



Pistachio oil (*Pistacia vera* L. cv. Uzun): Characterization of key odorants in a representative aromatic extract by GC-MS-olfactometry and phenolic profile by LC-ESI-MS/MS



Ahmet Salih Sonmezdag^a, Hasim Kelebek^b, Serkan Selli^{c,*}

^a Department of Gastronomy and Culinary Arts, Faculty of Fine Arts, University of Gaziantep, Gaziantep, Turkey

^b Department of Food Engineering, Faculty of Engineering and Natural Sciences, Adana Science and Technology University, Adana, Turkey

^c Department of Food Engineering, Faculty of Agriculture, Cukurova University, 01330 Adana, Turkey

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ABSTRACT

Volatile, aroma-active, and phenolic compounds of pistachio oil obtained from cv. Uzun were investigated in the current study. To obtain a representative aromatic extract, three of the most widely used extraction methods were compared using a representative test; the solvent-assisted flavour extraction (SAFE) aromatic extract from pistachio oil was found to be the most representative. A total of 50 aroma compounds were determined in pistachio oil and it was found that terpenes, aldehydes, and alcohols were the most abundant volatile compounds. Applying GC-MS-olfactometry and aroma extract dilution analysis (AEDA) resulted in a total of 14 aroma-active areas being detected in the extract of pistachio oil. In the phenolic fraction obtained by the LC-ESI-MS/MS method, a total of 12 phenolic compounds was found in the pistachio oil, of which seven compounds were reported for the first time. Eriodictyol-7-O-glucoside and protocatechuic acid were the most dominant phenolic compounds.

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1. Introduction

The pistachio nut is one of the most commonly used tree nuts in the world due to its special organoleptic characteristics (Acena, Vera, Guasch, Busto, & Mestres, 2010). This nut has been used as a savory snack or as a major ingredient in many traditional desserts, pastries, fermented meats, and puddings throughout human history. Today, pistachios are cultivated as an important agricultural commodity in Iran, Turkey, the United States, Syria, Greece, Italy, and Spain (Rodriguez-Bencomo et al., 2015). Of all these nations, Turkey is the third biggest importer and exporter of pistachio nuts (FAO., 2015). In Turkey, pistachio is primarily cultivated in the southeastern region where the main cultivated pistachio variety is “Uzun”, due to its distinct flavour and uniform green kernels (Satil, Azcan, & Baser, 2003).

Beyond its economic value, pistachio is among the 50 foods with the highest antioxidant potential. It is also known to contain considerable amounts of phenolic compounds (Halvorsen et al., 2006). Previous studies have highlighted that not only the kernels but also the skin, hull, flowers, leaves, and oil of pistachio contain discrete amounts of phenolic compounds (Goli, Barzegar, & Sahari,

2005; Martorana et al., 2013; Tomaino et al., 2010). The main phenolics in pistachio oil are benzoic acid derivatives, which have been known to combat colorectal, breast cancer, and heart disease (Saitta, La Torre, Potorti, Di Bella, & Dugo, 2014).

A complex mixture of volatiles, which is comprised mainly of aldehydes, pyrazines, alcohols, ketones, esters, terpenes, lactones, and carboxylic acids, is responsible for the oil aroma of nuts (Bail, Stuebiger, Unterweger, Buchbauer, & Krist, 2009). Within these a limited number of volatile compounds actually contribute to the characteristics of nut oil aroma. GC-olfactometry is considered a useful way to determine individual key odorants in samples. This technique makes it possible to precisely divide aroma compounds into aroma-active and non-aroma-active compounds depending on their amounts in foods (Schieberle, 1995). The success of GC-olfactometric studies depends on the extraction technique employed to extract volatile compounds from the studied sample. Therefore, it is necessary to check the representativeness of the odor of aromatic extracts with the original sample (Selli & Kelebek, 2011).

Most of the earlier papers focused on the physicochemical, color, antioxidant, and fatty acid properties of pistachio oils. To the best of our knowledge, the first study on volatile compounds of pistachio oil was published by Bail et al. (2009). These authors investigated the volatile compounds of pistachio oil from Austria

* Corresponding author.

E-mail address: sselli@cu.edu.tr (S. Selli).

and Turkey using SPME-GC-MS, and reported that the dominating compound analyzed was acetic acid in the headspace of both pistachio oils. Ling, Yang, Li, and Wang (2015) analyzed physicochemical characteristics and volatile compounds of cold-pressed pistachio oil from raw and two roasted kernels prepared by conventional and microwave roasting treatments. Among the 43 volatile compounds identified, major compounds included limonene, α -pinene, myrcene, hexanoic acid, and nonanal in the pistachio oil.

A limited number of papers have used GC-O studies to identify the key odorants of edible oils (Pollner & Schieberle, 2016; Tairu, Hofmann, & Schieberle, 2000). To the best of our knowledge, no data are present in the extant literature about the use of GC-MS-olfactometry and LC-ESI-MS/MS applied to the characterization of aroma-active and phenolic compounds of pistachio oil. Therefore, the aim of the present study is: *i*) to assess the representativeness of pistachio oil aromatic extracts obtained from three different extraction methods using similarity and intensity tests; *ii*) to characterize the aroma-active compounds by the application of aroma extract dilution analysis (AEDA); and *iii*) to determine the phenolic compositions by LC-ESI-MS/MS.

2. Materials and methods

2.1. Pistachio oil

The oil sample of Uzun cultivars of *P. vera* was obtained from a pistachio farming area in the southeastern Turkish province of Gaziantep during the summer of 2014. This region has a borderline Mediterranean climate which means hot and dry summers and warm and wet winters. Pistachio oil sample was obtained by a cold press machine (Ekotok-1, Izmir/Turkey). The extraction was implemented three times and then the sediments in the expressed oil were separated by filtration. The temperature of pistachio oil was measured throughout the operation with digital thermometer and it was continuously held at under 40 °C. A total of 2 L of the pistachio sample was obtained after a 30-min extraction. Once a centrifugation step was implemented to eliminate the remaining solid residues from the samples, the oil was transferred to small dark bottles and stored at 4 °C prior to analysis.

2.2. Chemicals

Water used in this study was purified by a Millipore-Q system (Millipore Corporation, Saint-Quentin, France). The following standard aroma compounds were purchased from the sources listed in parentheses: α -pinene, hexanal, β -pinene, myrcene, limonene, methylpyrazine, α -terpinolene, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 1-octen-3-ol, (*E,E*)-2,4 nonadienal, benzaldehyde, linalyl acetate, bornyl acetate, 2-furanmethanol, γ -butyrolactone, γ -hexalactone, nonanal, isovaleric acid, δ -octalactone, *p*-cymen-8-ol, 2-phenylethyl alcohol, 2-acetylpyrrole, heptanoic acid, phenol, octanoic acid, eugenol, nonanoic acid, and vanillin (Sigma-Aldrich, Steinheim, Germany); acetic acid, nonanal, butanoic acid, pentanoic acid, and 4-nonanol (Merck, Darmstadt, Germany). The standard phenolic compounds included gallic acid, protocatechuic acid, catechin, caffeic acid, *p*-coumaric acid, eriodictyol-7-O-glucoside, syringic acid, ferulic acid, rutin, naringin, eriodictyol, and luteolin purchased from Sigma-Aldrich (St Louis, MO). Dichloromethane, sodium sulfate, and LiChrolut were supplied by Merck (Darmstadt, Germany). All chemicals and solvents used in this study were of analytical and chromatography grade purity.

2.3. Extraction of volatile compounds

In the present study, three different extraction methods were used to isolate volatile compounds in the pistachio oil sample. These include simultaneous distillation/extraction (SDE), solvent-assisted flavour evaporation (SAFE), and purge-and-trap (PT) extraction techniques. The main goal of using the different extraction methods was to obtain a representative aromatic extract of oil for GC-MS-O analysis.

2.3.1. Simultaneous distillation/extraction (SDE)

A Likens-Nickerson apparatus (Neubert-Glas, Geschwenda, Germany) was employed to extract the volatile compounds of the pistachio oil by SDE. This method has already shown its reliability for the extraction of volatile components of different olive oils. For the extraction, 40 mL of pistachio oil, 100 mL of pure water, 25 mL of 30% NaCl, and 40 μ g of 4-nonanol as the internal standard were placed into a 500-mL distillation flask, and 40 mL of dichloromethane solvent was pipetted into a 100-mL distillation flask. Both flasks were placed in a heater in which the extraction was performed for approximately three hours. The temperatures of the sample mixture and the dichloromethane flasks were maintained by a water bath at 70 °C and 50 °C, respectively. After the dehydration by anhydrous sodium sulfate, the resulting organic extract was condensed to 5 mL in a Kuderna-Danish concentrator (Sigma-Aldrich, St. LouisMO) and then to 200 μ L under a gentle stream of pure nitrogen.

2.3.2. Solvent-assisted flavour evaporation (SAFE)

The volatile compounds of pistachio oil were also isolated using SAFE (Glasblaserei Bahr, Manching, Germany) under vacuum (10^{-3} Pa; Vacuubrand DCP 3000, Wertheim, Germany). A 40-mL volume of pistachio oil and 40 mL of dichloromethane were placed into a 500-mL flask. The content was stirred at 4 °C for 30 min under nitrogen gas and then centrifuged at 4 °C, 1000 rpm, for 15 min. The organic phase (solvent) was slowly fed into the dropping funnel of the transfer head. Separation of the mixture occurred when contents of the sample were dropped into the distillation vessel (10 mL/min) that was partially submerged in a warm water bath of 38 °C. To ensure a continuous temperature throughout distillation and to avoid condensation of the volatiles, the system was completely thermostated with water (4 °C). Separated volatiles passed through the separation head into a receiving vessel where they condensed and froze because of the sudden drop in temperature under the liquid nitrogen in the cooling trap. Once the separation was complete, the receiving vessel was removed and allowed to thaw out at room temperature for 30 min. After dehydration by anhydrous sodium sulfate, the resulting organic extract was concentrated to 200 μ L (Chetschik, Granvogl, & Schieberle, 2008; Engel, Bahr, & Schieberle, 1999).

2.3.3. Purge-and-trap system (PT)

The PT extraction process consisted of a source of nitrogen controlled by a flow-meter (LZT 4-M, Union-Tek Instrument, China). The needle of the source of N₂ and the cartridge were installed through the septum to purge and trap the aroma compounds. As adsorbent, 200 mg of Lichrolut EN resin from Merck was chosen as the most suitable material for aroma compound retention according to the literature. The temperature of the vial sample was controlled by a thermostat; 3 g of sample were transferred into a 20-mL vial, then the sample was pre-incubated at the extraction temperature (60 °C) for 10 min. The purge-and-trap process was conducted for 90 min with a nitrogen flow of 500 mL/min. After the purging process, the compounds retained in the cartridge were eluted with 6 mL dichloromethane. After dehydration by anhydrous sodium sulfate, the pooled organic extract was reduced

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