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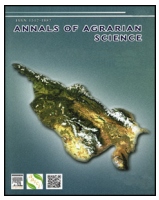

Sequential extraction of bioactive compounds from tangerine (Citrus Unshiu) peel

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ABSTRACT

Tangerine (Citrus Unshiu) is one of important agricultural crops in Georgia. As the statistical data for year 2016 manifest, the total harvest exceeds 100 thousand tons. Tangerine peel, agro-industrial waste of production of juice concentrates and jams present rich sources of valuable bioactive compounds. Due to their high bioactive compound content, tangerine peels could be applied by both pharmaceutical and food industries. In spite of this, citrus peel is usually treated as by-products or waste, resulting in environmental pollution. Citrus peels are promising source of essential oil, carotenes, natural flavanones such as hesperidin and pectin. Stepwise, sequential utilization of tangerine peel, which provides an opportunity for selective extraction of the aforementioned bioactive compounds and allows one to manage nonstandard tangerine (amounting to a third of the harvest) in a rational manner. Hence, the present study is undertaken to elaborate a feasible and effective method of sequential extraction of above mentioned bioactive compounds from tangerine peel. The first step is supercritical CO_2 extraction of essential oil. Optimal conditions for β-carotene free tangerine oil are 100 atm pressure, $35C^0$ temperature, and 15 min equilibrium time. The principal compound in Citrus Unshiu peel essential oil is d-limonene. Stepwise extraction requares correct extraction sequence of target products. Acetone (7%) was used as co-solvent in the second step of extraction. The β-carotene is soluble in acetone, whereas hesperidin is not. Optimal parameters are 150 atm, 40 °C and 1 h equilibrium, and 1 h extraction time in dynamic conditions. Methanol (7%) was used in the third step of extraction. Optimal parameters were: 250 atm pressure, 60 °C temperature, 1 h equilibrium and 30 min dynamic extraction time. At least pectin was extracted from residue of tangerin. Stepwise supercritical fluid extraction of bioactive compounds from agro-industrial waste materials is simple, effective, eco friendly separation method, which provides high quality of target products and needs one standard technological equipment.

Introduction

Tangerine (Citrus Unshiu) is one of the important agricultural crops in Georgia. By 2016 statistical data the total harvest exceeds 100 thousand tons. Tangerine peel, agro-industrial waste of juice, concentrates and jams production is a rich source of valuable bioactive compounds.

The main goal of the present study was to develop the feasible process of sequential extraction of valuable bioactive compounds from tangerine peel and to best manage nonstandard tangerine, which is 1/3 of the harvest.

Citrus peels are promising source of essential oil, carotenes, natural flavanones and pectin.

In the last decades numerous researchers have investigated the fundamentals and process application of supercritical (SC) fluids. SC-CO_2 extraction has been used for extraction bioactive compounds from natural products. Besides achieving high yield and quality, supercritical fluid extraction (SFE) can be operated under a wide range of conditions to selectively extract specific target products [1]. It is possible to make sequential fractionation of target products from plant matrix via a single system employing CO_2-based fluids [2,3].

Four different products: essential oil, carotenes, hesperidin and at least pectin were extracted from tangerine peel, by increasing the polarity of the supercritical fluid using different solvents as a modifier of CO_2 in stepwise extraction method.

Citrus oils are mixtures of very volatile components as terpenes and oxygenated compounds [4]. Limonene (1-Methyl-4-(1-methylethenyl)-cyclohexene, $C_{10}H_{16}$), a monoterpene, is the major component of lime and other related citrus essential oils [5]. These oils are used in the pharmaceutical, perfumery and food industries [6]. Studies with SFE of

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mandarin oils have shown good yield results in comparison with other extraction processes [7].

β-Carotene, α-carotene and β-cryptoxanthin are provitamin A carotenoids. Structurally, the retinol ($C_{20}H_{30}O$), one of the major forms of vitamin A, is essentially one-half of the β-carotene (1,3,3-Trimethyl-2-[3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-1-en-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaen-1-yl]cyclohex-1-ene, $C_{40}H_{56}$) molecule. Consequently, β-carotene is the most potent provitamin A; carotenoids have been credited with other beneficial effects on human health: enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration [8,9].

Recently, natural food colorants are preferred than synthetic ones. Today, in many of industrially produced food β-carotene (beta-carotene) is used as a natural food colorant.

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 flavones are very interesting for pharmaceutical and food industry. Citrus peels are rich source of natural flavanones such as hesperidin, narirutin and naringin. It is known that hesperidin (2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S, 6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R, 6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one, $C_{28}H_{34}O_{15}$) is the most abundant flavanone in tangerine peel [10].

Hesperidin may be associated with potential benefits in the prevention of many diseases, such as decreasing capillary permeability, anti-inflammatory, antimicrobial and anti-carcinogenic effects. Hesperidin also regulates hepatic cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductase [11–13]. This compound is effectively used as a supplemental agent in the treatment protocols of complementary settings. Its deficiency has been linked to abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and night leg cramps. Supplemental hesperidin also helps in reducing oedema or excess swelling in the legs due to fluid accumulation. A number of researchers have examined the radical scavenging properties of hesperidin using a variety of assay systems [14,15].

Pectin is a heteropolysaccharide, naturally occurring substance present in all plant tissue. Pectin exists in varying amounts in fruit cell properties (In the cell walls they serve as one of the main agents cementing the cellulose fibrils and may be linked covalently to other polymers. Intracellular pectins provide the channels for passage of nutrients and water [16]. The main use for pectin (vegetable agglutinate) is as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams, jellies or marmalades, which would otherwise be sweet juices [17].

Hence, the present study is undertaken to elaborate a feasible and effective method of sequential extraction of above mentioned bioactive compounds from tangerine peel.

Materialis and methods

Plant material

Ripe tangerine (Citrus Unshiu) was bought in the local market. The peels were manually removed and dried at ambient temperature for 14 days.

Chemicals and standards

All used chemicals were analytical grade. The certified analytical standard of β-carotene was supplied by Sigma-Aldrich (Germany). The HPLC/GC grade acetonitrile, methanol ethanol, acetone, ethyl acetate, acetic acid, dimethyl formamide, dimethyl sulfoxide, 1,2-dichloromethane, n-hexane were purchased from Sigma-Aldrich, Merck and Carl Roth (Germany).

Supercritical fluid extraction

Sequential supercritical fluid extraction method we used is eco friendly separation tool. Supercritical fluids (CO_2, etc.) as solvent have advantage such as excellent mass transfer and control of fluid density by temperature and pressure, which provides high selectivity of extracted products. Process need standard technological equipment and is fast [18].

Gas chromatography-mass spectrometry analysis

The chromatography-mass spectrometric (GC-MS) analysis was performed using Agilent Quadrupole GC-MS 790B1/5977A system (AG Technologies, USA). System control, data collection and data processing were accomplished using HP Chemstation software. The chromatographic condition was optimized using the fused silica capillary column – HP-5ms (30m × 0.32mm × 0.25 μm). The column oven temperature programmed 80 °C for 3 min then from 80 °C to 275 °C at the rate 45 °C/min; Helium was used as a carrier gas with a flow rate 1.5 mL/min. Injector and detector temperature was 220 °C and 250 °C, respectively. A sample of 1 μL was injected in the split mode with split ratio 100:1. The ionization mode – El; The mass resolution setting – normal; The source temperature – 230 °C. The run time was 7.33 min.

High performance liquid chromatographic analysis

The High performance liquid chromatographic (HPLC) analysis was performed using Agilent 1260 Infinity HPLC system (AG Technologies, USA). The output signal was monitored and processed using Chemstation software. All the measuring equipment for sample and standard preparations were appropriately calibrated and qualified. The analysis was carried out by the developed and validated HPLC method using a column - RP-18 endcapped Lichrocart 4 × 250 mm, 5 μm (Merck) with the binary gradient elution program of mobile phases (MP) A (a mixture of methanol, acetonitrile, dichloromethane and n-hexane 475:475:25:25 v/v) and B (a mixture of methanol and dichloromethane 70:30 v/v); The gradient program: 0–12 min 100% MP A and 0% MP B (isocratic), then 12–16 min MP A was changed from 100% to 0% (linear gradient), then 16–18 min from 0% to 100% (linear gradient) and finally, 18–20 min MP A 100% and MP B 0% (isocratic); The flow rate of elution was 1.5 mL/min, the UV detector wavelength was 455 nm for β-carotene; The injected volume was 50 μL; The column temperature was maintained at 40 °C. All the solutions were filtered through Durapore Polyvinylidene difluoride (DVDF), 0.45 μm membrane filters. Mobile phase B was used as diluent for standard and sample preparation. 2.5 μg/mL standard solution of β-carotene in diluent was used as the standard solution.

The concentration of β-carotene's content-**Cu**, μg/mL in sample solution was calculated by the following formula:

$$\boldsymbol{Cu} = \frac{Au \times W_1 \times D_1 \times P}{As \times 100}$$

where, Au-Peak area of β-carotene obtained from sample solution; As-Peak area of β-carotene obtained from standard solution; W_1 – Weight of β-carotene standard, μg; $\mathbf{D_1}$-Dilution factor of standard/sample solution; P – Purity of the standard, %.

The content of β-carotene – X in 1 g of the dried sample (waste material of tangerine) was calculated by the formula:

$$\boldsymbol{X} = \frac{Cu \times V \times D_2}{W_2}$$

where, Cu-the concentration of β-carotene in sample solution, μg/mL; V – The volume of extract, mL; D_2-Dilution factor of extract solution; W_2 –