Supercritical Carbon Dioxide Extraction of Mandarin (Citrus deliciosa tenore) From South Brazil[a]

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Citrus oils, like most oils, are very complex mixtures containing aliphatic, mono- and sesquiterpenoid compounds belonging to different chemical classes (hydrocarbons, esters, alcohols, carbonyl compounds, etc.) with widely varying distribution. The volatile fraction of citrus oils, with more than 100 components, normally represents 94-98% of the total oil. Most of these compounds are present in low levels.[1]

Several studies to quantitatively determine the composition of mandarin oil have been done. In these studies, the variation of results is wider than with any of the four major citrus oils (orange, lemon, grapefruit and lime). As such, with respect to mandarin oils, the interpretation of results must be based on the source of each particular oil. The major component of mandarin oils, d-limonene, varies from 65-94% of the total oil. Eighteen alcohols present in mandarin oils were quantified and most were found in concentrations smaller than 0.1%. The major alcohol is linalool. Terpinen-4-ol and α-terpineol, which are two possible indicators of oil quality, varied markedly in content. The terpinen-4-ol level ranged from 0.06-0.30% and the α-terpineol level from 0.03-1.10%. The level of thymol, considered an important flavor compound in mandarin, varied between 0.04% and 0.20%. Esters, ketones and acids are present only in trace quantities in mandarin oil.[2]

Citrus oils are an expensive material used in the perfume and flavor industries. In general, the oil is isolated from citrus-fruit peel by a cold-press machine. However, fresh cold-pressed oil deteriorates easily and develops off-flavor because of the lability of the monoterpene hydrocarbons, including limonene.[3] Steam distillation or solvent extraction can also be used to refine citrus oils. However, these processes present some disadvantages, such as low yields, the formation of degradation products (due to high operation temperatures) and solvent residues. Supercritical fluid extraction (SFE) has been found to be advantageous alternative process for refining citrus oils because of its low operating temperature and the absence of solvent residues.[4]

The extraction of volatile compounds contained in vegetable matter, responsible for its fragrances, is a promising field for the industrial application of the supercritical fluid processing. Indeed, there is considerable interest in replacing the steam distillation and solvent extraction processes traditionally used to obtain these products.[5]

SFE is an advance separation technique based on the enhanced solvating power of substances such as carbon dioxide above their critical point.[6] The solvating properties of a supercritical carbon dioxide phase can be varied over a wide range by changing its pressure and temperature. It can be used for the highly selective extraction of valuable compounds from plant material at low temperature, without the aid of organic solvents.[7] Therefore, it is possible to obtain even thermally labile compounds in a very pure state.[8,9] Due to these benefits, the commercial application of SFE CO_2 (extraction)for the isolation of volatiles from the plant material has increased dramatically.[10]

In this paper, we report the influence of extraction parameters (temperature, pressure, CO_2 flow, equilibrium time and extraction time) on the composition of a volatile

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concentrate of mandarin obtained by supercritical carbon dioxide extraction.

Material and Methods

The unripe mandarins were collected in March, 1999, in São Sebastião do Cái, in the state of Rio Grande do Sul, Brazil. The peels were manually removed and dried at 36°C for 10 days. The material was stored in a room with controlled temperature and humidity.

Sample preparation: SFE experiments were performed with two types of material: broken mandarin peel with particles of 0.25 cm^2, and mandarin peel processed in a cutting mill (TECNAL, type Willye TE650). After flaking, the particle size was 2.0 mm.

SFE: For the SFE of mandarin peel, a Hewlett Packard 7680 extraction module was used. This system consisted of a nozzle/trap assembly that acted as a controllable variable restrictor, allowing for an instantaneous depressurization of the supercritical fluid and the de-coupling of flow and pressure. The material to be extracted was loaded into a self-sealing extraction cell (a 7.0 mL thick-walled stainless-steel thimble).

Experimental

Samples of 1.0 g of mandarin peel were extracted with supercritical carbon dioxide according to the described procedure. Experiments with both materials' conditions (broken and milled) were performed under different values of the extraction perameters. Different experiments to optimize the extraction conditions were performed. The parameters evaluated were: temperature, pressure, CO_2 flow, extraction time and equilibrium time. The initial tests were carried out to find the ideal temperature/pressure for the extraction. The operation ranges tested for this pair of parameters were 40-60°C and 90-110, respectively. All other variables were kept constant: CO_2 flow, 2.0 mL/min; equilibrium time, 10 min; and extraction time; 30 min. In the further experients, the best condition met for the pair temperature/pressure was used, and the following variations were tested: carbon dioxide flow (0.5, 1.0 and 3.0 mL/min), extraction time (10, 20 and 40 min), and equilibrium times (0, 20 and 30 min).

GC Analysis

GC analyses were performed with a Hewlett Packard 6890 Series, equipped with an HP-Chemostation data processor, fitted with an HP-Innowax bonded phase capillary column (30 m x 0.32 mm, 0.50 µm film thickness), column temperature, 40°C (8 min) to 180°C at 3°C/min, 180-230°C at 20°C/min, 230°C (20 min); injector temperature 250°C; split ratio 1:50, detector temperature 250·C; carrier gas H2 (34 Kpa), volume injected 1 µL.

GC-MS Analysis

The GC/MS analyses were performed in the gas chromatograph using a mass selective detector Hewlett Packard 6890/MSD5973, equipped with HP Chemstation software and Wiley 275 spectra data. A fused silica capillary column HP-Innowax (30m x 250 µm), 0.25 µm film thickness was used. The temperature program was the same as in the GC analysis: interface 280·C; split ratio 1:100; carrier gas He (56 Kpa); flow rate: 1.0 mL/min; ionization energy 70 eV; mass range 40-350; volume injected 0.5 µl; solvent cut 3.5 min.

Results and Discussion

The results of the 18 initial tests with variation of temperature (40, 50 and 60°C) and pressure (90 and 110 bar) can be graphically observed in Figure 1. As already mentioned,

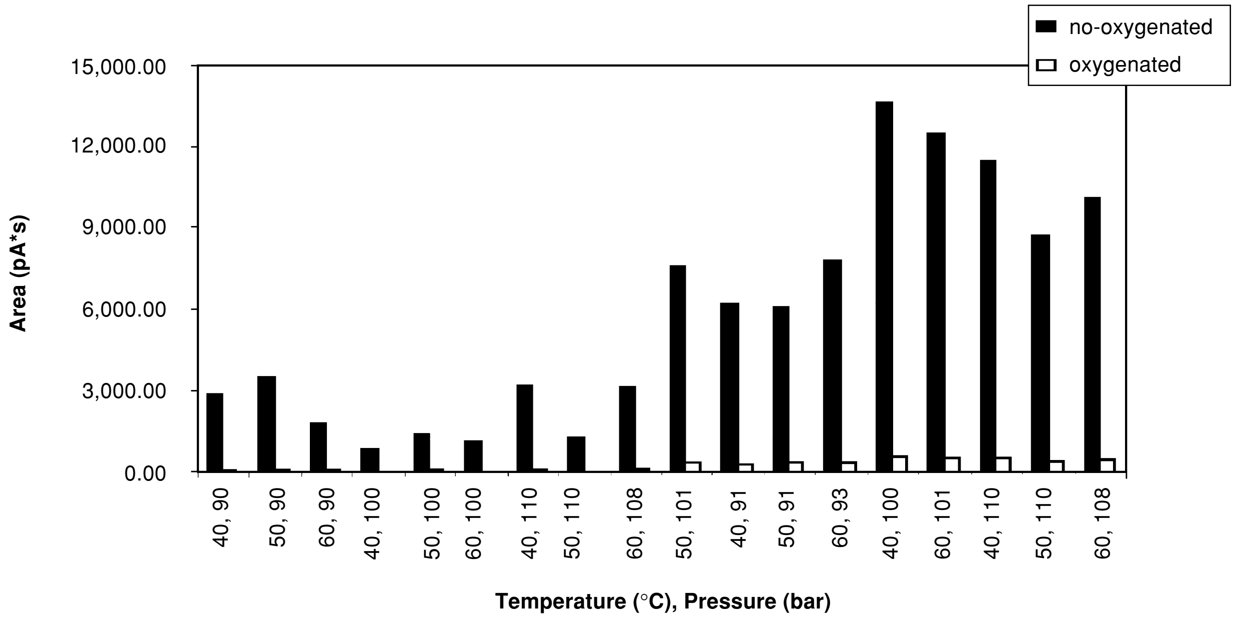

Figure 1. Total area obtained in GC analysis of the volatiles of *Citrus deliciosa* extracted by SFE under varying levels of temperature and pressure

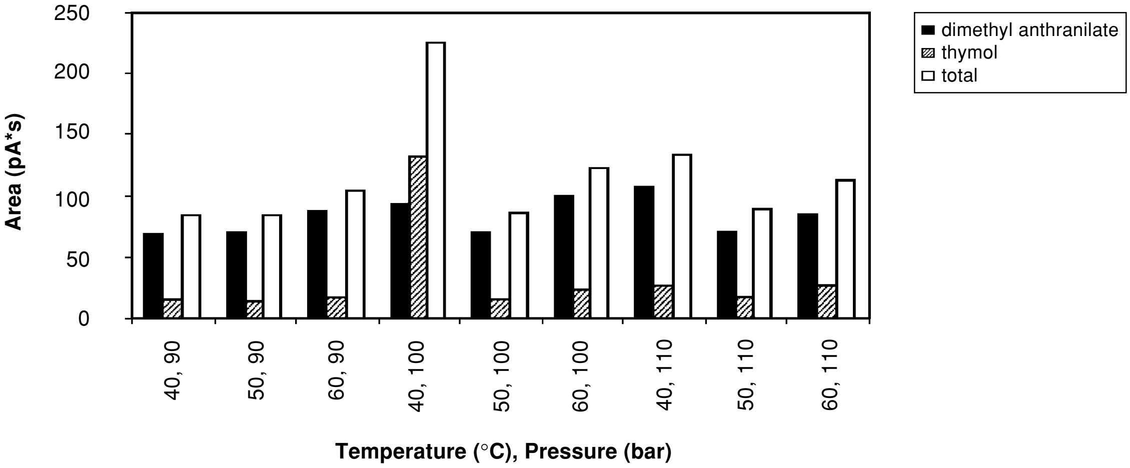

Figure 2. Total area obtained in GC analysis of oxygenated compounds of *Citrus deliciosa* extracted by SFE under varying levels of temperature and pressure

the definition of the optimal operation condition was based on the oxygenated compounds and content of the extract. It was found that, for the milled material, a temperature of 40°C and pressure of 100 bar were preferable because these conditions led to a higher level of oxygenated compounds and to a ratio of oxygenated and non-oxygenated compounds of 1:30.

With respect to the compounds of interest, thymol and dimethyl antharanilate, a considerable increase can be observed in the selected conditions of 40°C and 100 bar (Figure 2).

After definition of the best temperature/pressure, the next parameter studied was the equilibrium time; the time the sample is maintained under the selected pressure and temperature before the start of extraction processing. The aim of this test was to check the influence of the parameter studied on the solubility of the volatile concentrate. The results are presented in Figures 3 and 4.

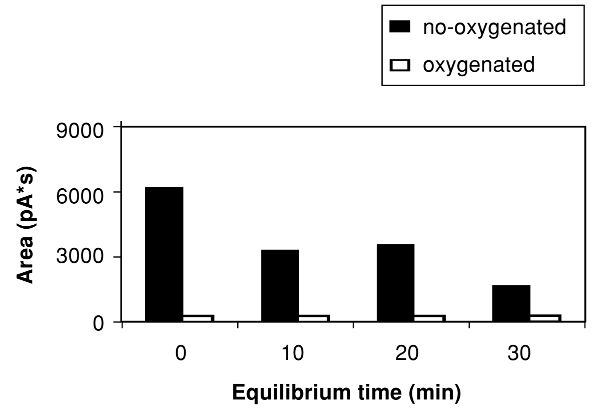

Figure 3. Total area obtained in GC analysis of volatile concentrates of mandarin extracted in different equalibrium times at 40°C, 100 bar

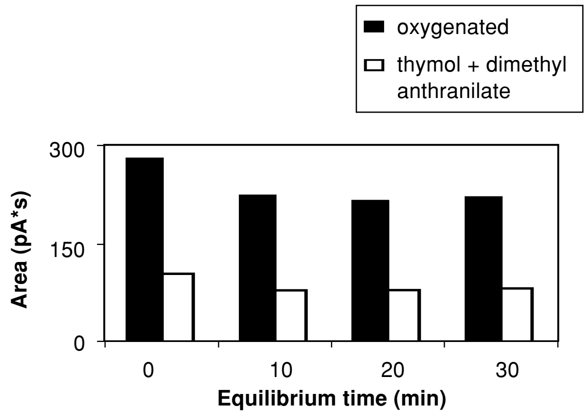

Figure 4. Total area obtained in GC analysis of volatile concentrates of mandarin extracted in different equalibrium times at 40°C, 100 bar

In the case mandarin, a short equilibrium time results in low extract yields (Figure 3), although this variation is less significant for oxygenated (Figure 4). This behavior can be explained by the higher solubility of oxygenated compounds in comparison to terpens in supercritical extraction, allowing a loss of the lighter compounds.

The influence of CO_2 flow can be observed in Figures 5 and 6. With the system at 40°C and 100 bar, in a dynamic process, similar results were obtained with CO_2 flows of 0.5 e 1.0 mL/min, as well as with 2.0 e 3.0 mL/min. In these figures, it is clear that a CO_2 flow of 2.0 mL/min increases the extraction of oxygenated compounds and also yields a good ratio of thymol + dimethyl antharanilate to total oxygenated substances. Thus, this flow was used in further experiments.

In the case of the effect of extraction time on the volatile concentrate composition of mandarin, no difference was

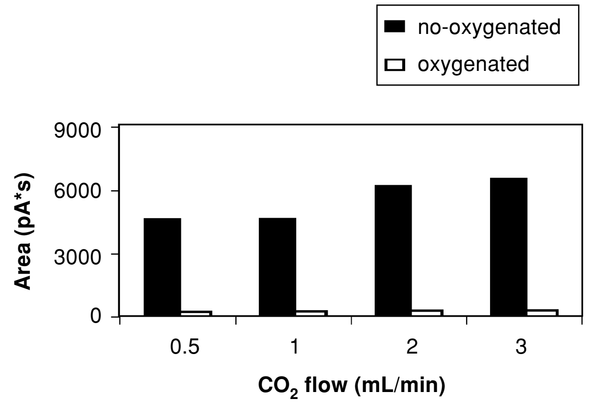

Figure 5. Total area obtained in GC analysis of the volatile concentrates extracted in different CO_2 flow at 40·C, 100 bar, in dynamic extraction

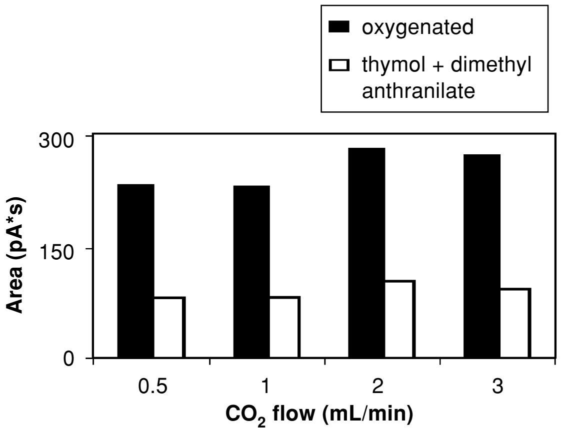

Figure 6. Total area obtained in GC analysis of oxygenated compounds in the volatile concentrates in different CO_2 flow at 40·C, 100 bar, in dynamic extraction

noticed for any condition studied. With the extraction times of 10, 20, 30 and 40 min, the results are similar (Figures 7 and 8). The small difference between the data obtained with 10 min and the other extraction times could be due to the loss of lighter compounds in longer time experiments.

The GC spectrum shown in Figure 9 exemplifies the results obtained in dynamic supercritical extraction of mandarin, with all selected conditions: 40°C, 100 bar, CO_2 flow of 2.0 mL/min, and extraction time of 10 min.

Conclusion

The results presented in this work show the importance of experimental studies for the evaluation of the quantitative influence of process parameters on the supercritical extraction of mandarin. Prior to performing further extrac-

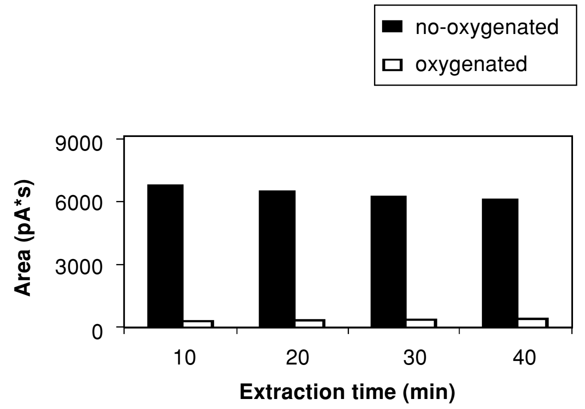

Figure 7. Total area obtained in GC analysis of the volatile concentrate extracted in different extraction times in dynamic extraction at 40°C, 100 bar, CO_2 flow of 2 mL/min

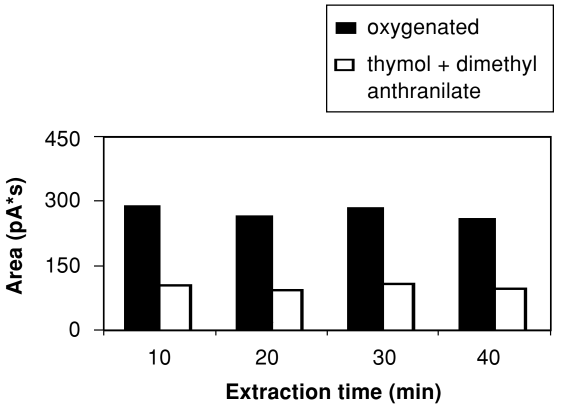

Figure 8. Total area obtained in GC analysis of the volatile concentrate extracted in different extraction times in dynamic extraction at 40°C, 100 bar, CO_2 flow of 2 mL/min

tion experiments, it was possible to find the most adequate conditions for maximizing the quantities of thymol and dimethyl anthranilate in the volatile concentrate. In the definition of the best value for each parameter, only the search for the optimal technical condition for the supercritical extraction of certain compounds with commercial interest was taken into account. Important aspects for this search, including the influence of the variables studied on the total yield of extract and the production cost were not analyzed and must be considered in the continuation of this work.

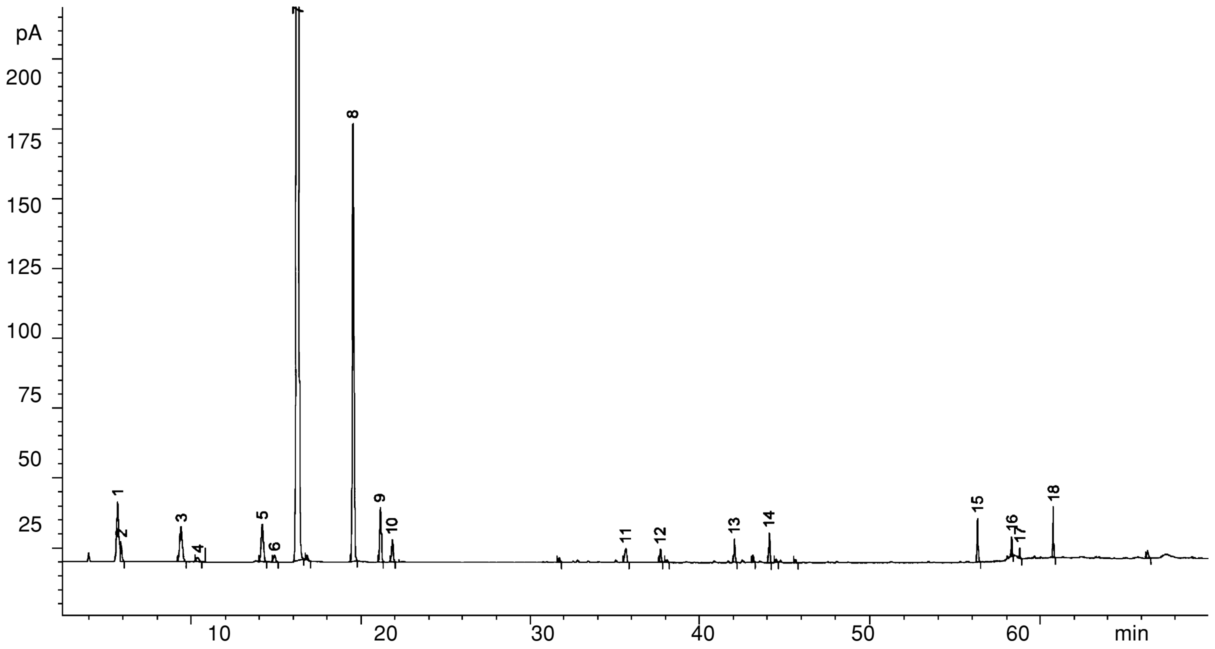

Figure 9. GC spectrum of the volatiles of mandarin extracted with supercritical CO_2 at 40°C, 100 bar, 10 min, 2.0 mL/min flow in dynamic extraction: (1) α-pinene; (2) α-thujene; (3) β-pinene; (4) sabinene; (5) mycrene; (6) α terpinene; (7) limonene; (8) γ-terpinene; (9) p-cymene; (10) terpinolene; (11) linalool; (12) terpinen-4-ol; (13) α-terpineol; (14) α-bisabolene; (15) dimethyl anthranilate; (16) thymol; (17) carvacrol; (18) α-sinensal

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