Combination of Supercritical CO$_2$ and Vacuum Distillation for the Fractionation of Bergamot Oil

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Supercritical CO$_2$ can be used to separate oxygenated compounds from essential oils. This technique still cannot replace vacuum distillation as an industrial process because of low recoveries and inconsistent results. In the present work, a comparison between the two methods was made in terms of composition, recovery, and color. Vacuum distillation and supercritical CO$_2$ are complementary processes for producing high quality oxygenated compounds with high recovery rates. The former is more suitable for removing monoterpenes at low fraction temperatures ($\leq$308 K), and the latter is more suitable for separating oxygenated compounds from pigments and waxes. Consequently, the two methods were combined. For supercritical CO$_2$ fractionation, the parameters of pressure, temperature gradient, and the ratio of solvent to feed were investigated for the fractionation of oxygenated compounds with high recoveries ($\geq$85%) and without other macromolecules, such as pigments and waxes.

INTRODUCTION

Bergamot oil is obtained by cold pressing the peel of the bergamot fruit (*Citrus aurantium Bergamia* Risso). A valuable citrus oil, bergamot oil is widely used in the food, cosmetic, and pharmaceutical industries (*1*). As compared with other citrus oils, bergamot oil is marked by a lower amount of limonene (25.6–53.0%) and higher amounts of linalool (1.7–20%) and linalool acetate (15.6–40.4%) (*2*). Generally, these volatile compounds make up approximately 93–96% of the bergamot oil. The characteristic flavor and pharmacological properties of citrus oils are mainly provided by the oxygenated compounds (OCs), which consist of alcohols, aldehydes, and esters, such as linalool, citral, and linalool acetate, respectively.

Monoterpenes (MTs), such as limonene and pinene, do not contribute much to the flavor and are relatively unstable in heat and light; thus, it is necessary to remove MTs to increase the product's shelf life. In addition, there are about 4–7% non-volatile compounds (NCs) that need to be removed. Waxes, pigments, and coumarins (citropten), for example, tend to precipitate, inducing turbidity in the product, and coumarins, as well as psoralens (bergapten and bergamottin), are phototoxic agents responsible for allergenic effects on human skin (*3*).

Over the past years, several researchers tried to remove MTs with supercritical CO$_2$ because the traditional method of vacuum distillation was thought to cause thermal degradation of the oils. For example, Budich et al. (*4*) carried out process scale-up experiments and concluded that supercritical CO$_2$ is competitive with conventional processes. Kondo et al. (*5*) reported that

terpeneless oil (limonene content < 1%) could be obtained at 333 K and 8.8 MPa, but the OCs recovery was only 45.13–65%. Reverchon and Iacuzio (*6*) applied supercritical CO$_2$ to desorb bergamot oil from silica gel. Although MTs with the high purity of 99.3% could be obtained, the purity of OCs was only 86.6%. From the literature, the main problem with the supercritical CO$_2$ process was the difficulty of simultaneously obtaining a high purity and a high recovery of OCs.

Although the new technique of supercritical CO$_2$ has been reported in the literature, the process of deterpenation continues to be conducted with vacuum distillation in most industries. Surprisingly little information on this matter can be found in the open literature (*7*). Consequently, we contacted several organizations producing citrus oils. Thermal degradation is not considered to be a main problem by most industrial experts because the actual temperature for vacuum distillation is not high. Generally, most of MTs can be removed at fraction temperatures lower than 313 K with pressures lower than 400 Pa. The main problem was the lower than required recovery of OCs, in details, after removing MTs from citrus oil; OCs retain together with pigments and waxes in a vacuum vessel, and then, the fraction temperature is increased to 328–333 K for concentrating OCs, but during this step, the recovery of OCs is generally lower than 70%. It is explained that the viscosity of the residual part in vessel increases with the decrease of the highly volatile components' content; consequently, mass and heat transfers become more difficult. If higher temperatures are employed, some pigments are distillated together with OCs, and in this case, thermal degradation is probably caused by high temperatures. Additionally, the process of supercritical CO$_2$ was thought to be feasible only if the production cost was acceptable

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Table 1. Boiling Points of Main Compounds

chemicals	formula	MW	boiling point (K) 1.013×10^5 Pa[a]	boiling point (K) 400 Pa
limonene	$C_{10}H_{16}$	136.23	448.7	308[b]
linalool	$C_{10}H_{18}O$	154.25	471.7	328–333[a]
linalool acetate	$C_{12}H_{20}O_2$	196.29	493	345.5[a]

[a] Cited from the database of Scifinder Scholar 2002 (American Chemistry Society). [b] Calculated with the software of Boiling Point and Vapor Pressure 7.02 (developed by Advanced Chemistry Development Inc., Toronto, Canada).

and if obviously better results (higher purity and recovery of OCs) were obtained than with vacuum distillation.

There are different opinions between literature and industrial information; thus, it is necessary to compare vacuum distillation with supercritical CO_2 fractionation. We thought that the problem in using the supercritical CO_2 process was mainly caused by the small solubility difference between MTs and OCs in supercritical CO_2. Fonseca et al. (8) measured related vapor–liquid equilibrium data, which they compared with those found in the literature. His work indicated that the binary critical pressures of both CO_2–limonene and CO_2–linalool were close to 10 MPa at 323 K, which indicated that a successful separation could only be conducted at pressures lower than 10 MPa and temperatures higher than 323 K. Such low pressures and high temperatures cannot provide an acceptable solubility for commercial production. However, there is about a 20 K difference in the boiling points of limonene and linalool, as shown in **Table 1**. On the basis of this difference, vacuum distillation became the most common process for separating MTs from essential oil.

The goal of our research is to concentrate OCs with high purities and recoveries since they contribute to most of bergamot oil's valuable properties. In this work, vacuum distillation was applied to the fractionation of bergamot oil and then we compared this method with supercritical CO_2 fractionation in terms of the purity, recovery, and color of OCs. On the basis of comparison, we attempted to find a relatively reasonable procedure for obtaining OCs with high quality (high purity and recovery and without pigments or phototoxic compounds).

MATERIALS AND METHODS

Materials. Cold-pressed bergamot oil was supplied by Inoue Perfumery MFG. Co., Ltd. (Yamagata, Japan). Limonene [≥99% by gas chromatography (GC)], linalool (3,7-dimethyl-3-octanol, 98% by GC), linalool acetate (3,7-dimethyl-1,6-octadien-3-yl acetate, 97% by GC), bergamottin [≥98% by thin-layer chromatography (TLC)], bergapten [5-methoxypsoralen, ≥98% by high-performance liquid chromatography (HPLC)], and citropten (5,7-dimethoxycoumarin, ≥99% by HPLC) were obtained from Sigma-Aldrich Japan K.K. (Tokyo, Japan).

Vacuum Distillation. According to the literature (9), an apparatus for vacuum distillation was established and mainly composed of a flask (300 mL), distillation column (150 mm), water bath, condenser, fraction collectors, buffer bottle, and vacuum pump.

Supercritical CO_2 Fractionation. An apparatus for supercritical CO_2 fractionation was described in our previous paper (10). The experimental setup consisted of a countercurrent contact column (2.4 m × 20 mm i.d.) and a separator (600 mL) for the top product. The column was packed with stainless steel 3 mm Dixon Packing (Naniwa Special Wire Netting Co., Ltd., Tokyo) over a length of 1.8 m, and the column temperature was separately controlled by eight proportional–integral–differential controllers.

GC-MS Analysis. The samples (solved in ethanol about 0.02–0.04 g/mL) were analyzed with GC-MS (Hewlett-Packard-5890 series, Palo Alto, CA), coupled with a mass selective detector (HP 5972). The column used was a HP-5MS phenyl methyl siloxane capillary (30 m × 0.25 mm i.d.; film thickness, 0.25 μm). The GC conditions were as follows: oven temperature 343 K for 1 min, then programmed from 343 to 403 K at 3 K min^{-1} and from 403 to 503 K at 10 K min^{-1} and then at 503 K for 5 min; injector temperature, 523 K; injection volume, 1 μL; the split ratio, 85:1; total carrier gas (helium) flow rate, 24 mL/min; and ionizing energy, 70 eV. As for the main components, limonene, linalool, and linalool acetate were identified by comparison of mass spectra and retention times with those of pure standards. For other components, the probability-based matching algorithm was employed for finding the most probable match in the reference library (NIST library of mass spectra and subsets, HPG 1033A). The weight composition was calculated from the GC peak area.

HPLC Analysis. On the basis of the method reported by Subra et al. (1), the content of the phototoxic compounds was determined with HPLC (CLASS-LC10/M10A, SPD-10A detector, Shimadzu Inc., Tokyo, Japan) using a silica gel column 150 mm × 4.6 mm (packing: inertsil SIL 150A 5 μm, GL Sciences Inc., Tokyo, Japan), the mobile phase was chloroform/heptane (20:80, v/v) at 1.0 mL/min, and the UV wavelength was 313 nm. The results were calculated with the external standards of bergamottin, bergapten, and citropten, respectively.

Ultraviolet Spectroscopy. The spectrophotometer (UV1200, Shimadzu Inc.) was employed for determining the color differences between samples. To select the optimum wavelength for determination, the bergamot oil was scanned in the wavelength range of 200–800 nm. The determination wavelengths were set to be 355 and 650 nm, at which there were two peak values, 2.833 and 0.309, respectively.
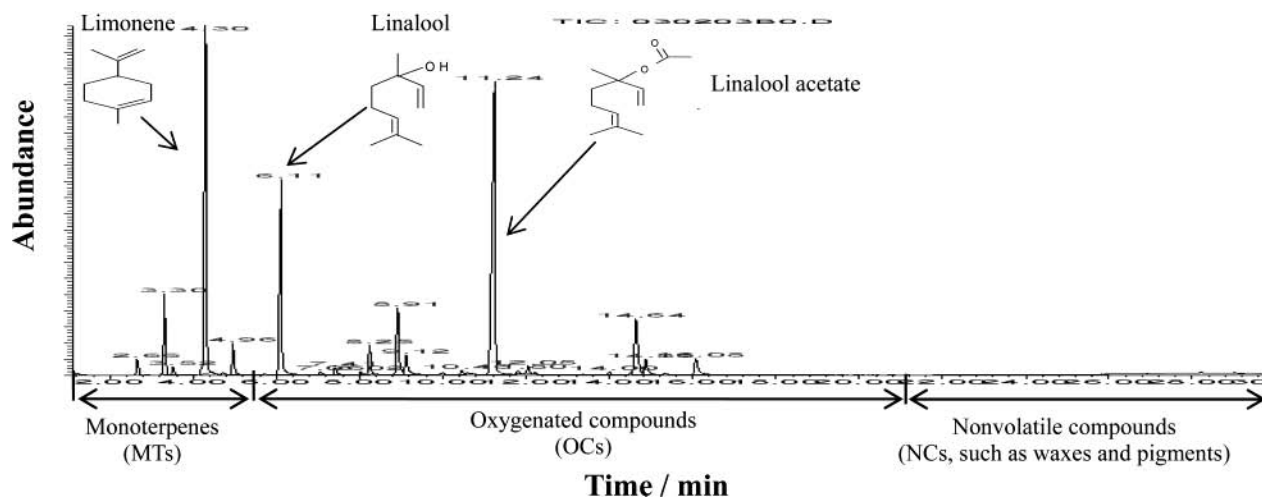
Figure 1. GC-MS analysis of bergamot oil.

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Table 2. Composition of Bergamot Oil Analyzed with GC-MS

peak	retention time (min)	identified substance	area (%)	group
1	2.65	α-pinene	1.01	MTs
2	3.30	β-pinene	5.31	
3	3.52	β-myrcene	0.52	
4	4.30	limonene	24.58	
5	4.96	3-carene	2.27	
6	6.12	linalool	14.20	OCs
7	7.06	citronellal	0.30	
8	7.41	terpineol-4-ol	0.62	
9	8.02	decanal	0.26	
10	8.25	octyl acetate	1.96	
11	8.92	α-terpineol	5.26	
12	9.12	γ-terpineol	1.92	
13	10.45	neral	0.28	
14	11.25	linalool acetate	30.69	
15	11.80	geraniol	0.34	
16	12.05	sesquiterpene (probably *iso*-caryophyllene)	0.69	
17	14.00	citral	0.24	
18	14.64	carvone	6.21	
19	14.88	geranyl acetate	1.60	
20	16.08	neryl acetate	1.74	
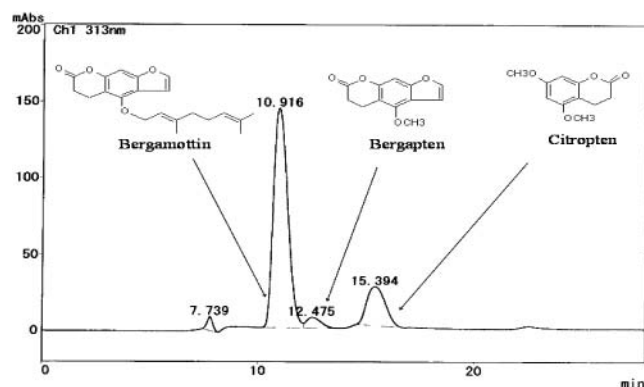
Figure 2. HPLC analysis of phototoxic compounds in bergamot oil.

RESULTS AND DISCUSSION

Composition of Bergamot Oil. The composition of bergamot oil (Inoue Perfumery MFG. Co., Ltd.) determined using GC-MS is shown in **Figure 1**. Totally, 20 compounds were identified in the bergamot oil and the results are listed in **Table 2**. The composition was 33.69% MTs and 66.31% OCs. The main compounds were limonene (24.58%), linalool (14.20%), and linalool acetate (30.69%). In the present work, the limonene was used to represent MTs, whereas linalool and linalool acetate

stood for OCs. In addition to MTs and OCs, there were a few NCs, most of which were not determined using the existing GC-MS conditions.

The results obtained with HPLC are shown in **Figure 2**. The bergamottin, bergapten, and citropten contents were 1.54, 0.106, and 0.175%, respectively.

Vacuum Distillation. Approximately 150 g of bergamot oil was distillated at 350−400 Pa, and the fractions at different temperatures were collected. The collected samples were weighted with an electronic balance and analyzed with GC-MS. The color was represented with UV/vis absorbance values at 355 and 650 nm. The results are listed in **Table 3**, and the GC-MS profiles of the fractions at different temperatures are shown in **Figure 3a−e**.

Vacuum distillation was very effective in removing MTs from bergamot oil, and the operation was conducted very simply by controlling the fraction temperature at or below 308 K.

After the highly volatile compounds were removed at this temperature, the MT content in the residual part was lower than 1%. Simultaneously, most of the linalool and linalool acetate was retained in the residual part, and their recoveries were higher than 95%. However, the separation of OCs from NCs was not satisfactory due to higher operation temperatures, as shown in **Figure 3c−e**. In details, when the fraction temperature was lower than 338 K, the total recovery of linalool acetate was not high, only 75.28%, although a fraction with higher linalool acetate (65.98%) and lower linalool (9.36%) portions could be obtained at 328−338 K, as shown in **Figure 3e**. The fraction's color was light yellow, and the absorbance value of this fraction was also higher than those of other fractions at lower temperatures. This change was thought to be caused by certain pigments codistillated with OCs or some new compounds generated by chemical reactions. The two tendencies were not good for the OCs' quality.

Our previous research (*11*) with supercritical CO_2 indicated that it was not easy to optimize the separation performance in terms of both the selectivity and the recovery. For example, at 8.8 MPa and 333 K, terpeneless oil was obtained, but the OCs recovery was only 45.13−65%, which was not really comparable to that of vacuum distillation.

We concluded that vacuum distillation is more suitable for deterpenation, while using supercritical CO_2 is possibly the more appropriate technique for separating OCs from NCs, because the solubilities of NCs, such as waxes and pigments, in supercritical CO_2 are generally very small at pressures below 20 MPa. Thus, it is possible to obtain waxless and colorless OCs using supercritical CO_2. Following these results, the terpeneless bergamot oil obtained with vacuum distillation was employed as the feed for supercritical CO_2 fractionation.

Table 3. Results of Vacuum Distillation (350–400 Pa)

			composition						UV/vis absorbance	
			limonene		linalool		linalool acetate			
sample	fraction temp (K)	mass ratio of sample to feed (%)	content (%)	recovery (%)	content (%)	recovery (%)	content (%)	recovery (%)	355 nm	650 nm
feed		100	24.58		14.20		30.69		2.833	0.309
fraction 1	298–308	26.06	82.67	87.65	1.96	1.76	1.31	0.26	0.224	0.029
residual[a]		65.07	0.33	0.87	20.91	95.82	46.63	98.87	0.224	0.761
fraction 2	308–318	13.85	0.64	0.36	45.60	44.48	32.17	14.52	1.796	0.033
fraction 3	318–328	20.76	0.29	0.24	27.45	40.13	57.70	39.03	1.969	0.338
fraction 4	328–338	10.11	0.23	0.095	9.36	6.66	65.98	21.74	2.834	0.579
total (2–4)				0.70		91.27		75.28		

[a] Residual part, after removing fraction 1 (298–308 K).

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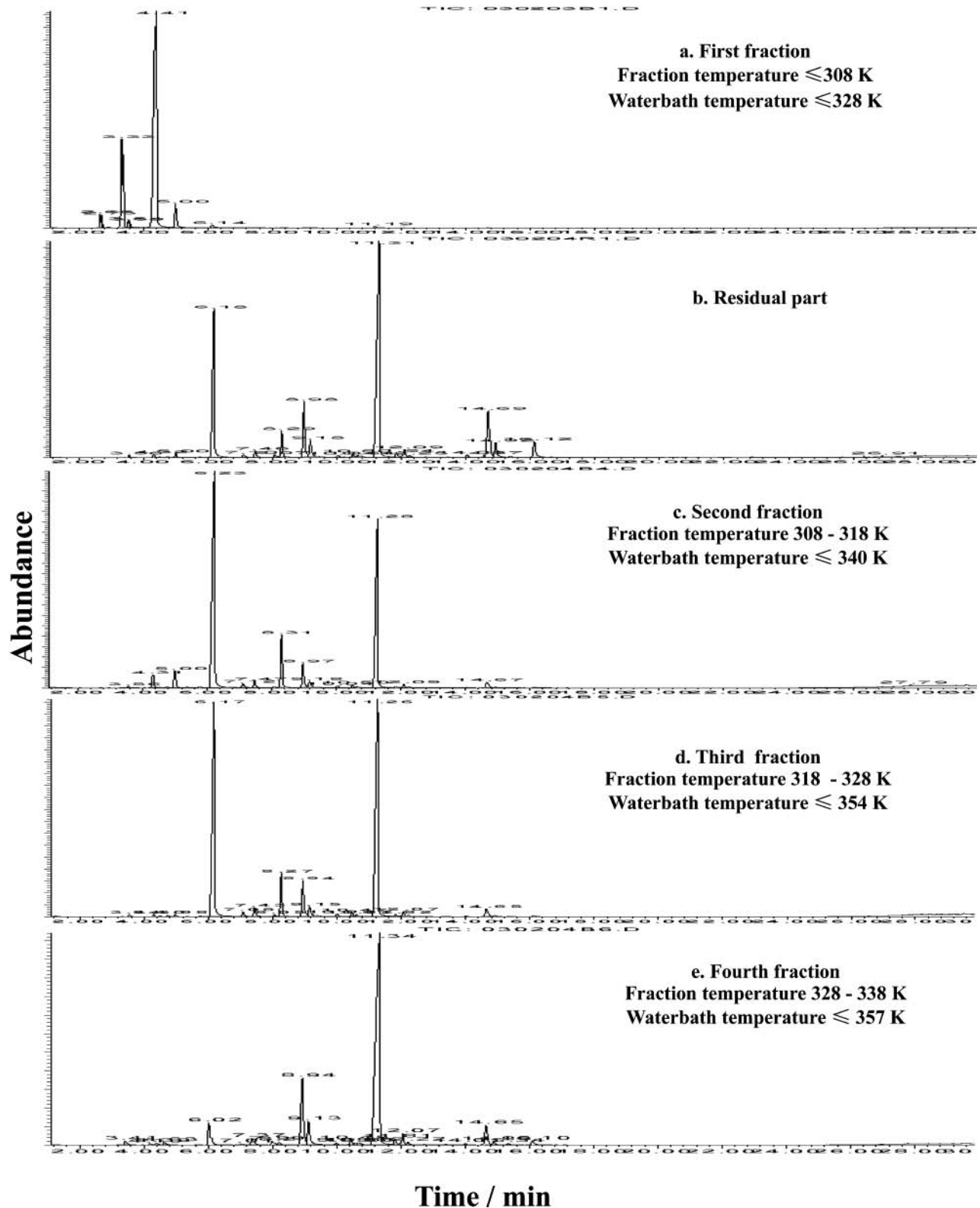*J. Agric. Food Chem.,* Vol. 52, No. 16, 2004   **5165**

Figure 3. GC-MS profiles of different fractions.

Supercritical CO$_2$ Fractionation. *Pressure.* The batch operation was carried out with four different column pressures, 8.5, 10.5, 12.5, and 14.5 MPa. Other operation parameters included the linear distribution of the temperature gradient from 313 K at the bottom to 348 K at the top; feed, 40 g; and CO$_2$ flow rate, 0.33−0.38 g/s (11−12.7 L/min). The extraction yield curve for OCs is shown in **Figure 4**, and analysis data are listed

in **Table 4**, in which the solubility data of OCs in CO$_2$ were calculated from the slopes of the yield curves.

Figure 4 shows that the extraction yield of OCs was greatly influenced by pressure, as higher pressure led to higher solubility and quicker extraction. For example, the solubility of OCs in CO$_2$ was approximately 5.85 g/100 g CO$_2$ at 14.5 MPa, while the solubility at 8.5 MPa was only 0.26 g/100 g CO$_2$. As a result,

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Table 4. Influence of Pressure on Fractionation Results

pressure (MPa)	OCs solubility (g/100 g CO_2)	OCs recovery (%)	absorbance (nm)	
			355	650
8.5	0.26	33.3	1.681	0.0534
10.5	0.98	91.2	1.969	0.0338
12.5	2.83	91.5	1.875	0.0327
14.5	5.85	92.7	3.017	0.0565

Table 5. Influence of the Temperature Gradient on Fractionation Results (12.5 MPa)

temperature gradients (K)	OCs solubility (g/100 g CO_2)	reflux ratio	OCs recovery (%)	absorbance (nm)	
				355	650
313–313	6.85		92.0	3.517	0.125
313–318	6.43	0.07	90.7	3.461	0.134
313–333	4.55	0.51	92.9	2.034	0.0533
313–348	2.83	1.42	91.5	1.875	0.0327

Table 6. Influence of S/F on Fractionation Results

CO_2 flow rate (g/s)	S/F	solubility (g/100 g CO_2)	recovery (%)	absorbance (nm)	
				355	650
0.25	22.5	2.93	65.9	1.739	0.0444
0.35	31.5	2.84	89.6	1.733	0.0383
0.45	40.5	2.35	92.2	1.774	0.0581
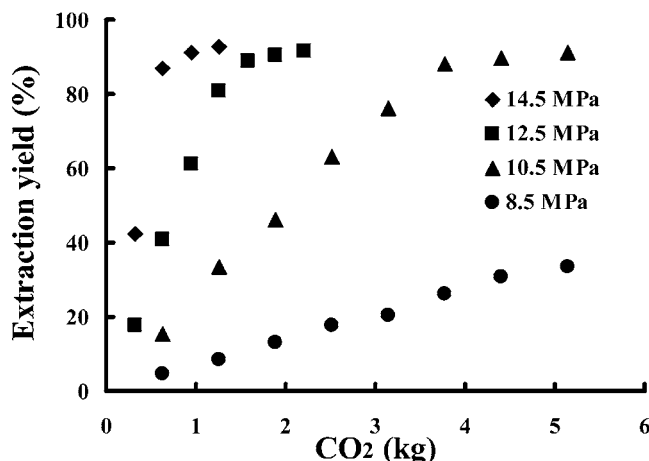
Figure 4. Influence of pressure on the extraction yield of OCs.

at 14.5 MPa, 92.7% of the feed was extracted with only 1.26 kg of CO_2, while at 8.5 MPa the extraction yield was only 8.4% with the same amount of CO_2. For UV/vis determination, the absorbance values at 8.5, 10.5, and 12.5 MPa were almost identical and smaller than those at 14.5 MPa, at which the sample was somewhat yellow and visually deeper in color than those at other pressures. A chemical reaction among different
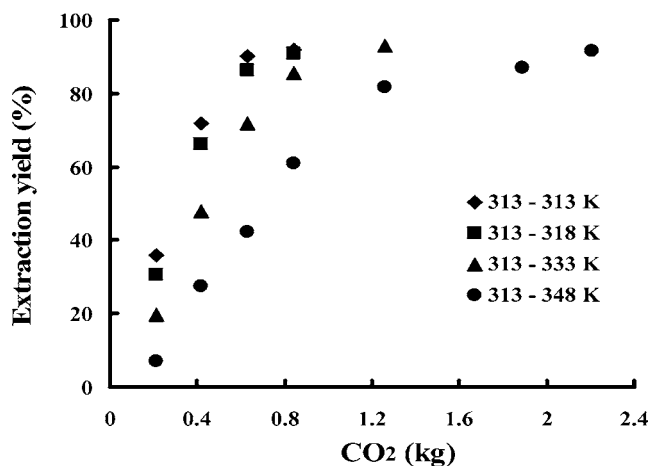
Figure 5. Influence of temperature gradient on the extraction yield of OCs (12.5 MPa).

solutes is probably not occurring in CO_2 inside the column; therefore, the color change was probably caused by pigments that were coextracted with OCs. Although the process was fastest at 14.5 MPa, 12.5 MPa was selected as the optimum pressure value for further experiments considering the color difference.

Temperature Gradient. **Figure 5** and **Table 5** show the results for different temperature gradients. The reflux ratio was defined as follows:

$$R_{333} = \frac{(S_{313} - S_{333})}{S_{333}}$$

where R_{333} is the reflux ratio when the temperature gradient is from 313 to 333 K, S_{313} is the solubility at 313 K, and S_{333} is the solubility when the temperature gradient is from 313 to 333 K. It was hypothesized that there was no internal reflux when the temperatures along the column were the same.

Table 5 illustrates that with the increase of the top temperature, the OCs solubility decreased and, simultaneously, the reflux ratio increased. As a result, the selectivity of the fractionation was improved. The color differences of the OCs obtained with different temperature gradients clearly indicated this trend. In addition, the gradients of the 313−333 and 313−348 K fractions did not show an obvious visual difference in color; both fractions looked like colorless water. Finally, the data demonstrated that the OCs recovery was not influenced by different temperature gradients.

Ratio of Solvent (CO_2) to Feed Flow Rate (S/F). After the optimum parameters were determined for the pressure (12.5 MPa) and temperature gradient (313−348 K), the experiment proceeded under continuous operation, and the feed was charged at the middle of the column. We investigated the influence of

Table 7. Results of Combining Vacuum Distillation with Supercritical CO_2 Fractionation

sample	mass (g)	contents of main compounds (%)			OCs recovery (%)	content of phototoxic compound (%)			absorbance (nm)	
		limonene	linalool	linalool acetate		bergamottin	bergapten	citropten	355	650
bergamot oil	100	24.58	14.2	30.69		1.501	0.0717	0.349	2.833	0.309
MTs	25–27	78–82	0.78–2.1	0.05–1.52		ND	ND	ND	≤0.224	≤0.029
deterpened oil	63–66	<0.79	19–22	40–48	95–98	2.03–2.35	0.10–0.12	0.48–0.60	3.8–4.0	0.6–0.8
OCs	59–62	<0.45	20–26	42–50	90–93	<0.03	<0.02	<0.03	≤2.0	≤0.06
residual of Se–CO_2 fractionation	4.0–7.0	<0.05	9.0–15.0	23.01–35.6		15–26	0.85–1.8	3.8–6.0		
				total recovery	85–89					

S/F, which was changed by adjusting the CO_2 flow rate with a fixed feed flow rate of 0.0111 g/s (40 g/h). The S/F was changed from 22.5 to 40.5. As shown in **Table 6**, the influence of S/F on color was negligible, and the OCs solubility at the S/F of 22.5 was larger than that at 40.5. At a high S/F, a great deal of CO_2 could dissolve more solutes than the amount provided by feed; thus, some CO_2 left the column in an unsaturated condition, and the calculated solubility was smaller than that of other S/Fs. In addition, phototoxic compounds were not determined in all top fractions of the supercritical CO_2 column; most of them were retained in the bottom paraffin.

Combination of Vacuum Distillation and Supercritical CO_2 Fractionation. In our research, the combination of vacuum distillation and supercritical CO_2 fractionation led to obviously better results than those obtained with one method. The first process of vacuum distillation can successfully remove MTs at a fractionation temperature of 308 K, when pressure is 350–400 Pa; the residual part is processed to obtain OCs with supercritical CO_2 fractionation, which is continuously conducted at 12.5 MPa and a temperature gradient set at 313–348 K; the S/F is at approximately 22.5. **Table 7** shows the comprehensive results of the whole separation procedure. The data indicated that the recovery of OCs remained at a relatively high level over both steps. The total recovery of OCs was generally higher than 85%, and most phototoxic components remained in the final residual together with pigments and waxes, which were retained in the fractionation column; therefore, the obtained OCs had almost no color and did not contain macromolecular impurities.

In conclusion, the vacuum distillation experiments clarified certain controversial opinions. The process of deterpenation can be performed successfully by vacuum distillation, which was not very suitable for further separation of OCs from NCs for recovery and fraction color. Supercritical CO_2 fractionation can satisfactorily overcome this difficulty. The two techniques are actually complementary processes for producing OCs of high quality (terpeneless, pigmentless, and waxless) and retaining the phototoxic components.

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