SMGroup

SM Analytical and Bioanalytical Techniques

Research Article

Volatile Compounds of Freshly Prepared Lemon Juice from the Region of Kalamata

Ioannis K Karabagias*

Department of Chemistry, University of Ioannina, Greece

Article Information

Received date: Sep 28, 2017
Accepted date: Nov 06, 2017
Published date: Nov 09, 2017

***Corresponding author**

Ioannis K Karabagias, Department of Chemistry, University of Ioannina, Greece, Tel: +306978286866; Fax: +302651008795; Email: ikaraba@cc.uoi.gr

Abstract

Background: Lemon is a favorable fruit due to its vitamin C content and numerous other applications in the daily diet

Aim: The aim of this study was to investigate the volatile profile of lemon juice prepared from home cultivated lemon trees in the region of Kalamata

Methods: Lemons (Maglini cv.) cultivated in home gardens were collected, washed and hand squeezed lemon juice was prepared. The volatile pattern was determined using solid phase micro extraction coupled to gas chromatography mass spectrometry (HS-SPME-GC/MS).

Results: Results showed that the volatile fraction of lemon juice was largely dominated by terpenoids. Limonene was the most abundant volatile compound followed by γ-terpinene, sabinene and neryl acetate.

Conclusion: Lemon juice from Kalamata is a rich source of terpenoids, especially limonene, and may be considered in adding valuable sensory characteristics or health benefits in other commercially available juices, food products, or specific pharmaceutical supplements.

Introduction

Citrus fruits are grown in areas with a mild climate, a suitable soil and sufficient humidity, free of frost. Lemon (*Citrus limon L.*) is cultivated for its fruits and especially for its juice. Lemon juice is a rich source of vitamin C and citric acid, while it is very useful in cooking, beverage drinks and pastry. Some typical regions in Greece, where lemon trees are cultivated, include: Messenia, Epirus, Corinthia, Aitolokarnania, Argolida, Crete, etc. [1].

From a medicinal point of view, lemons are considered precious fruits, because are widely used as anti-scurvy medicines, due to the high vitamin C content. Lemon juice may also be used as: antiemetic, refreshing, antipyretic, circulatory stimulant, mouth and throat disinfectant agent. The bark of small fruits is used in confectionery, for the preparation of spoon sweets or for the flavoring of other related products. From the bark, flowers and leaves of lemons are extracted the essential oils, which are widely used in perfumery [1].

The use of instrumental techniques such as gas chromatography/mass spectrometry or headspace solid phase micro extraction coupled to gas chromatography/mass spectrometry have contributed to a great extent on the accurate determination of volatile compounds in lemon products [2,3]. For example, the main volatile compounds found in lemon essential oil were monoterpenes, such as limonene, γ-terpinene and β-myrcene, along with limonene which is the most abundant volatile compound in lemon [4]. In addition, aldehydes, ketones and esters have been reported to contribute to the development of lemon aroma by using the aforementioned techniques [2,5].

Seeking a possible mechanism for the development of fruit aroma, it is documented that free volatile compounds are released from glycosides by acid or enzymatic hydrolysis during maturation, storage or processing of fruits [3,6].

Given that, there is no research study involving the volatile pattern of lemon juice prepared from house cultivated lemon trees in the region of Kalamata (prefecture of Messenia), the objective of the present work was to investigate the aroma profile of freshly prepared lemon juice from the dominant cultivar: “ Maglini”.

Materials and Methods

Lemon samples and juice preparation

20 kg of lemons (Maglini cv.) cultivated in house gardens were donated from local citizens of Kalamata during the harvesting year 2015-2016. The lemons were picked up at the peak of the

OPEN ACCESS

ISSN: 2573-3729

maturity which in a commercial level lasts between 1st November to 31th of May of each year. Lemons were hand squeezed using a hand juice extractor; the juice was filtered and was then collected in glass bottles. Samples for GC/MS analysis were divided in vials (in total 20 samples). All lemon juice samples of 4 mL volume, were stored in glass vials and maintained at -18 °C prior to analysis, which was accomplished in 10 days.

Reagents and solutions

Sodium chloride was purchased from Merck (Darmstadt, Germany). All materials used were cleaned with distilled water.

HS-SPME-GC/MS analysis

Headspace volatile compounds were extracted from lemon juice using a Divinyl Benzene/Carboxen/Poly Dimethyl Siloxane (DVB/CAR/PDMS) fiber 50/30 μm (Supelco, Bellefonte, PA, USA). The aforementioned fiber has been reported to have the best efficiency in extracting the volatile terpenoids from the headspace of lemon juice [2].

The fiber was conditioned by following the manufacturer's recommendations, prior analysis. Lemon juice samples of 4 mL along with 0.8 g of NaCl were placed in 15 mL screw-cap vials equipped with PTFE/silicone septa. Each vial was maintained at 40 °C in a water bath under stirring at 56 g-force (AREX heating magnetic stirrer, Velp Scientifica, Italy) for the headspace extraction. A cross-shaped PTFE-coated magnetic stirrer (diameter 10 mm) (Semadeni, Ostermundigen-Bern, Switzerland) was placed inside the vials. The sample preparation conditions were: 15 min equilibration time, 30 min sampling time and 4 mL sample volume and 40 °C water bath temperature.

Lemon juice samples were brought to room temperature daily prior to HS-SPME-GC/MS analysis. Blank runs were carried out before sample analysis, in order to avoid any source of contamination that could cause memory effects. Each sample was run in duplicate (n = 2).

Identification of volatile compounds

GC/MS instrumentation and conditions: A GC unit (Agilent 7890 A) coupled to a MS detector (Agilent 5975) was used to analyze the prepared lemon juice samples. A DB-5MS (cross-linked 5% PH ME siloxane) capillary column, having dimensions of 60 m x 320 μm i.d., x 1 μm film thickness, was used, with helium as the carrier gas (purity 99.999%), at 1.5 mL/min flow rate. The temperature for the injector and MS-transfer line were maintained constant at 250 °C and 270 °C, respectively. The oven temperature was held at 40 °C for 3 min and was further increased to 260 °C having a rate of 8 °C/min, for 6 min. Electron impact mass spectra were recorded at the mass range of 50-550. The ionization energy of the electron ionization system was 70 eV.

Mass spectral data processing: The identification of compounds was achieved using the Wiley 7, NIST 2005 mass spectral library. For the determination of linear retention indices a mixture of n-alkanes (C8-C20) dissolved in n-hexane, was employed. The standard mixture was purchased from Supelco (Bellefonte, PA, USA). Calculation of retention time indices was carried out for components eluting between n-octane and n-eicosane. Volatile compounds having ≥ 83% similarity with the Wiley mass spectral library were tentatively identified using GC-MS spectra. Each compound contribution was expressed as percentage (%) based on the ratio:

Table 1: Volatile compounds (VOCs, %) identified in freshly prepared lemon juice from the region of Kalamata.

RT	VOCs- IUPAC nomenclature	VOCs- Empirical nomenclature	Average	±SD	R_{exp}	R_{lit}	MOI
7.13	ethyl Acetate	Acetic ester	0.76	0.48	<800	614	MS
16.06	(1*S*, 5*S*)-2,6,6-Trimethylbicyclo[3.1.1]-hept-2-ene	α-Pinene	1.39	0.25	944	940	MS/KI
17.07	7-methyl-3-methylene- 1,6-Octadiene	β-Myrcene	1.42	0.46	986	989	MS/KI
17.21	4-methylene-1-(1-methylethyl)- Bicyclo[3.1.0] hexane	Sabinene	6.72	2.39	992	972	MS/KI
17.96	Para-mentha-1,3-diene	α-Terpinene	0.99	0.13	1025	1023	MS/KI
18.13	1-methyl-4-(1-methylethyl)- Benzene	p-Cymene	0.87	0.15	1033	1029	MS/KI
18.23	1-methyl-4-(1-methylethenyl)-Cyclohexene	Limonene	53.56	6.64	1037	1032	MS/KI
18.42	1,3,3-trimethyl- 2-Oxabicyclo[2.2.2]octane	1,8-Cineole	0.69	0.36	1059	1044	MS/KI
18.84	1-methyl-4-(1-methylethyl)-1,4-Cyclohexadiene	γ-Terpinene	12.42	3.72	1065	1065	MS/KI
19.50	1-methyl-4-(1-methylethylidene)-Cyclohexene	α-Terpinolene	1.21	0.22	1095	1093	MS/KI
19.55	3,7-Dimethylocta-1,6-dien-3-ol	Linalool	1.92	1.37	1097	1103	MS/KI
19.65	Nonanal	Pelargonaldehyde	2.21	0.64	1102	1106	MS/KI
19.75	1,3,3-trimethyl-Bicyclo[2.2.1]-heptan-2-one	Fenchone	0.77	0.49	1107	1097	MS/KI
21.61	4-methyl-1-(1-methylethyl)-3-Cyclohexen-1-ol	Terpinen-4-ol	1.64	1.26	1198	1185	MS/KI
21.83	2-(4-Methyl-1-cyclohex-3-enyl)-propan-2-ol	a-Terpineol	1.01	0.90	1209	1191	MS/KI
24.46	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (E)	Geranyl acetate	0.40	0.57	1350	1367	MS/KI
24.79	2,6-Octadien-1-ol, 3,7-dimethyl- acetate, (Z)	Neryl acetate	5.73	3.34	1369	1376	MS/KI

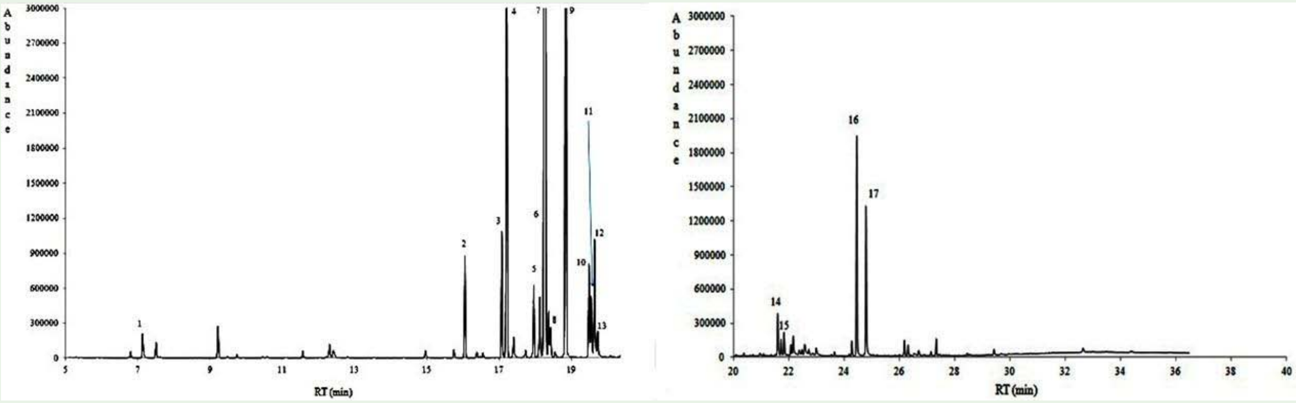

Figure 1: A typical gas chromatogram of freshly prepared lemon juice from the region of Kalamata.

$$Volatiles\,(\%) = \left(\frac{peak\ area\ of\ analyte}{total\ area}\right) \times 100$$

Statistical analysis: Statistical treatment of data was performed using the Microsoft office excel 2007 for Windows.

Results and Discussion

Volatile compounds of freshly prepared lemon juice

The volatile pattern of freshly prepared lemon juice from the Maglini cultivar was largely dominated by terpenoids. The most abundant terpenoids were limonene (53.56%), γ-terpinene (12.42%), sabinene (6.72%) and neryl acetate (5.73%) (Table 1) (Figure1).

Limonene (or dl-Limonene) is common constituent in cosmetic products. As the main odor constituent of citrus (Rutaceae), limonene is used in food manufacturing and some medicines, i.e. as a flavoring agent to mask the bitter taste of alkaloids used in certain drugs and as a fragrance material in perfumery, aftershave lotions, bath products, hand cleansers, etc [7]. It is also used, especially d-isomer which is the most active, as a botanical insecticide [8]. Limonene has a piney, turpentine-like odor. Some typical applications of limonene in natural and alternative medicine include its ability to relieve gastro esophageal reflux disease and heartburn [9].

Some other monoterpene hydrocarbons such as a-pinene, a-terpinene, a-terpinolene, b-myrcene, γ-terpinene were also identified. Such compounds have been reported previously to dominate the volatile pattern of lemon essential oils [5].

Oxygenated compounds such as linear aldehydes (nonanal) and monoterpene ketones (fenchone), monoterpene alcohols (linalool, terpinen-4-ol, a-terpineol), monoterpene esters (geranyl and neryl acetates) and monoterpene ethers (1,8-cineole), dominated the aroma of lemon juice.

Numerous of the volatile compounds of lemon juice from Kalamata have been reported previously in American lemon cultivars [3]. In addition, present results are in agreement with those reported previously in a study involving the volatile profile of 4 Italian lemon juice varieties [2]. Finally, compounds such as nonanal, α-pinene, p-cymene, limonene, γ-terpinene, α-terpinolene, linalool, terpinen-4-ol and α-terpineol have been recently identified in Spanish lemon fibres [10].

Apart from their use as flavor agents or other applications mentioned above, it should not be forgotten that terpenoids play an important role in the human defense against the carcinogenesis process at both the initiation and promotion/progression stages. In particular, monoterpenes are effective in treating early and advanced cancers, whereas limonene and perillyl alcohol have been shown to prevent mammary, liver, lung rodent cancers. Finally, *in vitro* data suggest that monoterpenes may be effective in treating neuroblastoma and leukemia [11].

Conclusion

The knowledge about phytochemicals of domestic products is of great interest to both: consumers and research community in a well built and developed society. Homemade lemon juice from Kalamata is a rich source of volatile terpenoids. Therefore, its use in a form of juice or in combination with other juices is heartily proposed. It should also be stressed that, apart from the characterization of this domestic product, the present study sets the basis for a new planned research involving the: i) Authentication, ii) Fruit juices adulteration control and iii) Beneficial health benefits after the consumption of home-made/cultivated citrus fruit juices.

References

1. Sakkas M. A review of the major tree crops in the Prefecture of Messinia, Diploma Thesis. Technological Educational Institute of Kalamata, Department of Agricultural Technology. 2005.
2. Allegrone G, Belliardo F, Cabella P. Comparison of volatile concentrations in hand-squeezed juices of four different lemon varieties. J Agric Food Chem. 2006; 54: 1844-1848.
3. Zhong S, Ren J, Chen D, Pan S, Wang K, Yang S, et al. Free and bound volatile compounds in Juice and peel of Eureka lemon. Food Sci Technol. 2014; 20: 167-174.
4. Fan G, Qiao Y, Yao X, Mo D, Wang K, Pan S. Free and bound volatile compounds in juice and peel of Jincheng oranges. Eur Food Res Technol. 2009; 229: 571-578.
5. Lota ML, de Rocca Serra D, Tomi F, Jacquemond C, Casanova J. Volatile components of peel and leaf oils of lemon and lime species. J Agric Food Chem. 2002; 50: 796-805.

6. Gunata Y, Bayonove C, Baumes R, Cordonnier R. The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. J Chromatogr A. 1985; 331: 83-90.

7. ‘’Limonene’’, cosmeticsinfo.org.

8. United States Environmental Protection Agency, EPA R.E.D. Fact Sheet on Limonene. 1994

9. Sun J. “d-Limonene: safety and clinical applications”. Alt Med Rev. 2007; 12: 259-264.

10. Marsol Vall A, Sgorbini B, Cagliero C, Bicchi C, Eras J, Balcells M. Volatile composition and enantioselective analysis of chiral terpenoids of nine fruit and vegetable fibres resulting from juice industry by–products. J Chem. 2017; 2017: 1-11.

11. Gould MN. Cancer chemoprevention and therapy by monoterpenes. Environ Health Perspect. 1997; 105: 977-979.