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RESEARCH ARTICLE

HS-SPME and microdistillation isolation methods for the volatile compounds of bergamot fruits cultivated in Turkey

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Abstract

Bergamot fruits are important for cosmetic, perfumery, food and drug industries. Bergamot essential oil is well characterized and is used extensively in many flavours and fragrances. In the present study, the bergamot fruits cultivated in Adana were collected for the isolation of the volatiles in the peels, by microdistillation and headspace-solid phase microextraction (SP-SPME) techniques. The fresh peels of the fruits subjected to microdistillation were analysed by GC and GC-MS systems, simultaneously. In the same time, to trap volatile compounds in the fresh peels HS-SPME technique was employed. As result, limonene (32.9, 36.8%), linalool (37.3, 16.6%) and linalyl acetate (7.8, 30.2%) were identified as the major compounds comparable in the peels obtained by microdistillation and HS-SPME, respectively.

Keywords: *Citrus x bergamia*, bergamot, microdistillation, HS-SPME, volatiles

Introduction

Citrus × bergamia Risso & Poit. belonging to the Rutaceae family is defined as a hybrid between a sour orange (*C. aurantium* L.) and lemon (*C. limon* L. Burm. f.) or formed as a mutation of the latter. In the same time, bergamot is thought to be a hybrid between a sour orange and lime (Navarra et al., 2015). The tree has been known for centuries in Mediterranean Region for its fruits and the essential oil due to the uses in perfumery, cosmetics, food and confectionary industries as well as in folk medicine. Bergamot essential oil is used in aromatherapy and it has become more and more popular for improving mood and mild symptoms of stress-induced disorders and facilitating sleep induction. Also, it is known as anti-inflammatory, antidepressant, antimicrobial, carminative, digestive, sedative and febrifuge (Karaca et al., 2007; Saiyudthong & Marsden, 2010; Forlot & Pevet, 2012; Navarra et al., 2015). The chemical composition of the essential oil is well defined by various studies (Dugo & Bonaccorsi, 2013; Costa et al., 2015). According to published data, monoterpene and sesquiterpene hydrocarbons, their oxygenated derivatives, aliphatic aldehydes, alcohols and esters were identified among the major components.

Bergamot is widely cultivated in the Mediterranean coasts of Turkey. The chemical compositions of the Turkish bergamot oils were identified by different authors (Baser et al., 1995; Kirbaslar et al., 2000; Kirbaslar et al., 2001). The previous studies focused on the chemical compositions of the oils produced by cold-pressing or steam distillations.

In the present study, two different techniques such as microdistillation and HS-SPME were employed for the fresh peels to isolate the volatile compounds, consequently analysed by GC, and GC-MS systems, respectively.

Materials and Methods

Plant source

The mature bergamot fruits were harvested from Subtropical Fruits Research and Development Centre at Çukurova University, Adana, Turkey in December, 2017. The fresh peels were separated from the fruits. Res. Assis. Ebru Duymuş (Çukurova University, Agricultural Faculty, Department of Soil Science and Plant Nutrition, Balcalı, Adana, Turkey) collected the fruits.

Isolation of the volatile compounds

Microdistillation

The volatiles were obtained by microdistillation of fresh peels (600 mg) using an Eppendorf MicroDistiller® with 10 mL distilled water per sample vial. The sample vial was heated to 108°C at a rate of 20°C/min and kept at this temperature for 90 min, then heated to 112°C at a rate of 20°C/min and kept at this temperature for 30 min. The sample was subjected to a final post-run for 2 min under the same conditions. The collecting vial, containing a solution of sodium chloride (2.5 g) and water (500 µL) plus *n*-hexane (300 µL) to trap volatile components, was cooled to -5°C during distillation. After the distillation was completed, the organic layer in the collection vial was separated and analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) systems, simultaneously. The distillation procedure was duplicate.

Headspace-solid phase micro-extraction (HS-SPME)

To trap volatile compounds in the fresh peels HS-SPME technique was employed. The manual SPME device (Supelco, Bellafonte, PA, USA) with a fiber precoated of a 65 µm thick layer of polydimethylsiloxane /divinylbenzene (PDMS/DVB-blue) was used for extraction of the bergamot volatiles. The vial containing the fresh bergamot peels (500 mg) was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the peels for 15 min at 40°C. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.

GC and GC-MS analyses

The GC analysis was carried out using an Agilent 6890N GC system. Flame ionization detector (FID) temperature was 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

Identification of the volatile compounds

The components of the sample were identified by comparison of their mass spectra with those in the in-house "Baser Library of Essential Oil Constituents", Adams Library (Adams, 2007), MassFinder Library (Hochmuth, 2008), Wiley GC/MS Library (McLafferty & Stauffer, 1989) and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. *n*-Alkanes (C_8-C_{21})

were used as reference points in the calculation of relative retention indices (RRI) (Curver et al., 1985). Relative percentage amounts (%) of the separated compounds were calculated from FID chromatograms.

Results and Discussion

Twenty-three (99.8%) and twenty-four (98.9%) compounds were identified yielded as 99.8% and 98.9% in the bergamot samples obtained by microdistillation and HS-SPME techniques, respectively. The detailed information was given in the Table 1.

Table 1. Volatile compounds of bergamot peels.

RRI	Compound	A %	B %
1032	α-Pinene	0.7	0.7
1035	α-Thujene	0.3	0.2
1118	β-Pinene	2.4	3.0
1132	Sabinene	0.5	0.6
1174	Myrcene	1.6	1.4
1188	α-Terpinene	0.2	-
1203	Limonene	32.9	36.8
1218	β-Phellandrene	-	0.3
1246	(*Z*)-β-Ocimene	0.5	0.1
1255	γ-Terpinene	3.5	6.2
1266	(*E*)-β-Ocimene	0.9	0.4
1280	*p*-Cymene	0.2	0.1
1290	Terpinolene	0.4	0.3
1553	Linalool	37.3	16.6
1565	Linalyl acetate	7.8	30.2
1611	Terpinen-4-ol	0.3	-
1612	β-Caryophyllene	0.3	0.2
1694	Neral	0.8	0.3
1706	α-Terpineol	2.9	0.1
1709	α-Terpinyl acetate	-	0.1
1737	Neryl acetate	1.3	tr
1741	β-Bisabolene	-	0.5
1742	Geranial	1.1	0.5
1765	Geranyl acetate	1.5	0.3
1808	Nerol	0.9	tr
1857	Geraniol	1.5	tr
TOTAL		**99.8**	**98.9**

A: the sample obtained by microdistillation; B, the volatiles obtained by HS-SPME; tr: < 0.1% of the sample.

In the microdistilled bergamot sample, linalool (37.3%), limonene (32.9%) and linalyl acetate (7.8%) were the main compounds. γ-Terpinene (3.5%), α-terpineol (2.9%), β-pinene (2.4%), myrcene (1.6%), geraniol (1.5%), geranyl acetate (1.5%), neryl acetate (1.3%) and geranial (1.1%) were the other characteristic compounds of the sample. The microdistillation technique is suitable for the isolation and analysis of materials in mg levels. It is reliable and very fast when compared to other distillation techniques (Ağalar et al., 2015).

Limonene (36.8%), linalool (37.3%) and linalyl acetate (30.2%) were found as major components in the bergamot fresh peels by HS-SPME. γ-Terpinene (6.2%), β-pinene (3.0%), and myrcene (1.4%) were > 1% of the sample. SPME is a simple, sensitive, rapid, solvent-free and eco-friendly technique for the extraction of analytes of the materials. This process has two steps: first, portioning of analytes between the extraction phase and the sample matrix and second step, desorption of concentrated extracts into an analytical instrument. In HS-SPME technique, a mass transfer begins after exposure to the vapour phase above the sample (solution, solid etc.) (Merkle et al., 2015).

Previously, three studies have been published on the volatile compounds of the Turkish bergamot fruits. Baser et al. (1995) reported that bergamot oil containing thirty-six compounds constituting ~99.4% of the fruits cultivated in Antalya was obtained by cold-pressing of the fresh fruit peels. The main compounds were found as linalyl acetate (37.39%), limonene (32.28%) and linalool (16.27%). Kirbaslar et al. (2000) identified forty-nine compounds yielding 99.4% of the oil obtained by cold-pressing. According to results, linalyl acetate (38.7%), limonene (23.7%), linalool (14.7%), γ-terpinene (4.7%), β-pinene (3.0%) and myrcene (2.0%) were found as major components. Kirbaslar et al. (2001) reported another study. In that study, bergamot fruits harvested in different times (December, January and February) were subjected to cold-pressing and steam distillation techniques. According to GC and GC-MS analyses, totally forty-seven components were fully characterized as monoterpenes, oxygenated compounds, sesquiterpenes, carbonyl compounds, alcohols and esters groups. The major compounds were limonene (37.2%), linalyl acetate (36.3%), linalool (7.9%), γ-terpinene (5.9%), β-pinene (3.9%) and myrcene (1.3%) in cold-pressed bergamot oil. In the steam-distilled oil, limonene (33.2%), linalool (20.1%), linalyl acetate (17.3%) were the majors.

For the isolation of the *Citrus* peel essential oils different techniques can be employed: distillations, cold-pressing, super critical solvent extraction (Gök, Kirbaslar, & Kirbaslar, 2015; Kademi, & Garba, 2017). In our study, microdistillation and HS-SPME methods for the isolation of bergamot volatile compounds in the peels were used for the first time.

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