Separation of phenolic compounds from plant materials using supercritical CO_2

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In the present work supercritical fluid extraction (SFE) using CO_2, was applied for the pre-treatment of mandarin peels. Two-step extraction, combining SFE and conventional extraction, was investigated. During the first extraction step supercritical CO_2 at temperatures 25, 40 and 60°C and pressures from 100 to 300 bar was applied to remove the non-polar components from the extracting material. During the subsequent step, conventional extraction of the residual material was performed with 70 % aqueous solution of acetone at optimal operating parameters (60°C, solid-to-solvent ratio 1:50 g/mL) which were investigated in prior extraction experiments.

The obtained results confirm earlier findings, that mandarin peels are a potentially good source of flavonoids. Pre-treatment with supercritical CO_2 removed the non-polar components and the polar polyphenols became more accessible. Results show that by using SFE with CO_2, the polymethoxylated flavones can be separated from flavanones.

INTRODUCTION

Flavonoids represent one of the main groups of polyphenolic components present in plants. They are a group of natural benzo-γ-pyran derivates and according to their structure they are divided into more subclasses. They are present as glycosides, aglycones or methylated derivates bounded or free in plant material [1,2]. In plants flavonoids play an important role in coloration of flowers and fruits and they also cooperate in some metabolic pathways [1,3,4]. Because of their high radical scavenging activities flavonoids are important also for human health [4]. Overall, flavonoids have been found to exhibit pharmacological properties such as antioxidative, antiallergic, anti-inflammatory, antidiabetic, gastro-protective, antiviral, antimicrobial and anticarcinogenic [1,3-6].

Citrus are an important agricultural crop of the Mediterranean area. Because citrus fruits contain several important nutrients, like vitamin C, dietary fibre, carotenoids and flavonoids they are an important part of a healthy diet [5]. Citrus fruits like oranges (*C. sinensis*), mandarins (*C. reticulata*), lemons (*C. lemon*) and grapefruits (*C. paradisi*) are important for the production of fruit juices and concentrates that are mainly used in food

industry for obtaining fruit drinks [7]. There are a lot of citrus peels produced as residues, which can be a potential source of pectin and natural flavonoids [8].

Two main groups of flavonoids which are present in citrus peels are flavanone glycosides and polymethoxylated flavones [5,9,10]. Flavanone glycosides are located mainly in a white part of peel – albedo, while the polymethoxylated flavones are located mainly in the flavedo – external colored part of citrus peel [10]. Studies have shown that citrus flavonoids play an important role in the prevention of degenerative and infectious diseases. Due to their anticarcinogenic, antiatherogenic, antimicrobial and anti-inflammatory properties flavanones and polymethoxylated flavone are very interesting for pharmaceutical and food industry [4,5,6].

It was previously observed that the pre-treatment with non-polar solvents (e.g. hexane) improves the extraction of polyphenols from plant materials [11-14]. Supercritical fluid extraction (SFE) with CO_2 provides an alternative to the pre-treatment of the plant materials, replacing toxic organic solvents. It was reported [15] that pre-treatment of grape marc material with supercritical CO_2 improved subsequent extraction of polyphenols with organic solvent. It was also shown that the exposure of plant material to supercritical fluids can lead to swelling of plant material, which may lead to increased diffusion in solid phase and better extraction yield [16].

In the present work SFE using CO_2, was applied for the pre-treatment of mandarin peels. A two-step extraction, combining SFE and conventional extraction, was investigated. During the first extraction step supercritical CO_2 was applied to remove the non-polar components from the extracting material. During the second-step, conventional extraction of the residual material was performed at optimal operating parameters, which were previously investigated.

MATERIALS AND METHODS

Chemicals and reagents

Hesperidin (Cat.No. 52040) and didymin (Cat.No. 36814) were purchased from Fluka; narirutin (Cat.No. 1130 S), sinensetin (Cat.No. 1326 S) and tangeretin (Cat.No. 1033) were purchased from Extrasynthese. All standards were HPLC grade. Acetone, methanol and anhydrous acetic acid were provided by Merck. Milli Q water produced by Milli-Q plus apparatus was used for HPLC analysis. Carbon dioxide (CO_2) was purchased from Messer.

Preparation of material

Mandarin peels were collected from fruits bought at the local supermarket. Peels were dried by hot air flow (40–50 °C) and stored in a dark and cool place. Dried peels were grounded before use and moisture content was determined by Karl Fisher Titrator (Mettler 99 Toledo DL31) and was equal to 7.1 % (w/w).

Conventional extraction experiments

For conventional extraction a 70 % (v/v) aqueous solution of acetone was used. Dry mandarin peels were weighed in a glass flask and solvent was added in different solid-to-solvent ratios (1:20, 1:30, 1:50 g/mL). After specific time of mixing (1, 1.5 and 2 h) by using a magnetic stirrer at different temperatures (20, 40 and 60°C), the solution was separated by filtration, the residual material was returned in the flask and the procedure was repeated with fresh solvent.

The extract was separated from each solution by evaporation of the solvent. After drying, the extract was weighed and stored in a cool place before HPLC analysis.

Supercritical extraction with CO_2

Supercritical extraction with CO_2 was performed by semicontinuous apparatus [17]. 40 g of grounded material was charged into the extractor. The extractor was placed into a water bath and the temperature was regulated and maintained at constant level. CO_2 was continuously pumped with a high pressure pump through the preheating coil and over the bed of material in the extractor. The product precipitated in the separator (glass trap), where the separation was performed at 1 bar and at room temperature. The solvent flow rate was measured with a flow-meter (ELSTER HANDEL GmbH, Mainz, Germany). The product collected in the glass traps, was weighed and stored in a dark and cool place before HPLC analysis.

HPLC analysis of extract

The composition and the content of flavanones (narirutin, hesperidin) and the presence of polymethoxylated flavones were determined by HPLC method. The standard solutions were prepared by dissolving standard samples in methanol. 10 mg of extract were dissolved in 10 ml of methanol, and the solution was homogenized in the ultrasound bath and filtrated before analysis. The HPLC system [2] consisted of a Varian 9012 pump and Varian diode array detector 9065. Column C-18 250×4.6 mm Microsorb 100 stationary phase with 5 μm particle size was used. The mobile phase consisted of two solvents: A: methanol, and B: 2 % (v/v) acetic acid in Milli-Q water. The method started with linear gradient from 25 % A to 40 % A in 3 min, then changed to isocratic for 5 min at 40 % A, followed by linear gradient from 40 % A to 70 % A for 30 min and 2 min isocratic at 70 % A. The method continued with linear gradient from 70 % A to 80 % A in 10 min and finished with isocratic for 5 min at 80 % A. The flow rate was 0.85 ml/min and detection was performed at 282 nm. The quantification was made with external standard.

RESULTS

Table 1: Results of the experiments of conventional extraction of mandarin peels.

Experiment	1	2	3
T / °C	20	60	40
t / h	2	1.5	1
R(g/mL)	1:20	1:50	1:30
Nr. of stage	3	3	3
Total yield of extraction/ %	49.6	48.8	46.4
Content of HES mg/g of dry mat.	41.0	49.5	36.9

Conventional extraction experiments

Results of the experiments of conventional extraction of mandarin peels are shown in Table 1. The table presents the extraction conditions: temperature and time of extraction, solid-to-solvent ratio (R) and number of extraction stages, the total yield of extraction and content of the main flavanone, Hesperidin (HES), which was determined by HPLC analytical method.

Regarding the results of the total yield of extraction and the amount of HES extracted the optimal extraction conditions are: temperature 60 °C, time of extraction 1.5 h, solid-to-solvent ratio 1:50 g/mL of 70 % aqueous solution of acetone and extraction in three stages.

Supercritical extraction with CO_2

Results of supercritical extraction with CO_2 are shown in Figure 1. The diagram presents the yield of extraction (in %) as a function of the specific amount of solvent (kg of CO_2 per kg of material, mandarin peel). It can be noticed that the highest yield of SFE of mandarin peels was achieved at 25°C and 300 bar (1.32 %), while the lowest yield, 0.26 %, was obtained at 60°C and 100 bar.

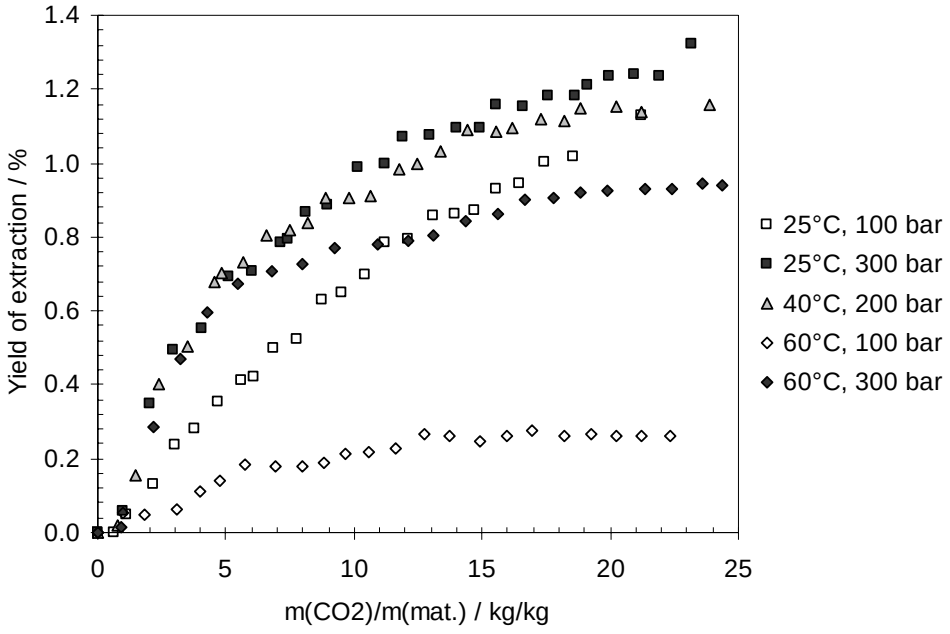

Figure 1 : Yield of SFE of mandarin peels with CO_2 as a function of solvent amount (kg CO_2 / kg of material).

In the extract of mandarin peels obtained by SFE polymethoxylated flavones were detected as main flavonoid. Tangeretin was identified by comparison to pure standards. These results are in agreement with data from the literature [18, 19]. The highest amount of TAN extracted from mandarin peel was obtained at 60°C and 300 bar. Table 2 presents the conditions and results of the SFE extraction experiments.

Table 2: Results of SFE of mandarin peel with CO_2.

SFE conditions					
T / °C	25	25	40	60	60
p / bar	100	300	200	100	300
Total yield of extraction/ %	1.13	1.32	1.16	0.26	0.94
Total content of TAN mg/g of dry material	0.079	0.077	0.084	0.013	0.119

Influence of pre-treatment with SFE

The residual material after supercritical (SC) CO_2 extraction was used for a subsequent conventional extraction, which was performed at the optimal conditions listed above, and the influence of pre-treatment of raw material by SC CO_2 was studied. The results, where the pre-treatment with SC CO_2 was performed at 60°C and 300bar, are presented in Figure 2. By applying SC CO_2 for pre-treatment of mandarin peels, the total yield of conventional extraction was increased from 48.8 to 50.5 %. The highest increase of the yield was observed for the 1st stage of conventional extraction, where the yield was increased for 1.4 %.

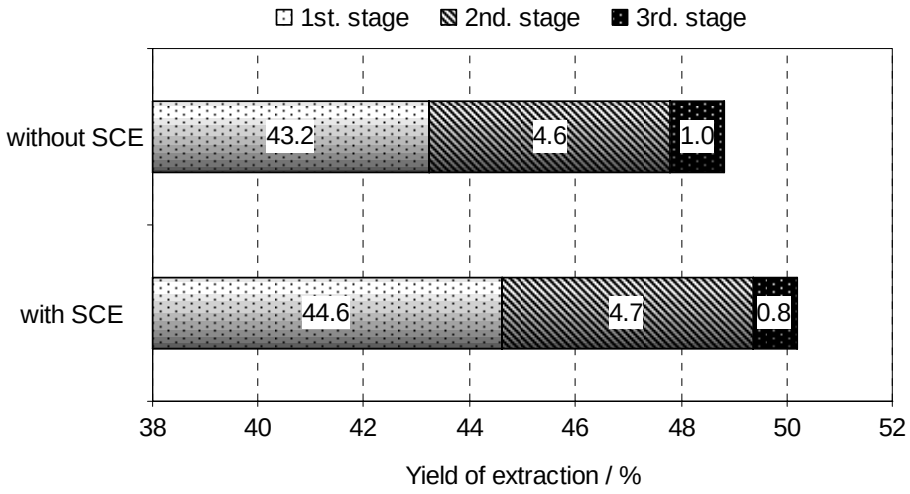

Figure 2: Total yield of conventional extraction of mandarin peels: influence of pre-treatment of material with SC CO2 at 60°C and 300 bar. (Conventional extraction: 70 % acetone, 60°C, 1.5 h extraction time, 1:50 g/mL solid-to-solvent ratio)

CONCLUSIONS

The present study investigates the application of SFE using CO_2 for pre-treatment of mandarin peels prior to conventional extraction. It was shown that the total yield of conventional extraction was increased when using SC CO2 for pre-treatment of material. Based on the presence of mainly TAN in SFE extracts and HES in extracts of conventional extraction it was concluded that SC CO_2 extraction could be used for separation of polymethoxylated flavones from flavanones present in mandarin peel. An additional set of experiments is planned in the future, where SC CO_2 will be used for treatment of conventional extract obtained by organic solvents, and the concentration and separation of flavonoids from mandarin peels will be investigated.

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