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
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 fiber was shaped before being introduced inside the vials. The vials were stored for 15 min equilibration time, 30 min sampling time and 4 mL sample volume and 40 °C water bath under stirring at 56 g-force (AREX heating magnetic stirrer). 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 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.

Table 1: Identification of volatile compounds

| RT | VOCs- | IUPAC nomenclature | VOCs- | Empirical nomenclature | Average | ±SD | Rexp | Rlit | MOI |
|----|-------|---------------------|-------|------------------------|---------|----|------|------|-----|
| 7.13 | ethyl Acetate | Acetic ester | 0.76 | 0.48 | <800 | 614 | MS/KI |
| 16.06 | (1S, 5S)-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-Mycrcene | 1.42 | 0.46 | 986 | 989 | MS/KI |
| 17.21 | 4-methylene-(1-(1-methylethyl) Bicyclo[3.1.0]hexane | Sabine | 6.72 | 2.39 | 992 | 972 | MS/KI |
| 17.96 | Para-memtha-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-methylethyl)-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-methylethylide) 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 | Terpinene-4-ol | 1.64 | 1.26 | 1198 | 1185 | MS/KI |
| 21.83 | 2-(4-Methyl-1-cyclohex-3-enyl)-propan-2-ol | α-Terpinol | 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 |

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.
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) (Figure 1).

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, aftshead 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 gastroesophageal reflux disease and heartburn [9].

Some other monoterpene hydrocarbons such as α-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.

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