T. Inui, D. Yonezawa and H. Abe

Development of supercritical CO_2 extracts for flavored beer

Different types of herbs and spices other than hops have been traditionally used for flavored beer such as Belgian style white. The distinct flavors in herbs and spices are typically derived from various terpenes. As oxygenated terpenes are easily eluted, certain terpenes, such as terpene hydrocarbons, are difficult to extract in the wort or beer and easily evaporate. Supercritical CO_2 (SC-CO_2) extracts were developed to provide distinct flavors to beer in a more efficient manner. Research was carried out to obtain the most appropriate temperature and pressure conditions to facilitate extraction and separation. Orange peels and coriander seeds, which contain more hydrophilic compounds, were used as starting materials. We found that a separation temperature below 20 °C efficiently increased the yield of key aromatic compounds without freezing, although the separation temperatures had to be set above 40 °C to prevent the water from freezing. Furthermore, the lower CO_2 density obtained a higher purity of key aromatic compounds, which means it provides the resulted extracts more essential oil with lesser impurities derived from each material, although the yield of extract was low. Additionally, the ratio of terpene hydrocarbons to oxygenated terpenes increased with CO_2 density, which resulted in changes in the aromatic characteristics. Finally, through a brewing trial, we find that the single unique SC-CO_2 extraction technique could be the most efficient method to provide beer the distinct aromatic characteristics of herbs and spices as compared to a general beer brewing method, wherein herbs and spices are added to a wort kettle, whirlpool, or fermentation tank.

Descriptors: supercritical CO_2, oxygenated terpenes, terpene hydrocarbons, orange peel, coriander seed, flavored beer

1 Introduction

Different types of herbs and spices other than hops have been traditionally used in the manufacture of flavored beer. In particular, orange peel and coriander seed have been traditionally used to flavor Belgian white ales.

Flavored beer is produced by adding herbs and spices in a wort kettle, whirlpool, or fermentation tank. The distinct aromas of herbs and spices are typically derived from various terpenes. Although oxygenated terpenes are easily eluted, certain terpenes, such as hydrophobic terpenes, are difficult to extract in the wort or beer and they easily evaporate or oxidize. Thus, conventional methods for extracting key aromatic compounds from herbs and spices are inefficient in terms of their yields, or large amounts of ingredients are required. Therefore, we attempted to develop beer using natural aromatic extracts which produce a more distinct aroma. Table 1 (see page 148) shows various manufacturing processes and features of aromatic extracts. All processes in the manufacture of flavor extracts consist of two operations: extraction and separation [1, 2]. The principal difference between the manufacturing processes is the extraction medium, namely water steam, animal fat, organic solvent, or supercritical CO_2 (SC-CO_2), among others. Depending on the extraction medium, separation method, and heat load during extraction, the resultant product extensively varies in terms of quality, cost, and storage stability. Among these manufacturing processes, we focused on SC-CO_2 extraction used with herbs and spices to provide distinct flavors to beer more efficiently.

When the pressure and temperature of carbon dioxide is above its critical point, the fluid is said to be "supercritical CO_2." In the supercritical zone, the fluid shows particular properties and exhibits intermediate characteristics that lie between the characteristics of a liquid and a gas. In particular, supercritical fluids possess liquid-like densities, gas-like viscosities, and diffusivities that fall between liquid and gaseous diffusivities. Carbon dioxide is the most widely used supercritical fluid because it is an odorless, colorless, highly pure, safe, cost-effective, nontoxic, nonflammable, and recyclable gas whose supercritical operation is possible at relatively low pressures and near room temperature, i.e. above a critical temperature of 32 °C and a critical pressure of 7.4 MPa [3]. Generally speaking, SC-CO_2 behaves like a lipophilic solvent; however, compared to liquid solvents, it has the advantage that its selectivity or solvent power is adjustable and can be set to values ranging from gas-like to liquid-like according to the pressure and temperature conditions of extraction. Many studies regarding SC-CO_2 extraction conditions to obtain aromatic compounds from various types of dried plants, such as hops [4,5,6], herbs, and spices [7,8,9,10,11,12,13], have been conducted; such studies compare SC-CO_2 extraction to other methods of extractions, i.e., extraction methods that use solvents and hydrodistillation, [14,15,16] although there are few studies

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Table 1 **Various manufacturing processes and features of aromatic extracts**

	Steam distillation	Expression	Effleurage, Maceration	Organic solvent extraction	Supercritical CO_2 Extraction
Extraction medium	Water steam	–	Lard, tallow	Petroleum ether, hexane	Supercritical CO_2
Separation	Difference in specific gravity between oil and water	Centrifugation	Volatilization after ethanol extraction	Deep cold separation after ethanol extraction	Vacuum release & centrifugation
Heat load	High	Low	Medium	Low	Low
Extraction ratio	Medium	Medium	Low	High	Low
Quality	Medium	Fresh, w/ impurities	Medium	Undrinkable	Good
Manufacturing cost	Low	Low	High	Low	High
Storage stability	Good	Poor	Good	Good	Good

regarding SC-CO_2 separation conditions. Furthermore, several evaluations have been conducted without CO_2 gas recycling, though not in the same manner as actual production. Therefore, to obtain the preferred aromatic quality and a good yield of extracts from herbs and spices for flavored beer, the objective of the study was to examine the optimal conditions for SC-CO_2 extraction. These were determined by the temperature and pressure of not only the extraction process, but also the separation process. The separation step uses the point of yield and profiles of key aromatic compounds related to the distinct aromatic characteristics derived from each herb and spice, by using equipment with CO_2 recycling.

2 Materials and methods

2.1 Chemicals

Carbon dioxide (99.9 % purity) was purchased from Showa Denko Gas Products Co., Ltd. The following chemicals were obtained from commercial sources and all purities were confirmed by gas chromatography (GC): α-pinene (>95.0 %), β-pinene (>95.0 %), β-myrcene (>99.0 %), limonene (>95.0 %), γ-terpinene (>95.0 %), borneol (>70.0 %, contains ca. 20 % isoborneol), valencene (>95.0 %), caryophyllene (≥98.0 %), 1.8-cineol (>98.0 %), camphor (>90.0 %), linalyl acetate (>99.0 %), neryl acetate (>99.0 %) and geranyl acetate (≥99.0 %) from the Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); anhydrous citric acid (≥99.0 %), citronellol (≥98.0 %), geraniol (≥98.0 %), nerolidol (≥98.0 %), nootkatone (≥99.0 %), and decanal (>97.0 %) from Nacalai Tesque, Inc. (Kyoto, Japan); α-terpineol (≥90.0 %), trans- and cis-linalool oxide (>97.0 %) and p-cymene (>99.0 %) from Sigma-Aldrich Japan (Tokyo, Japan); and pure R-linalool from Fluka, Sigma-Aldrich Chemie (Buchs, Switzerland). A total of 500 mg of Bond Elut Jr C18 from Agilent Technologies Japan, Ltd. (Tokyo, Japan) was used in solid phase extraction. Deionized water, sodium sulfate, anhydrous citric acid (≥99.0 %), and methanol (Guaranteed Reagent, 99.8 %) from Nacalai Tesque, Inc. (Kyoto, Japan) were used for preconditioning the solid phase. Dichloromethane for pesticide residue and polychlorinated biphenyl (PCB) analysis from Nacalai Tesque, Inc. (Kyoto, Japan) were used as the solid phase extraction solvent.

2.2 Starting raw materials

Commercially available diced orange peel and powdered coriander (*Coriandrum sativum* L.) seed were used as raw materials. The raw materials were vacuum packed and stored in a refrigerator until use.

2.3 Experimental process

First, to determine the essential oil volatile composition of each material, organic solvent was used in the extraction and the material essential oil compositions were examined using gas chromatography – mass spectrometry (GC/MS). Next, SC-CO_2 extracts of orange peel and coriander seed were prepared under various conditions and their sensory characteristics and chemical compositions were evaluated. Subsequently, to evaluate each extract's qualities in flavored beer and compare them with the cases of adding the materials into the wort kettle at the end of wort boiling, model solutions adding orange peel, coriander seed,

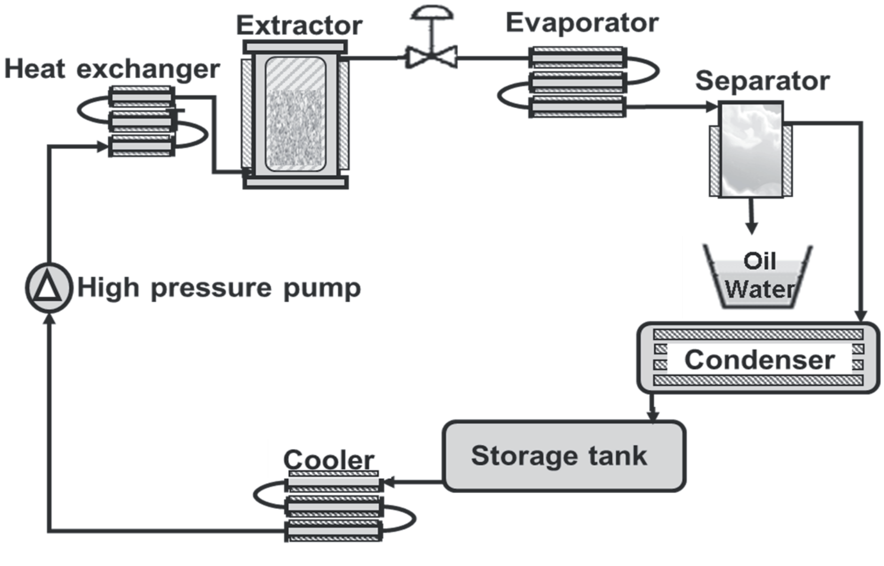

Fig. 1 **Diagram of supercritical CO_2 extraction equipment used in this study**

and their SC-CO_2 extracts were prepared using a heating mantle. Thereafter, their sensory characteristics and chemical compositions were evaluated. Finally, brewing trials were conducted to compare the qualities of the flavored beer brewed with raw orange peel and coriander seed to their SC-CO_2 extracts by sensory evaluation and chemical analysis.

2.4 Liquid-liquid extraction of aromatic components from starting materials

Each 1.0 g of starting material was extracted with 20 ml diethyl ether: n-pentane (2:1) under magnetic stirring overnight. After removing the residues via filtration, the organic phase was dried over Na_2SO_4, and concentrated to 200 µL using a Vigreux column (bp 40 °C/0.7 mmHg). In this study, the chemical compositions of the starting materials were determined using the area normalization method from the total ion chromatogram (TIC) area, obtained from GC/MS, to compare the compositions of the orange peel and coriander seed.

2.5 The diagram of SC-CO_2 extraction equipment and preparation of SC-CO_2 extracts

Figure 1 shows a diagram of the SC-CO_2 extraction equipment used in this study. The extractor scale was 2 liter. CO_2 gas was condensed up to its supercritical fluid state and recycled after separating the extracts from the CO_2 fluid. The collected extracts contained water which was removed using a centrifuge. In this study, to obtain the most preferable aromatic qualities of the extracts and a high yield of aromatic compounds, the pressure and temperature of the extractor and separator were varied. Diced orange peel and powdered coriander seed were used as starting raw materials and processed under five or four conditions as shown in table 2. Each extraction experiment was performed four times and each obtained sample was mixed excluding the first extraction sample, because the first extraction was unstable with regards to pressure and temperature. The amount of filling materials, length of extraction time, and flow rate of CO_2 were all the same at each test level. Extraction conditions such as pressure and temperature were set at these values and resulted in each CO_2 density. Under these conditions, T1, T2, T3, and T4 are supercritical phases and only T5 is a liquid phase. Two different temperatures, 50 and 18 °C, were applied to separate the orange peel extracts. Separation of every coriander seed extract occurred at 18 °C. The pressure of the separation process at each experimental level was automatically determined by temperature setting.

2.6 Preparation of the model solutions flavored with raw orange peel and coriander seed and their SC-CO_2 extracts

To compare the sensory characteristics and the chemical compositions that contribute to the aroma in beer using raw material and SC-CO_2 extracts, model solutions were prepared [17]. Each 0.2 g of starting material, or extract produced from the same amount of raw material, was added to 100 mL of a 20 mM citric acid buffer solution (pH 5.3) heated at 98 °C using a heating mantle with a reflux condenser, and was left to stand for 5 min. After filtration, the model solutions were used for sensory evaluation and chemical analysis.

Table 2 **Chemical compositions of raw materials via mixture of diethyl ether and n-pentane extraction**

Area (TIC, %)			
Orange Peel		**Coriander seed**	
β-Pinene	0.21 %	β-Pinene	0.04 %
Sabinene	0.09 %	β-Myrcene	0.23 %
β-Myrcene	1.31 %	Limonene	0.78 %
Limonene	88.3 %	trans-4-Thujanol	0.05 %
γ-Terpinene	5.06 %	γ-Terpinene	2.75 %
o- or *m*- or *p*-Cymene	0.28 %	t-Butylbenzene	0.42 %
Terpinolene	0.21 %	δ-Terpinene	0.20 %
δ-Elemene	0.07 %	Linalool oxide	0.07 %
α-Copaene	0.09 %	β-Terpineol	0.07 %
β-Cubebene	0.04 %	Camphor	5.31 %
Linalool	0.17 %	β-Terpineol	0.09 %
β-Elemene	0.22 %	Linalool	77.5 %
Humulene	0.05 %	cis-β-Farnesene	0.07 %
α-Terpineol	0.11 %	4-Terpineol	0.16 %
Germacrene D	0.12 %	Myrtenyl acetate	0.18 %
Valencene	0.07 %	Isoborneol	0.77 %
α-Selinene	0.15 %	Geranyl acetate	5.60 %
δ-Cadinene	0.08 %	Citronellol	0.07 %
α-Farnesene	0.28 %	Geraniol	2.68 %
Limonene glycol	0.07 %	Hodiendiol I	0.26 %
Benzyl benzoate	1.31 %	2,6-Dimethyl-1,7-octadien-3,6-diol	0.15 %
Octacosane	0.09 %	Palmitic acid	0.53 %
Palmitic acid	0.65 %	Squalene	0.41 %
Benzyl (E)-cinnamate	0.33 %	Oleic acid	1.60 %
Linoleic acid	0.67 %		
Total	100.0 %		100.0 %

2.7 Solid phase extraction of aromatic components from model solutions and beer samples

The internal standard, borneol, was used to decrease the variation in the extraction rate via solid phase extraction (SPE) or other sample treatments. It was added to 10 mL of each sample to achieve a final concentration of 50 µg/L. The solution was then loaded onto the solid phase (Bond Elut Jr C18 500 mg) that had been preconditioned with 10 mL of methanol followed by 10 mL of deionized water. Subsequently, the solid phase was washed with 10 mL of deionized water and dried with N_2 gas for 10 min. The retained compounds were eluted with 10 mL of dichloromethane and the eluate was concentrated to exactly 100 µL using a calibrated tube with a vacuum rotary evaporator below 25 °C and 250 mmHg for approximately 15 min. Identification of the constituents was based on a comparison of the retention times to those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons. As a further step, computer matching of the samples against a commercial (National Institute

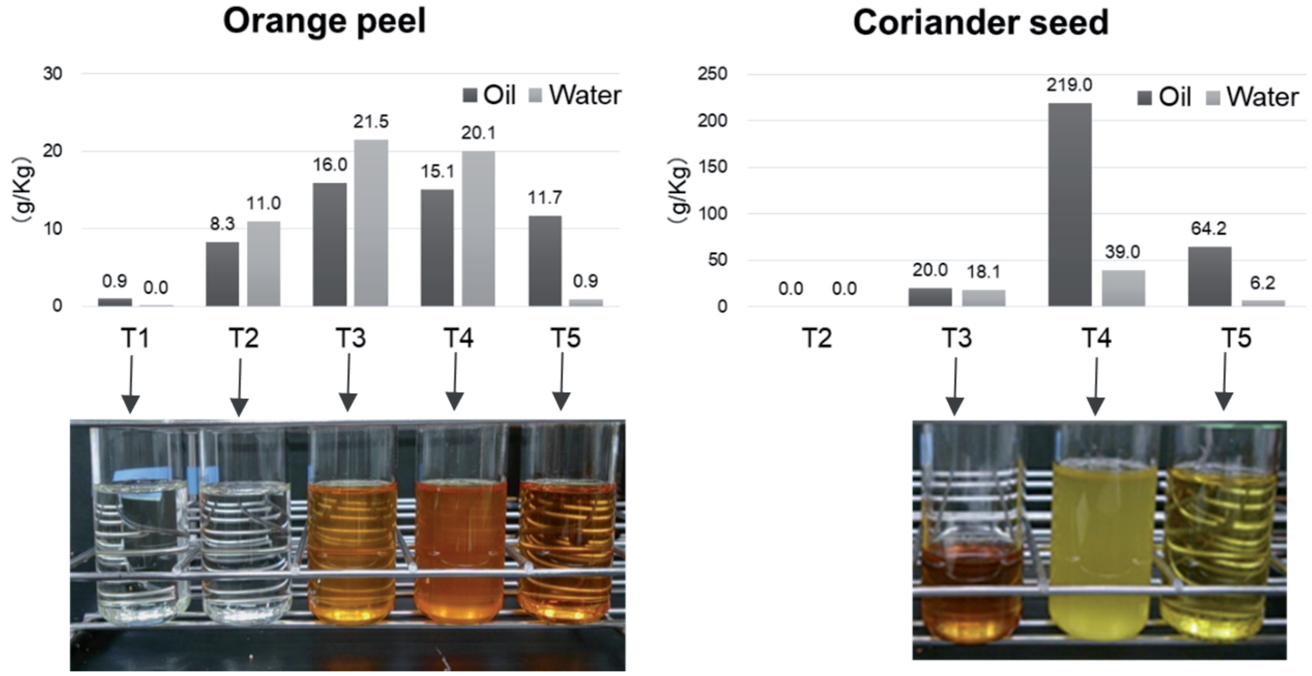

Fig. 2 **Resultant oil appearances and oil and water yields per kilogram of material weight obtained from orange peel and coriander seed. The extraction condition of each test level (T1–T5) is indicated in table 3**

of Standards and Technology and Wiley) library was also carried out. To create the calibration curves, a mixture of identified compounds in each starting material was prepared using the standard reagents dissolved in each sample, treated with the solid phase, and concentrated as previously mentioned. For all compounds, the calibration curves were linear ($R^2 > 0.95$) , and passed through the origin. The concentration range of the calibration curve was from 0.5 to 200 µg/L. Each sample was diluted to within this concentration range and quantitated.

2.8 Analytical instruments

A GC/MS system consisting of a 7890A gas chromatograph with a 5975C mass spectrometer (Agilent 138 Technologies, Palo Alto, CA) was used for the analysis of aromatic compounds. The column was a polar VF-WAX (with a 60 m × 0.25 mm inner diameter and 0.5-µm film thickness). The column was maintained at 40 °C for 1 min and then programmed to change from 40 to 250 °C at 6 °C/min where it was maintained for 5 min. The injection volume was 0.2 µL with splitless mode. Quantification was conducted in the selected ion monitoring mode (SIM). The ions used for quantification are indicated in tables 4 and 5 and the ion for internal standard, borneol, is m/z 95.

2.9 Brewing trials

Trials were conducted in a pilot scale brewery (100 L) with a malt ratio of 100 %. The same amount of CO_2 hop extract was added to each wort at the beginning of boiling. The timing of the addition of raw materials for the control and the extracts for testing was determined to have the highest impact on the aroma of each beer sample through preliminary estimation and the results of the model solutions. For the control sample, 220 g of diced orange peel and 33 g of powdered coriander seed were added to the whirlpool, and for the test samples, the T3 extract of the orange peel and T4 extract of the coriander seed, extracted from the same amount of raw materials used for the control, were added in the conditioning tank following filtration. Fermentation was performed using ale yeast below 18 °C for 4 days. After fermentation, each fermented wort was stored at 0 °C for 3 days. Filtration and bottling were completed at a pilot scale after adjustment of the carbon dioxide pressure.

2.10 Quantitative descriptive analysis of aromatic characteristics in model solutions and beer samples

Quantitative descriptive analysis (QDA) was performed by eight to 10 well-trained panelists one time. A total of 25 mL of each model solution and 150 mL of each beer sample were served.

Table 3 **Experimental conditions of SC-CO_2 extraction**

Materials		Orange peel				
			Coriander seed			
Conditions		T1	T2	T3	T4	T5
The amount of filling material (Kg)		1				
Extraction time (hr.)		1.5				
S/F(-): the ratio of the mass of solvent to the feed mass		45 (CO_2 flow speed: 30 kg/hr., extraction time: 1.5 hr)				
Extraction	Temperature (°C)	50				10
	Pressure (MPa)	9		15	30	6.5
	CO_2 density (kg/m³)	293	293	702	871	888
Separation	Temperature (°C)	50	18			
	Pressure (MPa)	5.5	4.8			

Table 4 Chemical compositions of the resulting SC-CO_2 extracts by weight of orange peel extract (mg/L) and those by weight of material (mg/kg)

		m/z	Aromatic compounds by weight of orange peel extract (mg/L)					Aromatic compounds by weight of material (mg/Kg)				
			T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Terpene hydrocarbons	α-Pinene	69	1616.16	3811.04	3354.23	2412.16	2913.99	1.62	34.49	58.53	39.74	37.29
	β-Pinene	69	210.09	421.45	353.09	258.69	302.83	0.21	3.81	6.16	4.26	3.87
	β-Myrcene	93	20809.75	30817.45	23387.91	18113.94	20091.86	20.90	278.88	408.11	298.39	257.09
	Limonene	68	604751.39	608100.75	376090.36	339031.79	392496.58	607.34	5502.96	6562.71	5584.91	5022.19
	γ-Terpinene	121	248.15	249.03	162.28	148.03	131.37	0.25	2.25	2.83	2.44	1.68
	Terpinolene	93	644.32	540.15	386.53	318.46	336.08	0.65	4.89	6.74	5.25	4.30
	p-Cymene	119	8.65	8.28	8.02	5.14	6.13	0.01	0.07	0.14	0.08	0.08
	Valencene	161	152206.83	105116.61	59213.57	51523.33	57425.19	152.86	951.24	1033.27	848.75	734.78
	Caryophyllene	147	1854.68	1209.81	720.38	626.02	692.49	1.86	10.95	12.57	10.31	8.86
Oxygenated terpenes	1.8-Cineol	81	200.60	184.91	146.16	111.80	127.04	0.20	1.67	2.55	1.84	1.63
	cis,trans-Linalool oxide	59	72.97	58.56	77.10	84.24	67.03	0.07	0.53	1.35	1.39	0.86
	Linalool	93	8148.34	5898.15	4016.10	3375.91	3324.96	8.18	53.37	70.08	55.61	42.54
	Camphor	95	18.33	10.65	7.77	6.04	6.81	0.02	0.10	0.14	0.10	0.09
	Linalyl acetate	93	223.27	129.89	94.97	89.10	98.07	0.22	1.18	1.66	1.47	1.25
	α-Terpineol	59	2045.79	1210.17	832.85	738.48	655.05	2.05	10.95	14.53	12.17	8.38
	Geranyl acetate	69	510.33	369.96	190.81	171.29	191.83	0.51	3.35	3.33	2.82	2.45
	Citronellol	69	1149.26	787.74	480.00	463.29	476.81	1.15	7.13	8.38	7.63	6.10
	Geraniol	69	211.10	144.28	96.99	92.87	76.33	0.21	1.31	1.69	1.53	0.98
	Nerolidol	69	266.18	177.66	79.83	77.80	85.71	0.27	1.61	1.39	1.28	1.10
	Nootkatone	147	2516.42	996.00	534.37	519.98	494.18	2.53	9.01	9.32	8.57	6.32
Aldehyde	Decanal	57	1563.85	1201.95	832.84	730.68	556.31	1.57	10.88	14.53	12.04	7.12
	Total		799276.44	761444.49	471066.13	418899.02	480556.66	802.70	6890.63	8220.02	6900.57	6148.96

The panelists evaluated the top note characteristics of the model solutions by smelling the samples. However, the panelists evaluated the retoronasal aroma of beer samples by swallowing. In this study, the juiciness and fresh scent of the orange peel, and citrusy and spicy characteristics of the coriander seed were used as the sensory descriptors for the QDA of the model solutions because these are the distinct key aromatic characteristics in each material.

In the sensory evaluation of the beer samples, the intensity of orange peel and coriander seed aromatic characteristics in each beer were scored. The intensities of the sensory descriptors were assigned scores of from 0 to 5 (0: none; 1: slight; 3: clear; and 5: intense). Statistical analyses were conducted using JMP version13.0 (SAS Institute Inc.).

3 Results and discussion

3.1 Chemical compositions of the starting raw materials

As shown in table 2, it was confirmed that the orange peel consisted of 88 % limonene and 5 % gamma-terpinene, which are hydrophobic terpene hydrocarbons. In contrast, the coriander seed contained 78 % linalool and several percentage points of camphor, geranyl acetate, and geraniol, which are hydrophilic oxygenated terpenes [18]. These detected compounds were reported to be the key aromas in each material [19, 20, 21, 22, 23]. From these results, it was supposed that the optimal conditions of SC-CO_2 extraction from the orange peel differs from that of the coriander seed.

3.2 Oil yield and appearance of resultant SC-CO_2 extracts

The resultant oil appearances and oil yields indicated a large difference as shown in figure 2. Orange peel oils T1 and T2 treated with a lower CO_2 density were colorless. In contrast, T3, T4, and T5 of both oils were colored orange or yellow and some were cloudy. It was concluded that a higher CO_2 density could promote the elution of pigments and waxes, thus resulting in highly lipophilic oils that contain more impure substances, as opposed to essential oils, which contribute to the key aroma.

The graphs of figure 2 show the oil and water yields per kg of material weight obtained from orange peel and coriander seed. Orange peel T1 showed the lowest oil yield. This result can be attributed to the lower CO_2 density and the higher separation temperature. The separation temperatures were set above 40 °C to prevent the water from freezing. However, it is clear that in the

Table 5 **Chemical compositions of the resulting SC-CO_2 extracts by weight of coriander seed extract (mg/L) and those by weight of material (mg/kg)**

		m/z	Aromatic compounds by weight of coriander seed extract (mg/L)			Aromatic compounds by weight of material (mg/Kg)		
			T3	T4	T5	T3	T4	T5
Terpene hydrocarbons	α-Pinene	69	930.88	126.31	196.58	99.88	520.65	237.51
	β-Pinene	69	304.22	34.04	55.93	32.64	140.31	67.58
	β-Myrcene	93	2132.84	83.07	112.48	228.84	342.43	135.90
	Limonene	68	2450.93	172.47	282.14	262.97	710.91	340.89
	γ-Terpinene	121	2898.39	192.82	314.35	44.38	89.94	45.22
	Terpinolene	93	307.34	9.95	15.74	310.98	794.78	379.81
	p-Cymene	119	1006.39	64.62	115.18	32.98	41.02	19.02
	Caryophyllene	147	1892.66	52.61	95.96	0.00	0.02	0.01
Oxygenated terpenes	1.8-Cineol	81	413.64	21.82	37.42	107.98	266.37	139.16
	cis,trans-Linalool oxide	59	149.14	7.45	12.73	11.18	22.14	11.19
	Linalool	93	132821.13	4144.09	5212.42	16.00	30.71	15.38
	Camphor	95	104.22	5.37	9.26	20.85	64.17	19.43
	Linalyl acetate	59	2960.38	123.85	207.12	14250.93	17081.80	6297.83
	α-Terpineol	69	143.67	4.59	7.92	317.63	510.52	250.25
	Citronellol	69	969.95	25.73	42.76	15.42	18.94	9.57
	Geraniol	93	20880.88	564.08	1049.43	203.07	216.85	115.94
	Neryl acetate	69	2522.19	66.71	98.19	104.07	106.04	51.66
	Geranyl acetate	69	11407.96	306.73	570.90	1.18	1.14	0.60
	Nerolidol	69	137.29	3.48	6.27	17.16	14.80	3.12
Aldehyde	Decanal	57	194.34	15.57	16.08	1224.01	1264.31	689.79
Total			184628.44	6025.35	8458.85	17302.15	22237.85	8829.84

case of T2, which was treated at a lower temperature, the separation of oil from carbon dioxide was more efficient compared to that of T1, although both were treated under the same CO_2 density. Moreover, T2 resulted in an increased oil yield without freezing. Additionally, the oil yield increased to some extent for both materials with increasing CO_2 density, although with liquid CO_2, in the case of T5, a lower oil yield was obtained than that of T4 despite the higher CO_2 density. Furthermore, in the case of T2, coriander seed yielded no oil. This was believed to be caused by the higher power needed to penetrate into plant materials plant materials such as the coriander seed. It was considered that, because the orange peel and the coriander seed have different physical properties [24] and chemical compositions, the characteristics of the oil yields under the same SC-CO_2 extraction conditions differed when using orange peel as opposed to coriander seed.

Tables 4 and 5 present the chemical compositions of the resulting SC-CO_2 extracts by the weight of the orange peel and coriander seed extract and those by weight of raw material, respectively. From the results of the chemical compositions by weight of orange peel extracts, the conditions of a lower CO_2 density, i.e. T1 and T2, showed a higher purity of key aromatic compounds, although T1, which was separated at a higher temperature, obtained less alpha and beta pinene and beta myrcene. T3, T4, and T5, which were treated with a higher density of CO_2, showed less key aromatic compounds in the same manner. These results indicate that these extracts contain other compounds such as long chain fatty acids, carotenoids, and waxes which are highly lipophilic. At the same time, from the results of the chemical compositions by weight of materials, under the conditions of a higher CO_2 density, T3 contained the most key aromatic compounds. However, T4 and T5, which were treated with a higher CO_2 density compared to that of T3, showed less key aromatic compounds than those of T3. It is believed that an excessive CO_2 density promotes the extraction of highly lipophilic compounds and results in a decreasing yield of key aroma. However, a higher CO_2 density was required to obtain the extracts from the coriander seed and T2 was unable to obtain it. However, in the case of coriander seed, T4 and T5, with an excessive CO_2 density, decreased the purity of the key aromatic compounds by weight of extract, similar to that of the orange peel. Nonetheless, T4, which was treated by SC-CO_2 with the highest CO_2 density, showed the highest yield of key aromatic compounds by weight of materials. From these results of two different materials, it was found that the optimal conditions for SC-CO_2 extraction were different for aromatic quality and yield of extract.

3.3 Sensory evaluation of the model solutions flavored with raw materials and their extracts

Figure 3 shows the results of the sensory evaluation of model solutions flavored with raw materials and their extracts. Apparently, the solutions flavored with both extracts showed higher scores for distinct characteristics: juiciness and fresh scent for orange peel and citrusy and spicy for coriander seed. In particular, juiciness in

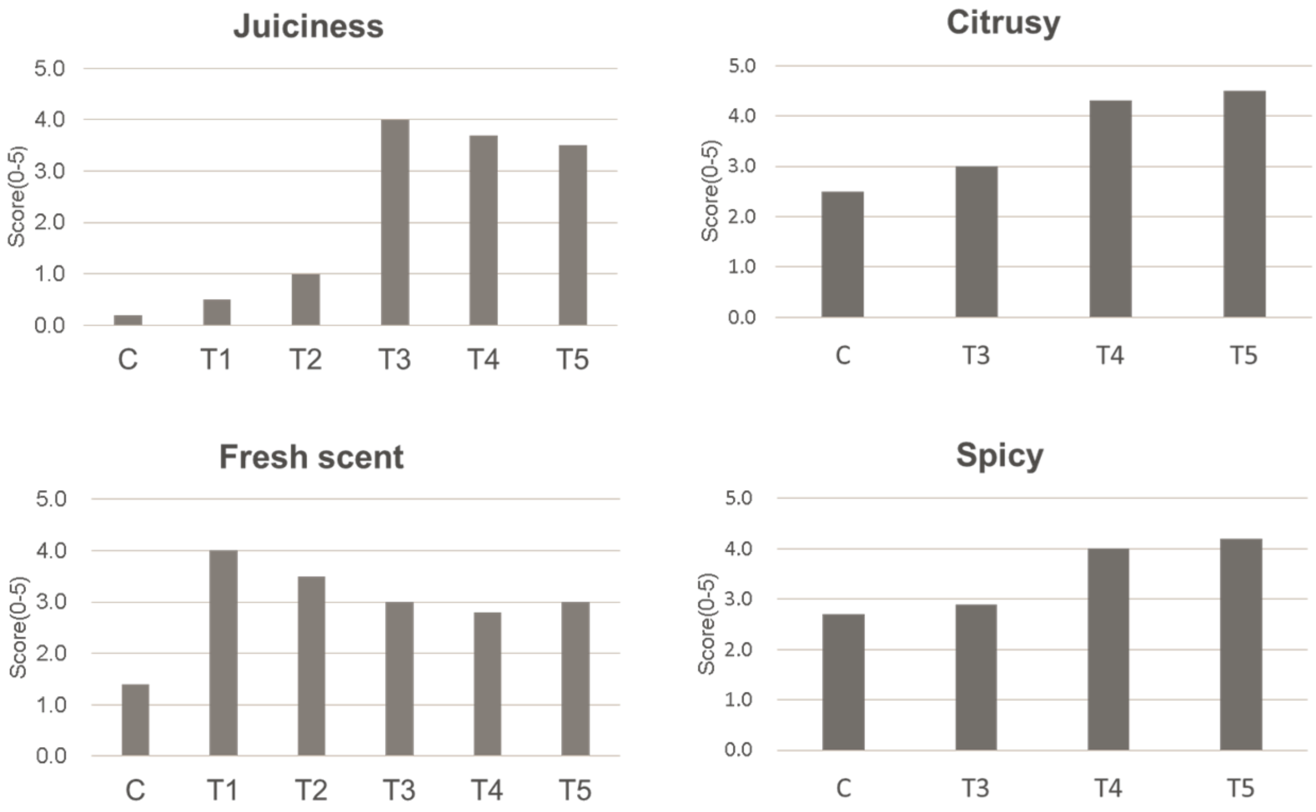

Fig. 3 **Results of the sensory evaluation of model solutions flavored with raw materials and their extracts. Juiciness and fresh scent for orange peel and citrusy and spicy for coriander seed**

Table 6 **Chemical compositions of model solutions flavored with orange peel and their extracts**

		Concentration in model solution (µg/L)					
		C	**T1**	**T2**	**T3**	**T4**	**T5**
Terpene hydrocarbons	α-Pinene	0.36	ND	2.92	14.90	16.13	17.97
	β-Pinene	0.11	0.02	0.37	1.63	1.51	1.59
	β-Myrcene	4.30	2.24	19.69	75.48	72.71	74.22
	Limonene	279.57	134.79	1879.78	6605.25	6535.35	6576.32
	γ-Terpinene	0.11	0.06	0.59	1.73	1.67	1.79
	Terpinolene	0.31	0.17	0.81	1.84	1.84	1.92
	p-Cymene	0.12	0.05	0.34	0.86	0.72	0.71
	Valencene	0.31	0.96	9.88	18.90	23.47	26.36
	Caryophyllene	ND	0.14	1.46	3.24	4.02	4.25
Oxygenated terpenes	1.8-Cineol	0.60	0.01	0.35	0.53	0.43	0.41
	cis,trans-Linalool oxide	75.44	6.12	42.68	52.16	44.38	38.43
	Linalool	21.55	0.39	1.48	5.27	6.90	3.61
	Camphor	0.16	0.03	0.09	0.10	0.08	0.10
	Linalyl acetate	0.42	0.03	0.21	0.38	0.30	0.32
	α-Terpineol	26.24	2.60	17.07	21.67	19.71	16.52
	Citronellol	1.43	0.17	0.67	0.97	0.94	0.77
	Geraniol	15.56	9.30	13.01	12.19	12.76	11.23
	Geranyl acetate	1.38	1.02	2.27	2.54	2.55	2.31
	Nerolidol	0.61	0.28	2.50	2.96	3.00	2.90
	Nootkatone	6.18	2.12	7.06	8.68	7.76	6.46
Aldehyde	Decanal	6.24	5.01	9.28	12.27	12.81	10.34
Total		440.98	165.49	2012.52	6843.55	6769.03	6798.52

Table 7 **Chemical compositions of model solutions flavored with coriander seed and their extracts**

		Concentration in model solution (µg/L)			
		C	T3	T4	T5
Terpene hydrocarbons	α-Pinene	0.17	3.21	52.60	49.75
	β-Pinene	ND	0.19	12.40	11.75
	β-Myrcene	3.34	15.89	35.18	27.96
	Limonene	1.86	12.10	93.25	88.39
	γ-Terpinene	0.11	8.57	75.02	69.52
	Terpinolene	0.26	1.41	5.17	4.87
	p-Cymene	0.15	3.40	30.60	34.90
	Caryophyllene	0.34	6.72	9.73	7.02
Oxygenated terpenes	1.8-Cineol	4.21	1.92	5.71	5.40
	cis,trans-Linalool oxide	814.61	523.34	784.01	712.37
	Linalool	14.51	3.29	5.11	5.78
	Camphor	34.38	19.84	40.79	38.21
	α-Terpineol	6.82	5.67	6.44	6.90
	Citronellol	0.69	0.13	0.12	0.13
	Geraniol	26.51	17.72	19.14	15.66
	Linalyl acetate	0.12	0.38	0.92	0.79
	Neryl acetate	ND	0.71	0.84	0.74
	Geranyl acetate	2.80	69.99	98.66	87.02
	Nerolidol	0.05	7.97	9.45	9.06
Aldehyde	Decanal	6.05	7.78	10.92	9.22
Total		916.97	710.22	1296.05	1185.44

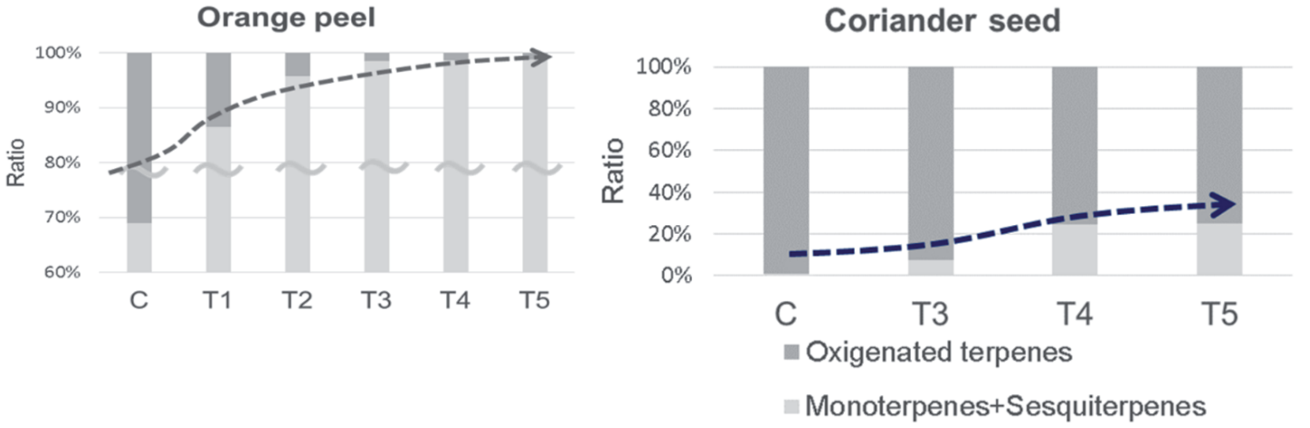

Fig. 4 **Proportion of oxygenated terpenes and terpene hydrocarbons in each model solution**

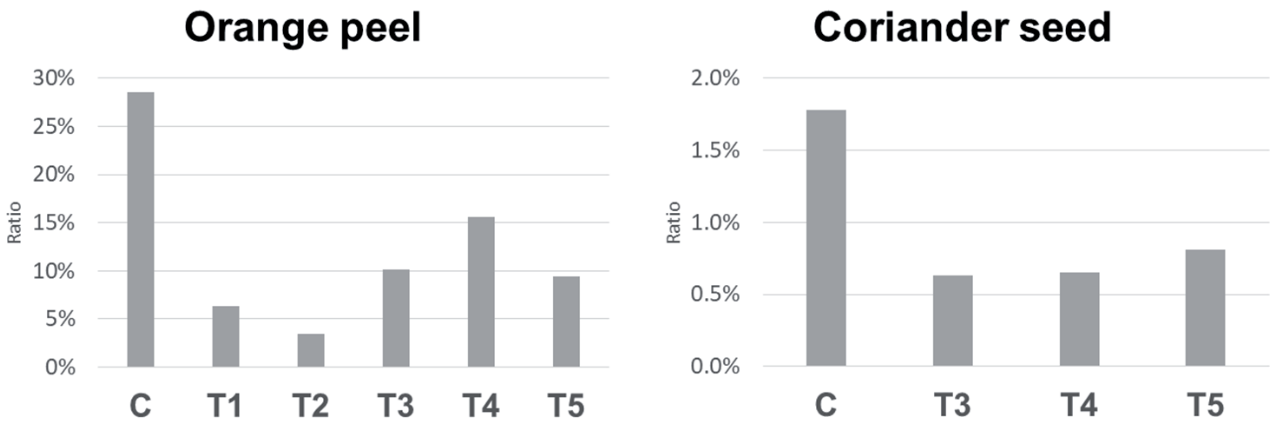

Fig. 5 **Ratio of cis,trans-linalool oxide to linalool in each model solution**

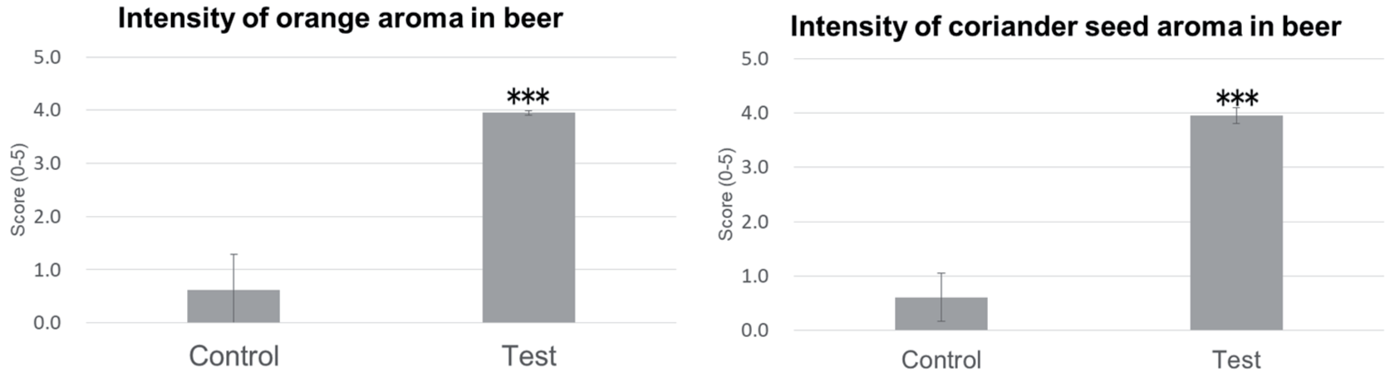

Fig. 6 Comparative results of the sensory evaluation of beer samples using raw materials vs. those using SC-CO_2 extracts. *** indicates the statistically significant difference at $P < 0.001$ analyzed via Tukey's test

the orange peel extracts, corresponding to a higher SC-CO_2 density, scored higher than the juiciness of other samples.

3.4 Chemical compositions of the model solutions flavored with raw materials and their extracts

Table 6 and table 7 show the chemical compositions of the model solutions flavored with raw materials and their extracts, respectively. Clearly, both control solutions flavored with raw materials contained a high concentration of oxygenated terpenes such as linalool, terpineol, geraniol, and citronellol, which are relatively soluble in water and contain a small amount of terpene hydrocarbons. However, the solutions flavored with extracts contained higher terpene hydrocarbons such as pinene, limonene, gamma terpinene, and valencene with a higher CO_2 density. These results were fully reflected by the results of the chemical composition of each extract by weight of material and are thought to be related to the sensory result that juiciness in the orange peel extracts, corresponding to a higher SC-CO_2 density, scored higher than the other samples.

Table 8 Chemical compositions of beer samples; Raw materials (control) vs. supercritical CO_2 extracts (test)

		Concentration in beer sample (µg/L)	
		Control	Test
Terpene hydrocarbons	α-Pinene	0.66	30.16
	β-Pinene	ND	3.58
	β-Myrcene	5.14	225.54
	Limonene	137.30	22500.51
	γ-Terpinene	0.21	9.61
	Terpinolene	0.32	5.55
	p-Cymene	0.15	6.81
	Valencene	0.13	7.95
	Caryophyllene	0.87	2.43
Oxygenated terpenes	1.8-Cineol	1.11	1.94
	cis,trans-Linalool oxide	13.00	3.62
	Linalool	175.26	197.85
	Camphor	4.37	4.83
	α-Terpineol	27.37	18.19
	Citronellol	2.11	4.98
	Geraniol	12.03	12.25
	Linalyl acetate	0.52	2.26
	Neryl acetate	1.75	3.13
	Geranyl acetate	8.92	20.58
	Nerolidol	ND	ND
	Nootkatone	6.34	11.62
Aldehyde	Decanal	12.22	23.30
Total		409.80	23096.67

3.5 Proportion of oxygenated terpenes and terpene hydrocarbons for each model solution and oxidation degree

The previous sensory results can also be proved by the proportion of oxygenated terpenes and terpene hydrocarbons in each model solution as shown in figure 4. As the yield of terpene hydrocarbons, such as mono and sesquiterpenes, considerably increased with increasing CO_2 density, SC-CO_2 extraction was more advantageous to orange peel than to coriander seed because orange peel's aroma mainly consists of terpene hydrocarbons. Furthermore, it was confirmed that there were fewer oxidants such as linalool oxides in solutions flavored with extracts as compared to the amount of oxidants in the control solution as shown in Fig. 5, which shows the ratio of linalool oxide to linalool. This result suggests CO_2 extraction prevents the solution from oxidation and maintains the fresh aroma. These results clearly reflect the differences of the sensory impact of each model solution as previously indicated.

3.6 Comparative results of sensory and chemical evaluation of beer samples using raw materials vs. those using SC-CO_2 extracts

Figure 6 and table 8 show the comparative results of the sensory and chemical evaluation of beer samples using raw materials vs. those using SC-CO_2 extracts, respectively. Apparently, the beer using SC-CO_2 extracts showed a significantly strong distinctive flavor for each material ($P < 0.001$). In addition, it also contained more terpene hydrocarbons and monoterpene acetate.

4 Conclusion

In conclusion, a lower separation temperature (< 20 °C) resulted in increased the yields of key aromatic compounds without freezing, although the separation temperatures had to be set above 40 °C to prevent the water from freezing. Furthermore, the lower CO_2 density resulted in a higher purity of key aromatic compounds, which means it provides the resultant extracts more essential oil with lesser impurities derived from each material, although the yield of extract was low. Additionally, the ratio of terpene hydrocarbons to oxygenated terpenes increased with CO_2 density, which resulted in changes in the aromatic characteristics. Finally, through a brewing trial, we find that this single unique SC-CO_2 extraction technique could be the most efficient method to provide beer the distinct aromatic characteristics of herbs and spices as compared to a general beer brewing method, wherein herbs and spices are added to a wort kettle, whirlpool, or fermentation tank.

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