

## Essential oil recovery from bergamot juice by supercritical and liquid carbon dioxide

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### Abstract

This study makes important observations about the alteration of fruit juice aroma compounds during extraction by CO<sub>2</sub>-SC. Supercritical carbon dioxide extraction is a promising technique for gentle stripping of fruit juice aroma compounds and deserves further consideration as alternative technique for gentle aroma stripping in industrial fruit juice processing. We have investigated the recovery of a useful fraction from bergamot juice. Usually this juice is a by-product of the citrus industry. The chemical composition of the fraction obtained by SC-CO<sub>2</sub> is very different compared to the hydrodistillate. In the hydrodistillate by Clevenger apparatus, the main compound was limonene (higher than 40% of the total peak areas) and other hydrocarbons, such as  $\gamma$ -terpinen (6%) were present. In the alcoholic fraction  $\alpha$ -terpineol (about 25%) and linalool (10%) were well represented, linalyl acetate (1-2%) and neryl acetate (0,5%) appeared in low quantity. The chemical composition of the volatile fraction extracted by SC-CO<sub>2</sub> at 80 bar and 50°C, showed a large amount of linalyl acetate (about 40% of the total peak areas). In this extract the limonene made up only 20% of the total volatile compounds and also other terpene hydrocarbons decreased. This process produces an extract which is rich in the desirable lower molecular weight flavor and aroma compounds and low in the undesirable higher molecular weight flavor and aroma compounds which could impart off-flavors.

### Keywords

*Citrus bergamia Bergamot juice*  
*Flavoring compounds*  
*Aroma recovery*  
*Supercritical CO<sub>2</sub> extraction*

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### Introduction

The aromatic fraction of the juices is made up of a large number of volatile compounds, whose volatility depends on their molecular structure (Ramteke *et al.*, 1990; Maccarone *et al.*, 1998). Generally the juices are concentrated to facilitate transportation and reduce costs (Jesus *et al.*, 2007). During concentration, the aromatic fraction is removed and added back to the juice at a later stage (Bagger-Jørgensen *et al.*, 2004; Onsekizoglu *et al.*, 2010). Today the most common method for the concentration of juices is evaporation, which is carried out at a temperature of 105°C. Many compounds are eliminated during concentration due to the high temperature, or undergo transformations in their molecular structure (Von Sydow and Karlsson, 1971; Nisperos-Carriedo and Shaw, 1990; Varming *et al.*, 2004).

Processes to separate and concentrate the aromatic fraction from the juice include membrane distillation, reverse osmosis (Calabrò *et al.*, 1994; Girard and Fukumoto, 2000; Laganà *et al.*, 2000; Cassano *et al.*, 2003; Jesus *et al.*, 2007; Cassano *et al.*, 2007a, 2007b). The aromatic fraction of citrus juice consists mainly of terpenes. These compounds, unlike oxygen

compounds, do not confer special flavors or aromas to the juice. Moreover, in the presence of air and at elevated temperatures they undergo chemical transformations which give the juice an unpleasant odor (off flavor).

The development of supercritical fluid extraction (SFE) technology might be able to alleviate some of the problems related to the classical aroma recovery process, since SFE does not require elevated temperatures. The extraction of the flavoring materials from fruit juice (Schultz and Randall, 1970; Randall *et al.*, 1971) and many other natural products (Garagay, 1981; Moyler, 1984; Selli *et al.*, 2004) has been carried out with supercritical carbon dioxide. This method shows great potential in replacing conventional methods (Meyer-Warnod, 1984). Fluid-liquid extractions have been carried out to fractionate the fat from milk, and to separate the aromatic fraction (lactones, ketones and aldehydes), which is of considerable interest in the food industry.

Among supercritical solvents, a special attention has been paid to the properties of carbon dioxide (CO<sub>2</sub>). Its convenient critical parameters (T<sub>c</sub> = 31°C, P<sub>c</sub> = 73.8 bar) permit processing at room temperature and under acceptable pressure (Figure 1). The low

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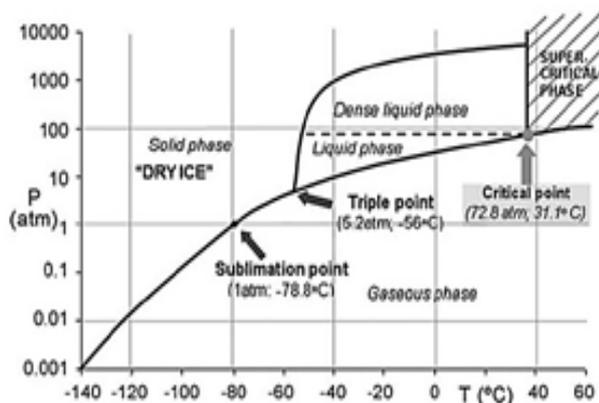


Figure 1. Carbon dioxide pressure-temperature phase diagram

(<http://www.osservatorioccs.org>)

temperature of the process allows most compounds to be extracted without causing damage. Furthermore, the solubility of many extracted compounds in  $\text{CO}_2$  vary with pressure, permitting selective extractions. Another advantage of  $\text{CO}_2$  is that it is non-toxic and non-flammable.

Supercritical fluids have been investigated by numerous researchers worldwide as their unique physico-chemical properties lead to applications in large domains: extraction from solid materials (Poiana *et al.*, 1994, Poiana *et al.*, 1998a, 1998b; Sicari and Poiana, 2017) or fractionation of liquid feeds are mainly devoted to food industries where the exceptional qualities of carbon dioxide are used in numerous developments.

The liquid-fluid extraction is not so intensively investigated as the solid-fluid processes. The development of a continuous liquid-fluid process is less costly and does not cause the technical problems which are encountered with solids. Some examples of liquid-fluid extraction use are: terpene removal from citrus fruit essential oil and detoxification of waste waters (Masaki *et al.*, 1995; Mitsuru *et al.*, 2000). For fractionation of a liquid mixture containing very similar compounds (or groups of compounds), a high performance, multi-stage, counter-current contactor is preferred; to increase the selectivity of separation, also a reflux of the extract can be used.

In the present work the extraction of the aromatic fraction of the bergamot juice by the use of a fractionating tower with a supercritical carbon dioxide flow was carried out. The aromatic fraction extracted was compared with that obtained by conventional separation. This possibility is very important in citrus juices. In these, in fact, the aroma will alter rapidly, while, once separated from the juice, however, it may be stored for a long time, to be reused at the appropriate time to give back to the

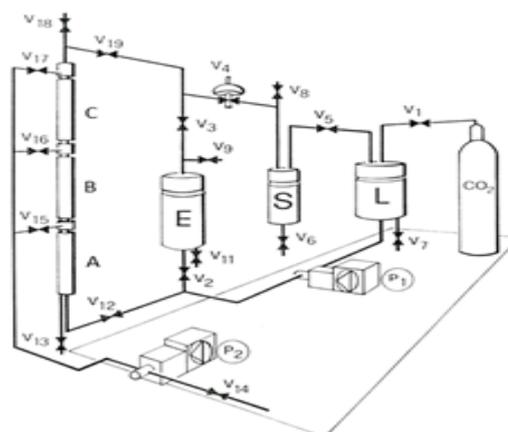


Figure 2. Plant extraction with supercritical carbon dioxide: Condenser (L)  $\text{CO}_2$ , separator (S), extractor (E), pumpa (P1) of  $\text{CO}_2$ , column filling pump (P2).

juices their typical note of freshness.

## Materials and Methods

### Reagents

Carbon dioxide ( $\text{CO}_2$ , 99.8% purity) was obtained from Air liquid Italia. Pentane of analytical grade was from Carlo Erba reagent (Milan, Italy). Standard compounds:  $\beta$ -myrcene, limonene,  $\gamma$ -terpinene, linalool, terpinen-4-ol,  $\alpha$ -terpineol, linalyl acetate, neryl acetate were purchased from Extrasynthèse (France).

### Bergamot juice

Fruit juice was obtained using a hand extractor, in order to avoid squeezing the flavedo and to prevent contamination of the juice with peel components.

The evaluation of the essential oil present in the freshly squeezed juice and in that collected from the fractionation tower was carried out following the AOAC (1990) methodology, using a Clevenger apparatus.

### Hydrodistillation of the essential oil

Bergamot juice 1,5 L was placed in a 2 L flask with distilled water (1 L) and boiled in an electric mantle and essential oils were distilled. The procedure was repeated three times. Total oil volume was recorded for each sample and the oil was stored at 4°C until gas chromatography (GC) and mass spectrometry (GC-MS) analysis.

### Extraction of essential oil by solvent

A 30 mL sample was blended with 50 mL solvent (pentane) and mixed thoroughly using a magnetic stirrer for 4 hours at room temperature. The solvent was then evaporated in an oven at 35°C and the extracts were dried until constant weight using a mild

flow of nitrogen.

#### *Extraction of essential oil by SC-CO<sub>2</sub>*

The continuous extraction experiments were performed in a laboratory plant for SFE of liquid mixtures (Müller GmbH - Coburg, Germania). A schematic diagram of the apparatus is shown in Figure 2. The plant is equipped with a fractionation column 3 meters high, with an internal diameter of 3 cm filled with Rasching rings. The column is divided into 3 segments, each of which is thermostatically controlled by a single water bath with digital temperature control. The introduction of the juice to be separated can be carried out at three levels: A (at 1 meter of height), B (2 meters) and C (3 meters). Preliminary tests indicated an injection point at the top of the first segment (at a height of 1 m) to be the best, allowing the remaining 2 m of column for the separation. A higher injection point resulted in water in the separator vessel, due to the solvent flow carrying it out of the column.

The CO<sub>2</sub> was pressurized by means of a piston pump (P) heated by a heat exchanger positioned in line before the column, at the same temperature as that set for the column. The temperature of the CO<sub>2</sub> was controlled by a sensor between the CO<sub>2</sub> heat exchanger and the column. The separator was heated to a temperature of 35°C by a thermostatic bath, and maintained at a pressure of 40-47 bar. The juice was fed into the column, by means of a Lewa high-pressure metering pump the rate of about 1 L/h, and the carbon dioxide was fed in at the bottom. The quantity of juice was approximately 4 liters for each work cycle.

#### *Gas chromatography*

The gas chromatographic analyses were performed by a Perkin Elmer 8600 gas chromatograph, equipped with a flame ionization detector. The column was a fused-silica capillary (30 m x 0.25 mm i.d., film thickness 0.25 µm) coated with a DB5 apolar stationary phase. Helium was the carrier gas at a flow rate of 1.1 mL/min. The column temperature was programmed as follows: initially, 70°C (2 min), rate of rise 3°C/min up to 180°C, then 5°C/min up to 290°C, final isotherm of 30 min. The injector and FID detector were at 250°C and 300°C respectively. A sample (1 µL), previously dissolved in hexane, was injected into the split mode with a split ratio of 1:25.

The composition was reported as a relative percentage of the total peak area. The GC analyses were performed in triplicate on three aliquots of every sample.

Table 1. Operating conditions and extraction indices

Extr.	Column temperature	Column pressure	Oil in starting material juice (mL/L)	Oil in refined juice (mL/L)
1	50	80	0.90	0.10
2	50	200	1.10	0.05
3	30	200	0.90	0.10-0.20

#### *Gas chromatography – mass spectrometry*

A Hewlett Packard 5971 Mass selective detector connected with a 5890 HP gas chromatograph was used. The separation was achieved by a HP-5 fused-silica capillary column (25 m x 0.2 mm i.d., film thickness 0.33 µm; Hewlett Packard). The column temperature was programmed from 70°C (8 min) to 150 (20 min) at 4°C/min, then 290°C (30 min) at 10°C/min. The flow rate of helium carrier gas was 1.1 mL/min. The sample dissolved in hexane was injected in splitless mode (ratio 1:50). The MS conditions were: ionization voltage, 70 eV; ion source temperature, 180-190°C.

#### *Compound identification*

The identification of the oil constituents was based on the comparison of their retention indices relative to (C9-C24) n-alkanes either with those of published data or with authentic compounds. Thus a C9-C24 series of straight-chain alkanes were dissolved in dichloromethane, and the solution injected into the gas chromatograph–mass spectrometer, operating under the same conditions described above (Tranchant, 1982; Adams, 2007). The sample components were identified by matching their mass spectra with those of the John Wiley & Son library and confirmed by their GC retention times.

#### *Statistical analysis*

Each sample was extracted in triplicate and the concentration of volatile compounds was determined as the mean value of three repetitions. Analysis of Variance (ANOVA) was carried out using SPSS 17 software. A probability value of  $p \leq 0.05$  was considered to denote the statistical significance difference.

## **Results and Discussion**

The injection of the liquid material into column could be done at 1 m (point A, v15 in Figure 2), 2 m (point B, v16 in Figure 2) and 3 m (point C, v17 in Figure 2) of tower high. The juice was injected into the separator column at the top of the first segment (at a height of 1 m), which preliminary tests had shown

Table 2. Compounds identified in the bergamot juice essential oil using gas chromatography mass spectrometry (GC-MS).

Compound	Kovats Indices	Hydrodisti	Pentane extract	SC-CO <sub>2</sub> 80 bar/50°C	Sign.
<b>Ilate</b>					
<b>β-myrcene</b>	982	1.28±0.02 <sup>b</sup>	1.02±0.41 <sup>c</sup>	1.62±0.48 <sup>a</sup>	**
<b>Limonene</b>	1031	60.05±3.54 <sup>a</sup>	43.05±2.85 <sup>b</sup>	41.08±4.29 <sup>b</sup>	**
<b>γ-terpinene</b>	1048	5.01±0.10 <sup>c</sup>	7.48±1.06 <sup>b</sup>	8.70±1.22 <sup>a</sup>	**
<b>linalool</b>	1088	8.67±1.21 <sup>c</sup>	11.09±1.55 <sup>b</sup>	11.50±1.43 <sup>a</sup>	**
<b>Terpinen-4-ol</b>	1158	0.06±0.01 <sup>a</sup>	0.01±0.01 <sup>b</sup>	0.01±0.01 <sup>b</sup>	**
<b>α-terpineol</b>	1173	0.95±0.05 <sup>a</sup>	0.01±0.01 <sup>b</sup>	0.02±0.01 <sup>b</sup>	**
<b>Linalyl acetate</b>	1245	1.42±0.23 <sup>c</sup>	23.32±3.08 <sup>b</sup>	27.41±2.08 <sup>a</sup>	**
<b>Neryl acetate</b>	1339	0.42±0.02 <sup>b</sup>	0.98±0.09 <sup>a</sup>	1.03±0.04 <sup>a</sup>	**
<b>Monoterpenes</b>		66.34	51.55	51.40	
<b>Terpene alcohols</b>		9.68	11.11	11.53	
<b>Esters</b>		1.84	24.30	28.44	

Values are mean ± SD of three sample oils, analyzed in triplicate. Different letters indicate significant

differences. Differences were evaluated by one-way analysis of variance (ANOVA) test completed with a multicomparison Tukey's test. \*\* p < 0.05 compared with the positive control.

to produce the best extraction. Due to the low aroma fraction in bergamot juice, two hydrodistillations (using Clevenger apparatus) were necessary to measure the fraction. The first was a hydrodistillation of the fresh juice; the second a hydrodistillation of the refined juice extracted from the bottom of the separator column. The aroma fraction was measured by subtracting the hydrodistillate of the latter from the hydrodistillate of the former.

Table 1 reports the processing parameters (temperature and pressure of CO<sub>2</sub>) and the essential oil content (aroma fraction) in the juices of the main trials, both in starting material and in refined. From the oil content it is possible to observe the high solubility of the aroma fraction. Almost 90% of the essential oil was extracted from the juice at extraction conditions of 80 bar, 50°C (CO<sub>2</sub> density of 220-230 kg/m<sup>3</sup>). The yield of extraction was increased when a higher CO<sub>2</sub> density was employed. The residual amount of the aroma fraction in refined juice obtained at 200 bar, 50°C and a solvent (CO<sub>2</sub>) density of 760-790 kg/m<sup>3</sup> was 4-5% of that contained in the starting material juice. At a low density value of CO<sub>2</sub> 90% of the aroma fraction amount was extracted the increase of the solvent density gave a small increase of the extraction yield.

A particular trend was observed when the CO<sub>2</sub> temperature was at lower values. When CO<sub>2</sub> was at 30°C, 200 bar and a fluid density of 890 kg/m<sup>3</sup>, the amount of oil measured in the refined juice was 0.1-0.2 mL/L, so 11-22% of that contained in the starting juice. The lower extraction yield compared to the extraction trials performed at 50°C confirm a difficulty of the extraction of the aroma fraction as reported by other authors (Schultz and Randall, 1970; Randall *et al.*, 1971) for citrus juices using

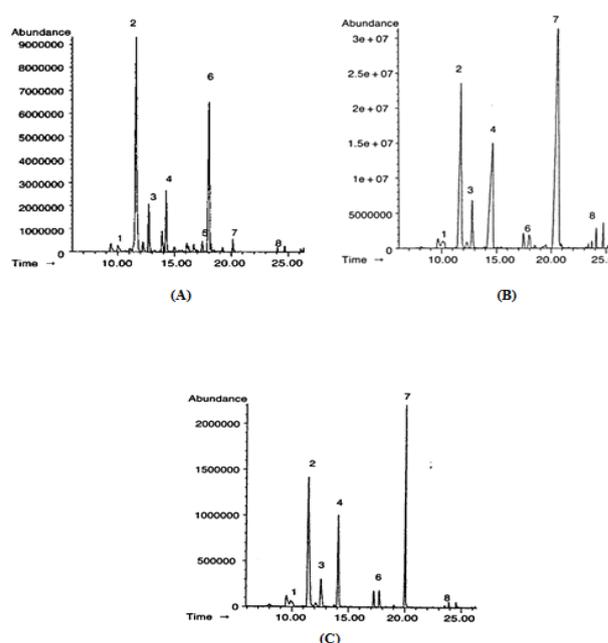


Figure 3. Citrus bergamia essential oil chromatogram carried out using a gas chromatography: A: Hydrodistillate, B: SC-CO<sub>2</sub> (supercritical carbon dioxide), C: Pentane extract. Components: 1. β-myrcene, 2. limonene, 3. γ-terpinene, 4. linalool, 5. terpinen-4-ol, 6. α-terpineol, 7. linalyl acetate, 8. neryl acetate.

CO<sub>2</sub> at a liquid (or near liquid) stage. Furthermore, at extraction temperatures lower than the critical point (31°C) the CO<sub>2</sub> shows a lower diffusivity compared to that of its critical state, which does not facilitate the solubility equilibrium between juice components and compressed CO<sub>2</sub>. At a higher temperature the equilibrium between the two phases is facilitated and so the solubility of some molecules in the CO<sub>2</sub> and consequently a higher extraction yield could be observed.

Figure 3 reports the gas-chromatograms of extracts obtained by hydrodistillation (Figure 3-A) and solvent extraction using supercritical carbon

dioxide (Figure 3-B) and pentane (Figure 3-C). The composition of volatile fractions were very different and they are strictly related to the extraction method. The quantity, stated as peak area percentage, of the main components identified are reported in Table 2. The oil obtained by hydrodistillation contained limonene as the most abundant component, this monoterpene hydrocarbon was measured as more than 40% of the total peak area. Another hydrocarbon present in this oil was  $\gamma$ -terpinene with an area ratio of 6%.  $\alpha$ -terpineol and linalool were the most represented alcohol components, while there was a very low content of linalyl acetate. The differences between the distilled oil and the SC-CO<sub>2</sub> extract of bergamot juice were evident. The most important component of the supercritical extract was linalyl acetate, its peak area was 40% of the total, while limonene was about 20%. In the SC-CO<sub>2</sub> extract linalool was present in a significantly higher amount than in the distillate. The pentane extract composition was similar to SC-CO<sub>2</sub> with only linalyl acetate being higher in the SC-CO<sub>2</sub> extract.

## Conclusion

In conclusion this study affirm that solvent extraction (by mean of pentane or supercritical carbon dioxide) gives an aroma fraction very similar to that of the fresh bergamot juice. Effectively, hydrodistillation modifies the composition of the volatile fraction of bergamot juice, probably due to the acidic condition of the medium and the temperature at which it is submitted. It is possible affirm that the extraction by mean of supercritical carbon dioxide are an important tool to obtain high quality value extracts from bergamot juice. This aroma fractions evidenced a composition as the fresh juice and so they can use as a natural ingredient as for juice restoration as for other usage. This could bring to a better exploitation of the whole bergamot crop.

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