added to essences in order to increase their stability. Essence 1218 has a very remarkable composition. The low concentration of oxygenous compounds as citral proves that the essence is made up of natural components and is not deterpenized. On the contrary, we observe a homologous series of hydrocarbons which is not formed in nature. These chemical compounds generally proceed from high temperature distillation of paraffins. Finally two plasticizers, di-(2ethylhexyl)-phthalate and di-(2ethylhexyl)-adipate, were observed. They are detected as concentrated impurities of the solvents used.

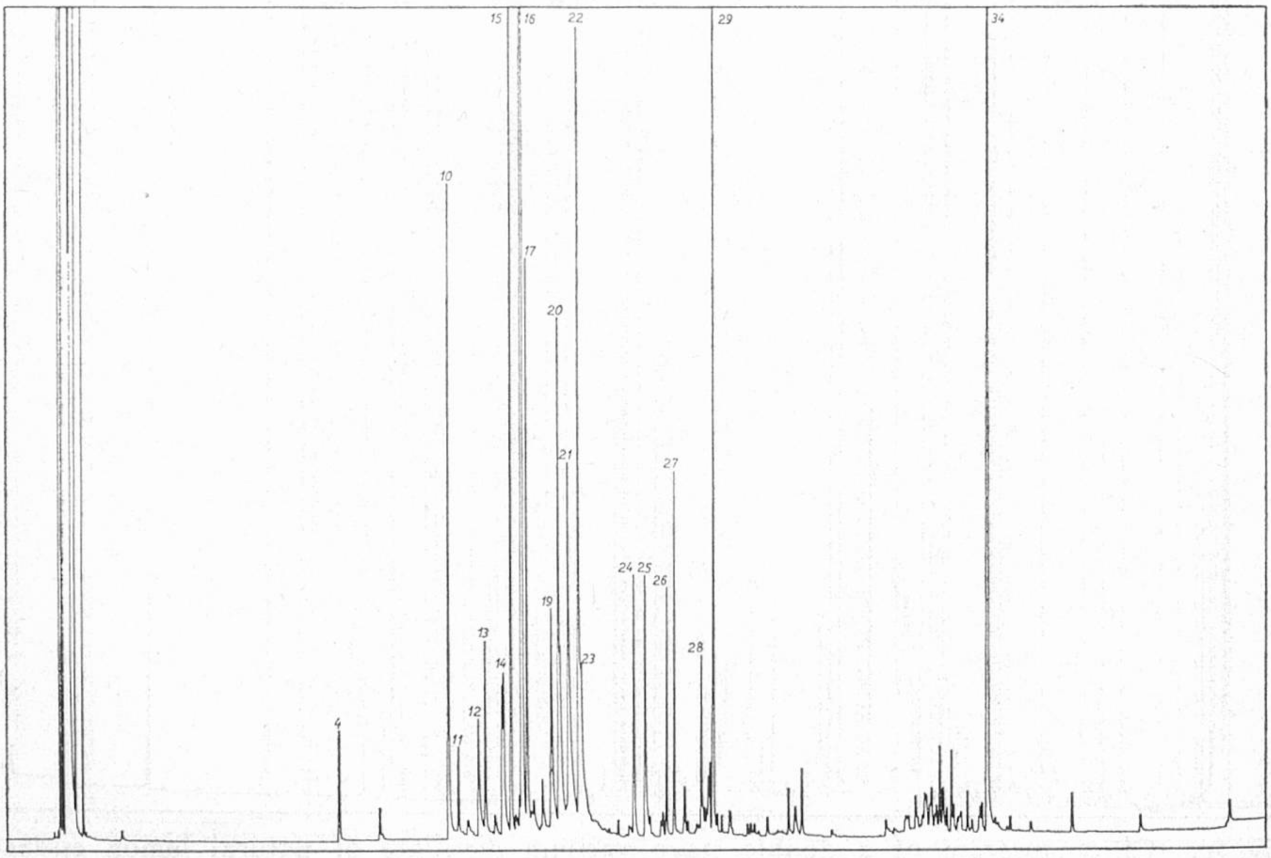
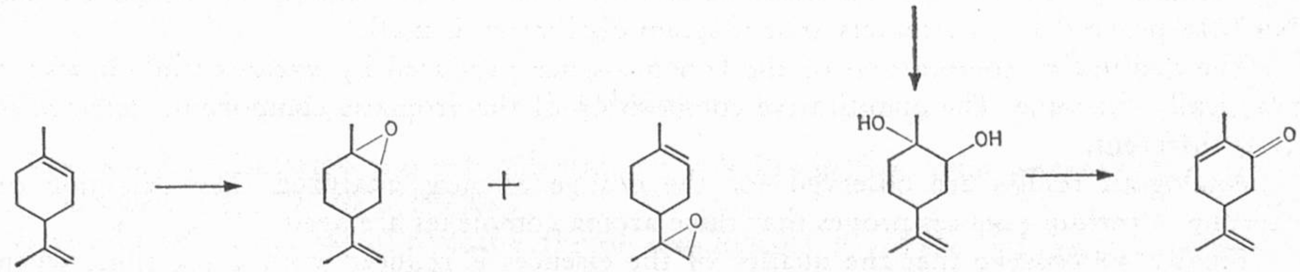


Fig. 5d. Chromatogram of a single stage vacuum distillate of natural lemon essence

Contrary to the lemon essences analyzed, the orange essences are principally composed of monoterpene hydrocarbons (86% to 90%). d-limonene which proves to be the principal component has a concentration which ranges between 72% and 88%. The other monoterpenes α-pinene, β-pinene, myrcene and γ-terpinene are found in smaller quantities.

As the trade price of oranges is lower than the other citron essences, a lot of adulterations are observed. The concentrations of the terpenes and aldehydes are primarily questioned. The decanal content is very important here. Table 7 shows the different quantities of decanal which were detected in the various orange essences analyzed.

The aromatic effect of the orange essences is also considerably influenced by the presence of esters. These compounds were exclusively detected in essences 1220 and 1222. The identification of sesquiterpenes such as valancene, α- and β-sinensal and nootkatone let us suppose that the essences isolated are concentrated. Although the antioxidation agents butylhydroxyanisole (tert.) and butylhydroxytoluene (tert.) are observed in the essence 1220, 1222 and 1224, the oxidizing product carvone is found in essence 1220. This product is formed by the oxidation of d-limonene as a result of aging of the essence.



d-limonene 2,2-epoxide 8,9-epoxide carvone

Finally we observe that orange essences also contain plasticizers.

Conclusion

From the four isolation techniques applied steam distillation is qualitatively and quantitatively most efficient. However the recoveries of the aroma components vary according to the kind of essence analyzed. In this way we noticed that the volatiles in pineapple essences are very well isolated by continuous liquid-liquid extraction, whereas this method is not convenient for lemon, orange and cola essences.

Comparison of the aromagrams illustrated in figures 5a—d shows that steam distillation does not lead to artefact formation. BHT, the aliphatic hydrocarbons and the plasticizers are high boiling substances which are not extracted by the isolation techniques A, B and C. Furthermore we notice that the quality of the essences is reduced with the aging time.

The natural lemon essences proceeding from various firms have practically identical qualitative compositions. Analyzing the results proceeding from orange essences we observe an analogous phenomenon. The proportional, quantitative presence of the detected components varies considerably. It depends on origin, crops seasonal conditions, aging time and furthermore on isolation procedures used. Those factors make it almost impossible to establish quality standards for natural essences. Therefore the detection of possible adulterations in lemonade soft-drinks is difficult.

Acknowledgment

We thank Mr. *G. Janssens* and Mr. *A. Vranken* for their technical assistance.

Summary

Volatile components of different natural essences are isolated by simple extraction, continuous liquid-liquid extraction, vacuum distillation and steam distillation. The isolation extracts are dried on anhydrous sodium sulphate and concentrated on a rotavapor. The flavour components are analyzed and identified by the computerized system gas chromatography — mass spectrometry. The results, summarized in tables or illustrated on chromatograms, show us that steam distillation is the most efficient technique to characterize essences added to soft drinks.

The data given and the chromatograms illustrated for the analysis of lemon essence No 1218 prove that no artefacts arise if steam distillation is used.

The qualitative composition of the lemon essence produced by various trade marks is practically the same. The quantitative composition of the aromatic components detected is quite different.

Analogous results are observed for the orange essences analyzed. The detection of carvone in certain essences proves that these aroma complexes are aged.

Finally we observe that the quality of the essences is reduced with aging time, when essences are kept at 4°C in contact with air.

Résumé

Les composants volatils de différentes essences naturelles ont été isolés par extraction simple, extraction liquide-liquide, distillation sous vide et entraînement à la vapeur d'eau. Après séchage et concentration, les extraits ont été analysés et les produits identifiées par le système chromatographie en phase gazeuse — spectrométrie de masse — ordinateur.

Les résultats, résumés en tableaux ou illustrés sur figures, montrent que l'entraînement à la vapeur d'eau est la technique la plus efficace pour caractériser les essences ajoutées aux boissons sans alcool.

Les résultats donnés et les chromatogrammes illustrés pour l'analyse d'essence de citron no 1218 ont prouvé qu'il n'y a pas de formation d'«artefact» quand l'entraînement à la vapeur d'eau est appliqué.

La composition qualitative des différentes marques d'essences de citron est presque identique. Par contre les proportions des différents composants aromatiques détectés varient pour chaque essence. Des résultats analogues ont été observés pour les essences d'orange. La présence de carvone dans certaines essences signifie que ces essences ont été conservées quelque temps.

Enfin nous observons que la qualité des essences diminue sensiblement en fonction du temps, lorsqu'on les conserve à 4°C en présence d'air.

Zusammenfassung

Die flüchtigen Anteile verschiedener natürlicher Essenzen wurden mittels üblicher Extraktion, kontinuierlicher Flüssig-Flüssig-Extraktion sowie Vakuum- und Wasserdampfdestillation isoliert. Nach dem Trocknen und Konzentrieren wurden die isolierten Extrakte anhand des kombinierten Systems «Gaschromatographie — Massenspektrometrie — Datenverarbeitung» analysiert und deren Komponenten identifiziert.

Die in Tabellen zusammengefaßten oder auf Chromatogrammen illustrierten Resultate zeigen, daß die Dampfdestillation die zweckmäßigste Technik ist, um alkoholfreien Getränken zugesetzte Aromen zu identifizieren.

Die bei der Analyse der Zitronenessenz Nr. 1218 erhaltenen Ergebnisse beweisen, daß bei der Anwendung der Dampfdestillation keine Artefacte auftreten. Die qualitative Zusammensetzung der Zitronenessenzen von verschiedenen Handelsmarken ist praktisch identisch. Die quantitative Zusammensetzung der nachgewiesenen Aromakomponenten ist verschieden.

Für die analysierten Orangenessenzen wurden analoge Resultate beobachtet. Der Nachweis von Carvon in gewissen Essenzen beweist, daß diese Aromakomplexe während einiger Zeit aufbewahrt worden sind.

Abschließend möchten wir noch bemerken, daß die Qualität der Essenzen stark mit der Aufbewahrungszeit abnimmt, wenn diese Essenzen bei einer Temperatur von 4°C in Luftanwesenheit aufbewahrt werden.

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