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SIMPLE MICROSCALE SUPERCRITICAL FLUID EXTRACTION SYSTEM AND ITS APPLICATION TO GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF LEMON PEEL OIL

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SUMMARY

The instrumentation and applications of a simple microscale supercritical fluid extraction system are described. The system consisted of an high-performance liquid chromatography pump with a pump-head cooling jacket, an extraction vessel made from a short empty column, 35 mm × 4.6 mm I.D., and a capillary restrictor. Lemon peel oil was extracted with sub- and supercritical carbon dioxide at various pressures and temperatures and collected in a micro-vial. The extract was analyzed by gas chromatography–mass spectrometry and the results were compared to those for a cold-pressed lemon oil.

INTRODUCTION

Although more than 100 years ago Hannay and Hogarth[1] reported that supercritical fluids showed solvating power, supercritical fluid extraction (SFE) was introduced by Zosel[2] only in the 1960s. Since then, the method seems to have been developed mainly as an industrial-scale extraction technique, as reported by many research groups[2–13], though SFE can be performed on the microscale with an extraction vessel of less than 1 ml in volume using high-performance liquid chromatographic (HPLC) components, as will be demonstrated in this paper. We call this technique micro-SFE. It has many advantages over a pilot plant SFE having an extraction vessel of one tenth to a few litres in volume: (1) ease of construction; (2) ease of operation; (3) small sample quantity; (4) low running costs; (5) on-line and/or off-line monitoring of extract by UV, IR, NMR, mass spectrometry (MS), etc.; (6) potential for sample pre-treatment in chromatographic analysis.

Citrus essential oil is a fairly expensive material used in the perfume and flavour industries. Since the composition of the oil has a crucial influence on the quality of the product, extensive studies have been carried out including the investigation of analytical methods[14–21], of seasonal and regional variations in the composition of

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oils[21–23], of the variation in composition using different extraction methods[21,24], including extraction with supercritical carbon dioxide[11,24], and of the mechanism of deterioration of the flavour[25–27].

In this paper, a simple extraction method with supercritical carbon dioxide and its application to the analysis of lemon peel oil are presented.

EXPERIMENTAL

In principle, an SFE system consists of an high-pressure pump, an extraction vessel, a back-pressure regulator and a separation vessel. Fig. 1 shows diagrams of different types of SFE system; system A is based on the pressure reduction that causes decreases in solubility, and system B is based on the temperature change that causes changes in solubility. It should be noted that, in a supercritical fluid, the solubility usually depends on the density effected by pressure changes.

Recent advances in HPLC instrumentation now readily permit us to build a

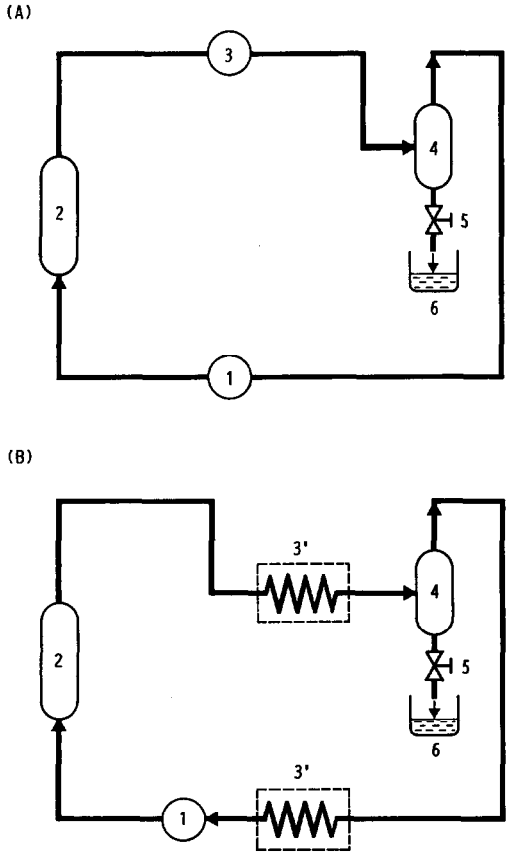

Fig. 1. Diagrams of different types of SFE systems having the extract separation based on pressure reduction (A) and on temperature change (B). Component: 1 = pump; 2 = extraction vessel; 3 = pressure regulator; 3′ = heat exchanger; 4 = separation vessel; 5 = collection valve; 6 = collection vessel.

micro-SFE system without any difficulty. As shown[28–31], an HPLC pump with a cooling jacket for the pump head can be used for delivery of liquefied carbon dioxide. A short empty column can be used as an extraction vessel, and a restrictor having proper flow resistance can serve as a device which generates a backpressure. A commercially available back-pressure regulator can be used to apply a suitable back-pressure without changing the mass flow-rate of the fluid, though fractionation of the extract is difficult because the dead-volume of such a back pressure is large, several tens of millilitres, in comparison with the volume of the extraction vessel, a few tenths to ten millilitres, so that the extract is left in the regulator. However, an UV detector can be placed between the extraction vessel and the back-pressure regulator, in order to perform on-line absorption monitoring of the extract as a function of the extraction time, or UV spectra can be obtained as a series along the time axis, *i.e.*, the three-dimensional UV spectrum, if a multiwavelength detector is employed. We shall call this type of data the *extraction profile*. Such an arrangement is very useful when examining extraction conditions.

Micro-SFE apparatus

We built two types of micro-SFE systems, diagrams of which are shown in Figs. 2 and 3. The JASCO Model BIP-1 (Tokyo, Japan) with cooling jacket was used as the carbon dioxide delivery pump. A cartridge-type extraction vessel was made of 35 mm × 4.6 mm I.D. × 1/4 in. O.D. stainless-steel tube with ordinary 1/4-in. HPLC

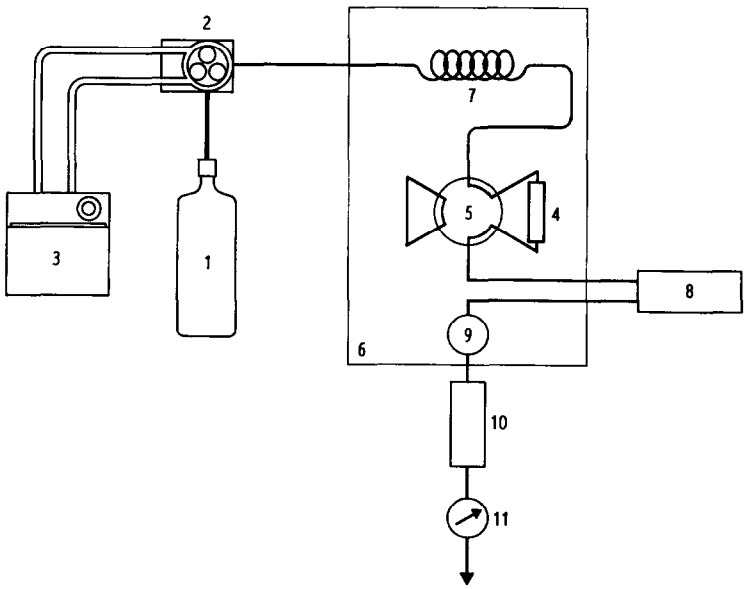

Fig. 2. Micro-SFE system for extraction profile monitoring. Components: 1 = carbon dioxide cylinder; 2 = carbon dioxide delivery pump with head cooling jacket; 3 = coolant circulating bath; 4 = extraction cartridge, 35 mm × 4.6 mm I.D. × 1/4 in. O.D.; 5 = six-port valve for changing the flow line; 6 = oven; 7 = 5 m × 0.5 mm I.D. × 1/16 in. heat exchanger coil; 8 = multiwavelength UV detector with high-pressure cell; 9 = back-pressure regulator; 10 = trap for mass flow meter; 11 = mass flow meter.

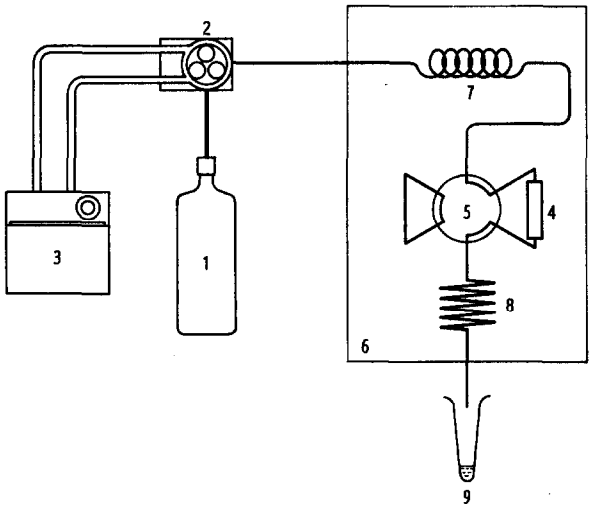

Fig. 3. Micro-SFE system for collecting extract. Components: 1–7 as in Fig. 2; 8 = flow restrictor; 9 = micro vial.

column end fittings. A Model 7000 six-port valve was used for changing the flow line (Rheodyne, CA, U.S.A.). An HPLC column oven (Model TU-100, JASCO) was used for elevating the temperature of carbon dioxide and the extraction vessel above its critical temperature. A 5 m × 0.5 mm I.D. × 1/16 in. O.D. stainless-steel tube was connected between the pump and the extraction vessel. It was coiled and kept in the oven to serve as an heat exchanger. A Model 26-3200-24 back-pressure regulator (Tescom, MN, U.S.A.) was used to change the back pressure while monitoring UV spectra of the extract, *i.e.*, the extraction profile, with a multiwavelength UV detector (JASCO MULTI-320) as shown in Fig. 2. A carbon dioxide mass flow meter (KOFLOC Model 2500; Kojima Flow Instruments Corp., Kyoto, Japan) was used for monitoring the mass flow-rate of carbon dioxide after the back-pressure regulator had reduced the pressure to atmospheric pressure.

For collecting the extract, a 250 mm × 0.25 mm I.D. stainless-steel tube was used, instead of the back-pressure regulator, as a flow restrictor as shown in Fig. 3. The restrictor tube was pinched with a pair of pliers to increase the flow resistance.

For the identification of the components of the oil, a GC–MS system, consisting of an HP-5790 gas chromatograph (Hewlett-Packard, CA, U.S.A.) and a JMS-DX300 mass spectrometer (JOEL, Tokyo, Japan) was used.

Materials

A lemon was purchased from a grocery store, and its yellow skin was carefully cut out from the fibre of the peel using a clean razor into 1 mm × 10 mm pieces each weighing about 30 mg.

In SFE, carbon dioxide is generally the preferred extraction medium and is widely used for the reasons mentioned previously[7,9,29]. We therefore used carbon dioxide as the extraction medium throughout the experiments described in this paper. Carbon dioxide in a cylinder with a siphon tube was obtained from Toyoko Kagaku (Kawasaki, Japan).

Procedure

Extraction profile monitoring with a multiwavelength UV detector. The hydraulic system shown in Fig. 2 was used for extraction profile monitoring. The extraction vessel containing a piece of lemon peel was connected to the flow line. For equilibration of the system, carbon dioxide was first delivered from the pump into the detector, bypassing the vessel, then vented to the atmosphere, via the back-pressure regulator, which controls the extraction pressure, through the mass flow meter. The extraction temperature was controlled by the oven in which the vessel, back-pressure regulator, etc., were installed. Pressure equilibration was tested with the detector baseline, which was displayed on a cathode ray tube (CRT). After equilibration, the six-port valve was switched to start the extraction. The spectral data for the extract shown on the CRT were monitored during the extraction and at the same time stored on a floppy disc for later use.

Collection of the extract. The procedure for collection of the extract is very similar to that for the extraction profile monitoring except for the hydraulic system used, shown in Fig. 3. The carbon dioxide pump was operated in the constant-pressure mode at a pre-set extraction pressure. The pressure of the fluid containing the extract was released as it flowed along the restrictor and the lemon peel oil was collected in a micro vial of capacity 200 μl. The extracton yield was calculated by dividing the weight of the oil collected by the peel weight before extraction.

Capillary gas chromatography–mass spectrometry (GC–MS). Oils extracted under various conditions were separated on a CBP 20 capillary column (Shimadzu, Kyoto, Japan) by using gas chromatography with flame ionization detection (FID), and the chromatographic peaks obtained were grouped into five major components with regard to compound types. Each component was identified by GC–MS analysis. The results obtained were compared each other, and also with analytical results for a commercial cold-pressed oil obtained by the same chromatographic method.

RESULTS AND DISCUSSION

Extraction profile

The extraction profile of the lemon peel is shown in Fig. 4. The extraction temperature was kept constant at 45°C, however the pressure was changed stepwise

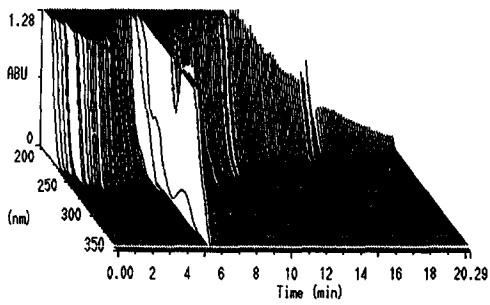

Fig. 4. Extraction profile of lemon peel. SFE conditions: temperature constant at 45°C; pressure changed stepwise from 90 to 110, 140 and 170 kg/cm^2 at intervals of 5.0 min.

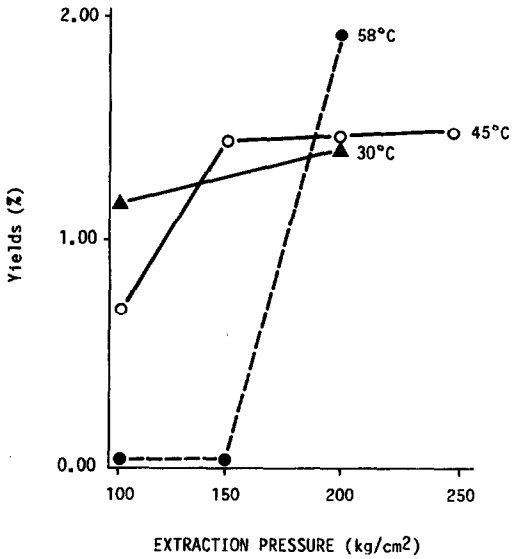

Fig. 5. Extraction yields of lemon peel oil under various SFE conditions. Temperatures (°C): 30 (▲); 45 (○); 58 (●).

from 90 to 110, 140 and 170 kg/cm² at intervals of 5.0 min, while maintaining constant the mass flow-rate of carbon dioxide. According to the profile, the extraction of the lemon peel oil starts at a relatively low pressure, 90 kg/cm². Therefore, we decided to examine the extraction pressure in the range 100–250 kg/cm².

Percentage yields of extracted oils under various conditions

The yields were obtained simply by dividing the mass of oil collected by the mass of sample before extraction. Fig. 5 shows the relationship between the percentage yield and the extraction pressure at different temperatures. At 58°C the oil was hardly extracted below a pressure of 150 kg/cm², however at 200 kg/cm² the extraction yield rapidly increased to 1.94%. Although this yield was the maximum among all the conditions examined, the extract had a slight off-flavour. Therefore, such conditions are not suitable from the viewpoint of the quality of the product.

The extraction at 45°C gave satisfactory results from the viewpoints of both the quality of the flavour and the efficiency of extraction over the relatively wide range of pressure, 150–250 kg/cm².

The yield at a pressure of 100 kg/cm² and at 30°C, which is a slightly below the critical temperature of 31.3°C, is higher than that at 45°C and 100 kg/cm², and a little lower than that at 200 kg/cm². The quality of the oils extracted under the above conditions was comparable with these extracted at 45°C and pressures of 150 and 250 kg/cm². This suggests that the extraction could be performed at room temperature with sub-critical or liquid carbon dioxide at comparatively low pressures around 100 kg/cm².

Considering the extraction efficiency and quality of the product, the conditions chosen were 30–45°C and pressures of 100–250 kg/cm². These conditions are similar

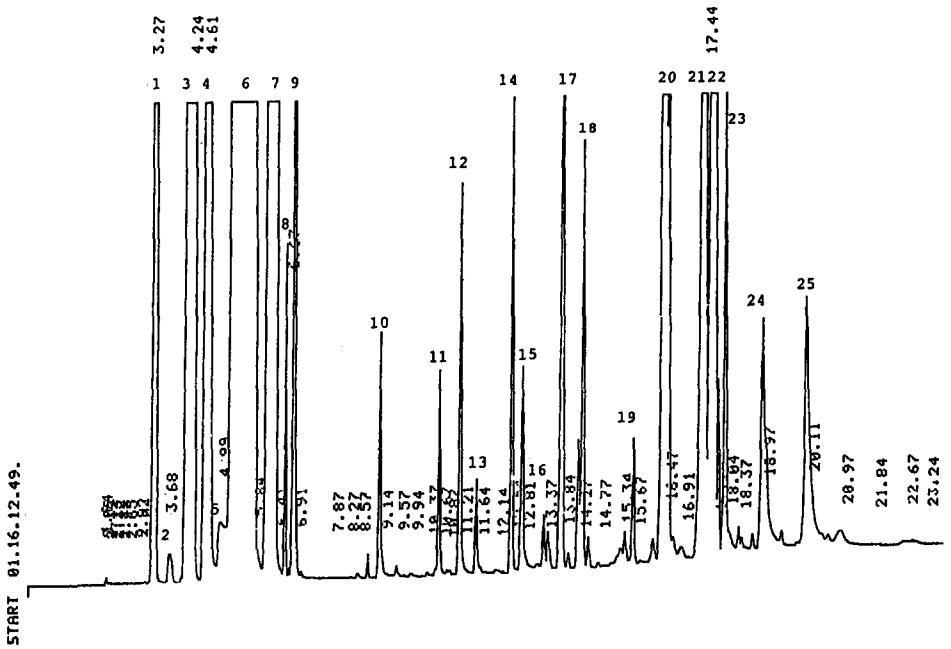

Fig. 6. Typical capillary GC chromatogram of lemon peel oil. SFE conditions: temperature, 45°C; pressure, 100 kg/cm². GC conditions: column, 25 m × 0.2 mm I.D. CBP 20; carrier gas, helium (1 ml/min); splitting ratio, 1/90; column temperature, 90°C for 2 min, 5°C/min to 175°C; detection, FID.

to those proposed by Coppella and Barton[24], 35–40°C and 77–85 bar (78.5–86.7 kg/cm²). The higher pressures in our case are accounted for by the difference in the state of the starting material, ours was solid, theirs was liquid already extracted from lemon peel. It is considered that the higher pressure is required for carbon dioxide to diffuse into the oil cells through the cell membrane.

Comparison of components of extracted oils and cold-pressed oil

Fig. 6 shows a typical GC chromatogram of the oil extracted at 100 kg/cm² and 45°C. There are 25 major peaks and each peak area and the sum of the areas were used to calculate the content. Peak identification by GC–MS analysis was successful for 23 components, however peaks 13 and 16 were not identified. Table I lists the contents of the components of oils extracted under various conditions; those of a cold-pressed oil are also listed for comparison.

Apparently, the limonene content in cold-pressed oil is higher than that in any of the carbon dioxide-extracted oils. In order to facilitate the comparison, the components listed in Table I were grouped into several compound types, namely hydrocarbons, aldehydes, alcohols, esters and oxygen-containing compounds. The total amounts of these types of compounds and their major components are shown in Table II.

Even though the total hydrocarbon contents are very similar for all the oils, carbon dioxide contain less limonene than the cold-pressed oil, in accord with a

TABLE I

AMOUNTS (%) OF COMPONENTS OF OILS EXTRACTED WITH CARBON DIOXIDE UNDER VARIOUS CONDITIONS

Amounts calculated as: (peak area) · 100/(total area of 25 peaks assigned). Peak numbers correspond to those in Fig. 5. Peak 23 may have included citronellol.

Peak No.	*Component*	*30°C*		*45°C*			*58°C*	*Cold-pressed*
		100 kg/cm²	*200 kg/cm²*	*150 kg/cm²*	*200 kg/cm²*	*250 kg/cm²*	*200 kg/cm²*	
1	α-Pinene	1.52	1.19	1.44	1.13	1.51	1.49	1.77
2	Camphene	0.04	0.03	0.03	0.03	0.04	0.04	0.03
3	β-Pinene	11.47	9.18	9.95	8.97	11.39	10.54	9.68
4	Myrcene	1.40	1.40	1.45	1.39	1.38	1.48	1.80
5	α-Terpinene	0.09	0.07	0.09	0.09	0.10	0.10	0.09
6	Limonene	64.28	68.38	68.23	68.23	64.15	68.59	71.80
7	γ-Terpinene	11.29	9.80	9.85	9.63	11.35	9.60	6.35
8	*p*-Cymene	0.12	0.07	0.08	0.07	0.11	0.07	0.29
9	Terpinolene	0.57	0.50	0.55	0.57	0.58	0.47	0.51
10	Nonanal	0.10	0.11	0.08	0.09	0.11	0.10	0.08
11	Limonene oxide	0.11	0.10	0.09	0.09	0.11	0.09	0.02
12	*trans*-Sabinene	0.17	0.16	0.13	0.16	0.19	0.14	0.33
13	– hydrate	0.05	0.05	0.04	0.05	0.05	0.04	0.02
14	Citronellal	0.23	0.18	0.17	0.18	0.22	0.16	0.45
15	Octyl acetate	0.15	0.11	0.12	0.12	0.14	0.10	0.08
16	–	0.07	0.06	0.04	0.06	0.07	0.05	–
17	Linalool	0.56	0.56	0.44	0.56	0.58	0.58	0.07
18	Linalyl acetate	0.42	0.35	0.27	0.34	0.42	0.46	0.09
19	4-Terpineol	0.10	0.09	0.07	0.09	0.14	0.08	0.01
20	Neral	1.67	1.82	1.48	1.66	1.90	1.54	1.89
21	Citral	1.68	1.91	1.36	1.80	1.88	1.38	1.02
22	Neryl acetate	2.01	2.17	2.21	1.96	2.27	1.87	2.34
23	Geranyl acetate	0.39	0.44	0.37	0.50	0.36	0.35	0.51
24	Nerol	0.57	0.42	0.41	0.56	0.34	0.37	0.01
25	Geraniol	0.65	0.56	0.46	0.81	0.44	0.52	0.01

previous report by Calame and Steiner[11]. Regarding alcohols, the carbon dioxide extracts exhibited more than ten times higher contents than the cold-pressed oil, also in accord with the previous report.

Although the total amounts of aldehydes are similar in all the oils including the cold-pressed oil, the carbon dioxide-extracted oils contain more citral by a factor of 1.3–1.9 than the cold-pressed oil, which is not in accord with the previous report. However, this difference can be regarded as within the seasonal and regional variation of the citral content because the ordinary commercial cold-pressed oil and the lemon were from different sources and these materials are of totally different origins. According to Staroscik and Wilson[22], this variation was greater than a factor of 2. Another reason for the disagreement may be the different scales of the experiments: the previous workers[11] used a 4-l extraction vessel; we used an extraction vessel of 60 μl in volume. Therefore, only about 600 mg of the yellow skin of lemon peel were used in our system; in the previous study[11] whole peel including the fibre may have been used, but this is not clear.

TABLE II

COMPOSITIONS (%) OF COMPOUND TYPES AND MAJOR COMPONENTS

Compound type/ major component	*30°C*		*45°C*			*58°C*	*Cold-pressed*
	100 kg/cm²	*200 kg/cm²*	*150 kg/cm²*	*200 kg/cm²*	*250 kg/cm²*	*200 kg/cm²*	
Total hydrocarbons	91.00	90.83	91.84	90.72	90.85	92.56	92.67
Limonene	64.28	68.38	68.23	64.63	64.15	68.59	71.80
Total aldehydes	3.61	4.02	3.09	3.73	4.11	3.18	3.44
Citral	1.68	1.91	1.36	1.80	1.88	1.38	1.02
Total alcohols	1.88	1.63	1.38	2.02	1.50	1.55	0.10
Linalool	0.56	0.56	0.44	0.56	0.58	0.58	0.07
Total esters	2.97	3.07	2.97	2.92	3.19	2.78	3.02
Linalyl acetate	0.42	0.35	0.27	0.34	0.42	0.46	0.09
Oxygen-containing compounds*	8.57	8.82	7.53	8.76	8.91	7.60	6.58

* The total including those in the above four groups.

The total amounts of esters in the oils is also similar, however the major component linalyl acetate was richer in the carbon dioxide extracts by a factor of 3–5 than in the cold-pressed oil. It is unknown whether this is due to the different extraction methods, or to the different sources of material.

The oil obtained at a pressure of 200 kg/cm² and 58°C which had a slight off-flavour did not show any special difference in constituents that can be differentiated by the chromatogram. It is assumed that the reason for the off-flavour was the higher solubility of the fluid than that at lower temperature and pressure, thus ad-

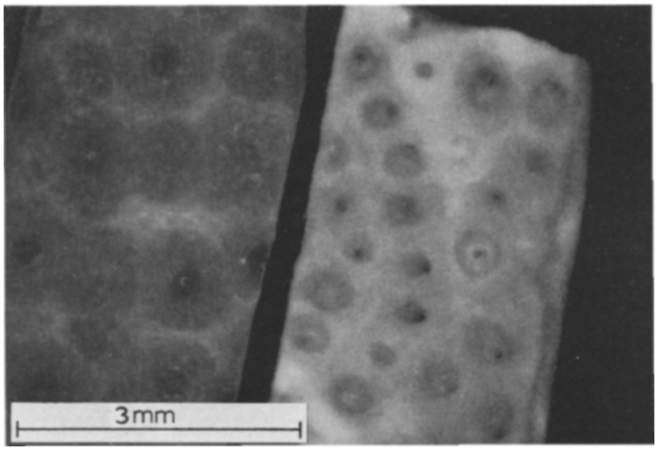

Fig. 7. Microscope photographs of lemon peels before and after extraction. The peel before extraction (left-hand side) shows oil cells through the skin. After extraction (right-hand side) oil was drawn out of the cells and they look like craters.

ditional undesirable aromatic compounds were extracted as well as the necessary compounds. The increase in the total yield lends support to this assumption.

Comparison of the appearance of the peel surface before and after extraction

Fig. 7 shows microscope photographs of the lemon peels before and after extraction. Before extraction, oil cells are seen through the skin. On the other hand, after extraction, oil was drawn out of the cells and they look like craters. The skin itself looks less transparent due to the lack of oil. These photographs suggest that the oil was not simply squeezed out by the pressure of carbon dioxide, but carbon dioxide diffused into the oil cells, dissolved and drew out the oil from the cells, *i.e.*, the oil was extracted.

CONCLUSION

Micro-SFE is a very simple and easy extraction technique. It is much easier to perform, as we have demonstrated, than one might expect. In addition, by changing the extraction conditions, temperature and pressure of carbon dioxide, extracts of different components can be obtained as though different solvents had been used. Furthermore, it should be stressed that an extract is obtained frozen in solid carbon dioxide, and separation of the extract from carbon dioxide can be carried out simply by leaving it at room temperature. There is no need to heat the extract, which is very desirable for extraction of thermally unstable substances from natural products.

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