Analysis of volatile flavour components by dynamic headspace analysis/gas chromatography-mass spectrometry in roasted pistachio extracts using supercritical carbon dioxide extraction and sensory analysis

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ABSTRACT
In this study, optimum process parameters for supercritical carbon dioxide (CO₂) (SC-CO₂) extraction of roasted pistachio (Pistacia vera L.) were investigated by depending on amount of the characteristic flavouring components (α-pinene, limonene-D, α-terpinolene and β-myrcene). The extracts were analysed by DHA/GC-MS for determination of the volatile compounds and the optimum process parameters were decided as 200 g, 350 Bar, 70°C, 75 g CO₂/min and 60 min. In the pistachio extracts obtained having the optimum process parameters, α-pinene, β-myrcene, limonene-D and α-terpinolene were detected as 24.47 g/100 g, 0.52 g/100 g, 2.25 g/100 g and 5.70 g/100 g among 31 volatile compounds in total, respectively. The fatty acid composition of the extract, which had the most desirable taste and flavour, was detected by gas chromatography using fatty acid methyl esters (FAME) preparation. The fatty acid composition analysis showed that the pistachio extract had included mostly oleic acid (67.51%, w/w) and linoleic acid (17.85%, w/w).

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Introduction
Pistachio (Pistacia vera L.) is widely cultivated in saline, dry and hot areas in the Middle East, Mediterranean countries and the United States. It is mostly grown in Iran and Turkey according to the FAOSTAT reports.[1–3] There are five types of pistachios which are ‘Uzun’, ‘Siirt’, ‘Kırmızı’, ‘Ohadi’ and ‘Halebi’.[4] Uzun is the mostly used type of pistachio nut in Turkey with its special flavour and texture for manufacture of cakes, biscuits, breads, candies, chocolates, ice creams, fermented meat, sauces, puddings and deserts.[5,6]

Pistachio is well known for its good nutritional properties and health promoting effects.[7–9] It contains sterols, vitamins, minerals, fatty acids and phenolic compounds.[10] Pistachio has more than 50% (w/w) oil content, which is mainly composed of oleic and linoleic acids.[11] Pistachio shows antifungal,[12] antimicrobial[13] and antioxidant[14] properties in addition and these properties of pistachio vary according to the region where the plant is grown. Pistachio can be considered as one of 50 functional foods having high antioxidant potential.[6]

Quality of pistachio mainly depends on its flavour, colour and texture. It is commonly consumed all over the world after being roasted because raw pistachio releases rather bland taste and odour. However, chemical constituents giving its characteristic flavour, like aldehydes, ketones, esters, pyrazines, terpenes and acids are improved by roasting.[15–18] Roasting has additional advantages
like improving the shelf life of the product by removing the aflatoxins\(^6\) and moisture about 5–9% in pistachio.\(^{19}\)

Information on the taste and aroma of the roasted nut extracts could lead to better characterization of these food ingredients. In a flavour research, sensory evaluation is essential because it has the advantage of describing sensory attributes with a high sensitivity. Descriptive sensory analysis (DSA), which is a common technique in flavour researches and has been successfully used for comparing odour and taste properties in food products, can also be used for pistachio extracts for better definition of their flavouring characteristics.

Pistachio extracts can be obtained by supercritical carbondioxide (SC-CO\(_2\))-extraction method,\(^{11}\) which is an environmentally benign technique and is beneficial for showing higher efficiency, avoiding use of solvent and retaining organoleptic characteristics of the starting material. In this study, we aimed to design the most efficacious SC-CO\(_2\)-extraction method for obtaining roasted pistachio extracts having the most desirable taste and flavour. For this purpose, various extraction parameters were optimized considering amounts of some critical flavouring components of the extracts (\(\alpha\)-pinene, limonene-D, \(\alpha\)-terpinolene and \(\beta\)-myrcene). Amounts of those components were detected in the extracts by dynamic headspace analysis/gas chromatography-mass spectrometry (DHA/GC-MS) for revealing their effects on the flavouring characteristics of the extracts when the flavouring characteristics were evaluated sensorially. Volatile compounds and fatty acid compositions of the extracts were also determined.

**Materials and methods**

**Materials**

Uzun-type roasted pistachios were obtained from Kızıroğlu Gıda San. Ve Tic. Ltd. Şti. (Turkey) and they were stored at 0–4°C until being used. CO\(_2\) for SC-CO\(_2\)-extraction system was purchased from Habaş (Kocaeli, Turkey). Starch was provided from Aromsa Flavours and the Food Additives San. ve Tic. A.Ş. (Kocaeli, Turkey).

**Sample preparation and the equipment**

Before experiments, roasted Pistachios were chopped into small particles using a planetary grinder (Arzum Elektrikli Ev Aletleri San. ve Tic. AŞ., Turkey). Roasted pistachio particles were sorted by certified test sieves (Retsch, Haan, Germany) by manually. Particle fraction obtained from the sieves between 3 mm and 5 mm was used in experiments. The powdered pistachios were stored at 0–4°C until the extraction process. The particle size of the feed material is very critical parameter for extraction processing.\(^{20}\) When the particle size is very small, the caking problem occurs, which is the tendency of the powders to form lumps, comes out in the extract or; on the other hand, when the particle size is very large, it results in channeling which can decrease the extraction yield.\(^{21}\) SC-CO\(_2\) extraction was performed by using a Waters SFE 1000 Supercritical Fluid Extraction System (Waters Corp., USA). This system has an extractor vessel of 1000 mL, maximum pressure of 700 Bar, a separator with an internal volume of 200 mL and a maximum CO\(_2\) mass flow rate of 200 g/CO\(_2\)/min. The extracts were stored at 0–4°C.

Parameters of the extraction were optimized including pressure, temperature, CO\(_2\) flow rate, time, feed amount and particle size, which are critical and valuable for the extraction process affecting yield. For this purpose, in every study one parameter was changed when the others were kept constant. This study was replicated three times.
**Determination of the volatile compounds**

The volatile compounds of the extracts were analysed by DHA/GC-MS. Their total ion chromatogram were obtained using a Gerstel DHS System (Germany) connected to an Agilent 7890A GC and 5975C MS equipped with an Inert MSD with Triple Axis Detector (Germany). A total of 1 g of pistachio extract was placed in a 20 mL standard headspace vial. It was placed in a tray of a Gerstel Multi Purpose Sampler. Desorbed compounds were injected automatically into a CP-WAX 52 GC column (60 m × 25 μm film thickness × 0.25 mm innerdia) (INNOWAX, Germany). The flow rate of the Helium carrier gas was adjusted to 1.2 mL/min. Each sample was injected in the splitless mode. The GC oven temperature was programmed from 40°C to 240°C at 5°C/min.

**Analysis of the fatty acid composition**

Oil was extracted from pistachios through SC-CO$_2$-extraction method and extracted oil samples were analysed by gas chromatography using fatty acid methyl esters (FAMEs) preparation.$^{[22]}$ FAMEs were analysed by a GC-MS, equipped with a flame ionization detector (FID) and a HP-innowax 19091N-136GC column (60 m × 0.25 μm film thickness × 0.25 mm innerdia). The oven temperature was programmed from 40°C to 260°C at a 5°C/min heating rate. The injector and detector temperatures were held at 260°C. A reference standard composed of a mixture of FAMEs (Supelco Inc.) was analysed under the same operating conditions to determine the peak identity. FAMEs value from the results of duplicate analyses were reported.

**Descriptive sensory analysis**

The pistachio extracts were evaluated by DSA done with an experienced assessor group (11 male and 12 female assessors aged 25–55) to determine the intensities between the tasting/flavouring attributes. Prior to DSA, the extracts were dissolved in a 2% (w/w) starch sauce and the assessors were informed about the method, analysis steps and the product information. Critical information like the brand of the products and their production and expiration dates were not given to the assessors. Evaluations were performed in the personal cabins of the Aromsa Sensory Analysis Laboratory. A positive pressure was applied to get rid of the several odours in those cabins, when the cabin temperature and relative humidity were kept at 21–22°C and 50–55% during the analysis. There were no objects that can disturb the concentration of the assessors in the cabins. A five-linear scale was used to evaluate the intensity of the sample taste. Instructions about the evaluation and the scale were also demonstrated on evaluation monitors. The samples were presented to the assessors as blank and coded with randomly selected three-digit numbers.$^{[23]}$

Nine attributes for taste were selected from previous studies on pistachio extract lexicon and were thoroughly defined for taste profiling (Table 1).$^{[24,25]}$ Flavour lexicons are used in a sensory evaluation to determine flavour profiles of food products.$^{[24]}$ Flavour is a combination of taste and aroma. Two principal methods exist to measure flavour of a food product. The first method uses a consumer panel composed of a large group of untrained people or through a small highly trained group of people known as a descriptive panel. Descriptive panels use lexicons, a list of defined terms, to describe products. The assessors discussed and found out characteristics of the samples and reference product for each attribute. At the beginning of each evaluation session, the assessors were calibrated with the blank starch sauce as the reference. Descriptive panels used defined lexicon of terms, and were trained to rate the intensity or the prevalence of each attribute that was present. At the beginning of each session, the assessors were calibrated to prevent fatigue. The pistachio extract was measured in separate sessions in triplicate by each assessor.
Results and discussion

Optimization of the process parameters

In this study, since flavours of the pistachio were taken into consideration, optimum process parameters for the SC-CO$_2$ extraction were decided by depending on amount of the flavouring compounds rather than the yield of the extraction process. α-pinene, limonene-D, α-terpinolene and β-myrcene were primary ones among these compounds.$^{12,27-28}$ All the extracts were analysed by DHA/GC-MS for determined characteristic odour of pistachio’s compounds (α-pinene, limonene-D, α-terpinolene and β-myrcene).

Optimization of the feed amount

The feed amount, particle size of the extracts and, temperature and pressure applied as the conditions for CO$_2$ extraction process are important parameters for a good mass transfer between CO$_2$ and particles of pistachio. For optimization of the feed amount, other parameters—pressure, temperature, CO$_2$ flow rate and process time—were kept constant at as 300 Bar, 60°C, 100 g$_{CO2}$/min, 120 min, respectively, while different feed amounts were tried (100, 150, 200, 250 and 300 g) as given in Table 2. As it is seen in Figure 1, maximum amounts of the flavouring compounds which give the characteristic taste and odour of the pistachio (α-pinene, limonene-D, α-terpinolene and β-myrcene) were obtained by 200 ± 2 g feed amount of the starting material. When the feed amount was higher than 200 g, amounts of the flavouring compounds were observed to decrease (Figure 1A).

Table 1. Flavouring attributes selected for DSA.

| Attribute* | Characteristics       |
|------------|-----------------------|
| Sweet      | Taste of candy        |
| Salty      | Taste of sodium chloride |
| Pistachio oil | Oily taste of extracted pistachio |
| Pistachio  | Characteristic taste of pistachio |
| Bitter     | Taste of caffeine solution |
| Shell      | Taste of roasted nut  |
| Roasted    | Taste of nonroasted pistachio |
| Raw        | Taste of rancidity and old fat |

Table 2. Different process parameters for SC-CO$_2$ extraction.

| Method | Feed amount (g) | Pressure (Bar) | Temperature (°C) | CO$_2$ flow rate (g$_{CO2}$/min) | Time (min) |
|--------|-----------------|----------------|------------------|----------------------------------|------------|
| 1      | 100             | 300            | 60               | 100                              | 120        |
| 2      | 150             |                |                  |                                  |            |
| 3      | 200             |                |                  |                                  |            |
| 4      | 250             |                |                  |                                  |            |
| 5      | 300             |                |                  |                                  |            |
| 6      | 200             | 150            | 60               | 100                              | 120        |
| 7      | 200             |                |                  |                                  |            |
| 8      | 250             |                |                  |                                  |            |
| 9      | 350             |                |                  |                                  |            |
| 10     | 400             |                |                  |                                  |            |
| 11     | 450             |                |                  |                                  |            |
| 12     | 200             | 350            | 50               | 100                              | 120        |
| 13     |                 |                |                  |                                  |            |
| 14     |                 |                |                  |                                  |            |
| 15     | 200             | 350            | 70               | 50                               | 120        |
| 16     |                 |                |                  | 75                               |            |
| 17     |                 |                |                  |                                  | 125        |
| 18     | 200             | 350            | 70               | 75                               | 40         |
| 19     |                 |                |                  |                                  | 60         |
| 20     |                 |                |                  |                                  | 90         |
| 21     |                 |                |                  |                                  | 180        |
Optimization of the process pressure

Pressure is one of the most important parameters that affect the yield and selectivity of the extraction process. Increasing the pressure at a constant temperature enhances the solubility of the feed sample.[20] In order to optimize the pressure, a series of experiments was performed by keeping the feed amount, temperature, CO$_2$ flow rate and the process time constant (200 g, 60°C, 100 gCO$_2$/min and 120 min, respectively) for different process pressures (150, 200, 250, 350, 400 and 450 Bar) (Table 2). As a result, the highest amounts of the flavouring compounds were achieved at 350 Bar pressure as it is illustrated in Figure 1B similar to the results of Ghaziaskar and Sheibani who obtained the pistachio oil by an optimum extraction pressure of 345 Bar.[29]

Optimization of the process temperature

When the temperature is increased at a constant pressure, the density of the supercritical fluid decreases and its solubility increases.[21] For optimization of the process temperature, heat-sensitive flavouring compounds should be taken into account. For optimization of the process temperature, feed amount, pressure, CO$_2$ flow rate and process time were kept constant (200 g,
350 Bar, 100 g\textsubscript{CO2}/min and 120 min, respectively) for temperatures 50°C, 70°C and 80°C as shown in Table 2. According to the results introduced in Figure 1C, 70°C was observed to be the optimum process temperature with the highest amount of the flavouring compounds.

**Optimization of the CO\textsubscript{2} flow rate**

The extraction yield is enhanced with increasing the flow rate of CO\textsubscript{2} which decreases the mass transfer resistance of particles.\textsuperscript{[30]} For optimization of the flow rate of CO\textsubscript{2}, different rates (50, 75 and 125 g\textsubscript{CO2}/min) were performed while the feed amount, pressure, temperature and process time were kept constant (200 g, 350 Bar, 70°C and 120 min, respectively) as introduced in Table 2. As it is shown in Figure 1D, ‘75 g\textsubscript{CO2}/min’ was confirmed to be the optimum flow rate.

**Optimization of the process time**

It was reported that when the process time was increased, the yield enhanced for SC-CO\textsubscript{2} of nuts of various species.\textsuperscript{[21,30]} In order to optimize the process time, the parameters like feed amount, pressure, temperature and CO\textsubscript{2} flow rate were kept constant (200 g, 350 Bar, 70°C and 75 g\textsubscript{CO2}/min, respectively) (Table 2) when the process time was changed (40, 60, 90 and 180 min) (Figure 1E). When the process time is increased, the yield was enhanced. However, amounts of the desired flavouring compounds were observed to decrease. Therefore, 60 min at which amounts of the flavouring compounds were the highest, was observed to be the optimum process time.

As a result based on the data obtained, the optimum process parameters for SC-CO\textsubscript{2} extraction of the roasted pistachios were decided as 200 g, 350 Bar, 70°C, 75 g\textsubscript{CO2}/min and 60 min. Furthermore, the volatile compounds and fatty acid composition of the extracts obtained under those optimized conditions were determined and the sensory evaluation was made.

**Determination of the volatile compounds**

Totally 31 compounds were detected in the pistachio extract obtained via SC-CO\textsubscript{2} method by DHA/GC-MS (Table 3). Among those compounds, there were 5 aldehydes, 13 monoterpenes, 2 ketones, 3 pyrazines, 2 alcohols, 4 organic acids and 1 lactone. The characteristic flavouring components of the pistachio which are \(\alpha\)-pinene, \(\beta\)-myrcene, limonene-D and \(\alpha\)-terpinolene were also observed among these components (24.47 g/100 g, 0.52 g/100 g, 2.25 g/100 g and 5.70 g/100 g, respectively). Volatile compound analyses were replicated three times.

**Fatty acid composition analysis**

The fatty acid profile of the pistachio oil obtained under the optimum conditions are shown in Table 4. Table 4 introduces that oleic and linoleic acids were measured as major fatty acids in the pistachio extract. Ghaziaskar and Sheibani 2008 announced that pistachio included oleic and linoleic acids more than 50%.\textsuperscript{[11]}

**Descriptive sensory analysis**

In this study, the pistachio extracts which were mentioned for optimizing extract conditions were evaluated by the assessors using sensory analysis in order to choose the best one. Thus, the chosen extract was also evaluated again by DSA. For the pistachio extract obtained by SC-CO\textsubscript{2} extraction, the characteristic tastes like oily, pistachio and roasted were sensed primarily and dominantly, on the other hand the sweet, shell, raw, salty, bitter and oxidized tastes were sensed weakly. The results are also illustrated in a cobweb diagram (Figure 2). According to data obtained from DHA/GC-MS analysis, rate of \(\alpha\)-pinene, \(\beta\)-myrcene, limonene-D and \(\alpha\)-terpinolene in the extracts was
confirmed to be available in the typical range as reported earlier.\textsuperscript{30} Thus, increase in the area of α-terpinolene was confirmed to provide a more desirable flavour by the accessors when increase in β-myrcene rate in the extracts caused to less desirable flavour combined to decrease in the rate of α-terpinolene.

**Conclusion**

The aim of the present study was to determine the optimum process parameters for SC-CO\textsubscript{2} extraction of Uzun-type roasted pistachios by detecting the volatile components of the pistachio extracts and by analysing the flavouring attributes. The optimum method was determined by

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**Table 3.** The flavouring compounds detected in the pistachio extracts by DHA-GC/MS.

| Compounds               | Area % |
|-------------------------|--------|
| Isobutyraldehyde        | 0.48   |
| Isovaleraldehyde        | 46.66  |
| Diacetyl                | 0.19   |
| Tricylene               | 0.36   |
| α-Pinene                | 24.47  |
| Camphene                | 1.61   |
| Hexanal                 | 0.31   |
| Pinene 2-β              | 1.42   |
| Delta-3-carene          | 1.04   |
| β-Myrcene               | 0.52   |
| Limonene D              | 2.25   |
| Isoamyl alcohol         | 0.41   |
| β-Ocimene               | 0.15   |
| P-cymene                | 0.20   |
| α-Terpinolene           | 5.70   |
| Acetoin                 | 0.31   |
| 2.5-Dimethyl pyrazine   | 0.18   |
| 2.6-Dimethyl pyrazine   | 0.26   |
| Nonanal+2-Ethyl-6-methyl pyrazine | 0.21 |
| α-para-dimethyl styrene | 0.13   |
| Acetic acid             | 9.32   |
| Furfural                | 0.18   |
| Benzoaldehyde           | 0.23   |
| 2.3-Butanediol          | 4.09   |
| γ-Butyrolactone         | 0.76   |
| 1,8-Menthadiene-4-ol    | 0.23   |
| P-Cymene-8-ol           | 0.09   |
| Octanoic acid           | 0.11   |
| Decanoic acid           | 0.11   |
| Palmitic acid           | 1.24   |

Italic values are the primary flavouring compounds which give the characteristic odour and taste of pistachio.

**Table 4.** The fatty acid composition (%) of the pistachio extract.

| Fatty acid composition | % total |
|------------------------|---------|
| Methyl myristate       | 0.16    |
| Methyl palmitate       | 9.86    |
| Methyl palmitoleate    | 0.69    |
| Methyl heptadecanoate  | 0.06    |
| Methyl stearate        | 1.78    |
| Methyl oleate          | 67.51   |
| Methyl linoleate       | 17.85   |
| Methyl linolenate      | 0.31    |
| Methyl arachate        | 0.14    |
| Methyl docosanoate     | Not detected |
| Methyl-10-octadecenoate | Not detected |
performing several trials with different values for each parameter while keeping the other parameters constant. As a result, the optimum process parameters for SC-CO$_2$ extraction of pistachio were chosen as ‘200 g, 350 Bar, 70°C, 75 g CO$_2$/min and 60 min’. DHA/GC-MS demonstrated totally 31 compounds in the extracts obtained at those optimum conditions, which were grouped as 5 aldehydes, 13 monoterpenes, 2 ketones, 3 pyrazines, 2 alcohols, 4 organic acids and 1 lactone. Among those, α-pinene, β-myrcene, limonene-D and α-terpinolene gave the characteristic taste and odour of roasted pistachio and their concentrations were detected as 24.47 g/100 g, 0.52 g/100 g, 2.25 g/100 g and 5.70 g/100 g, respectively. Fatty acid composition of the extract was observed that oleic and linoleic acids were the major fatty acids. The pistachio extracts were also analysed sensually and nine flavouring attributes were introduced by the Lexicon procedure. Among those attributes which were oily, pistachio and roasted, were observed to be sensed primarily and dominantly. Other attributes (sweet, shell, raw, salty, bitter and oxidized) were observed to be sensed weakly.

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