Supplementary Materials

Exploring the Chemical Composition and Antioxidant Properties of Apricot Kernel Oil

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Supplementary Methods

2.4. Volatile Compound Analysis by HS-SPME/GC-MS

An SPME fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, USA) was used. The fiber was preconditioned (1 h at 260 °C) in accordance with the manufacturer's specifications before usage. In a 50 mL glass vial, 3 g of AKO was added and sealed with a PTFE/silicone septum. The sample was left for 10 min at 40 °C while being stirred at 250 rpm to reach equilibration. Then, the fiber was inserted into the headspace, and extraction was carried out for 1 h. Finally, the fiber was placed in the injector of a gas chromatograph coupled to a mass spectrometer (GC-MS) for desorption of the compounds and further analysis. The GC-MS system used herein was an Agilent Technologies (Santa Clara, CA, USA) gas chromatograph model 7890A connected to a mass selective detector model 5975C and a capillary column Agilent J and W DB-1 (30 m × 320 mm × 0.25 mm). The helium carrier gas flowed at a rate of 1.6 mL/min (0.1 bar). The injector temperature was set at 230 °C, and the sample was inserted into the column using splitless mode. After 30 min at 30 °C, the temperature of the column was increased to 220 °C at a rate of 2 °C/min and kept at this temperature for 10 min. The detector was operated in the Scan mode, with a mass range of *m*/*z* 29–350. The settings of the detector were: acquisition mode electron impact (EI 69.9 eV); source temperature 230 °C; quadrupole temperature 150 °C. Each sample was analyzed three times with the above settings. Spectra were processed with the Agilent Chemstation (version B.03.02). By comparing the mass spectra of the various peaks to those in the Wiley W8N08 database (Wiley, New York, USA), the individual peaks were identified. The normalization approach (without correction factors) was used to calculate the percentage composition of the samples from the GC peak regions.

2.5. Essential Oil

2.5.2. Essential Oil Analysis by GC-MS

The GC-MS system used for the analysis was the same as the one described in Section 2.4. Samples (1 μL) were injected automatically with an Agilent 7683B autosampler injector in split mode (1:30) at 240 °C. After 5 min at 50 °C, the temperature of the column was increased to 230 °C at a rate of 2 °C/min. The settings of the detector were: a source temperature of 230 °C, quadrupole temperature of 150 °C, scan acquisition mode with an electron impact (EI, 69.9 eV), and a mass range of *m*/*z* 50–550. Peaks were identified by comparison of the mass spectra with the spectra found in the Wiley (W8N08 and 7th edition, John Wiley & Sons, New York, USA) and NIST 11 (National Institute of Standards and Technology, Gaithersburg, MD, USA) databases. The normalizing method (without the use of correction factors) was used to calculate the percentage composition of the samples from the GC peak regions.

2.6. Quality Indicators for AKO Samples

2.6.1 Fatty Acid Composition by GC-FID

A GC coupled to a flame ionization detector (FID) was used for the analysis of methyl esters. A gas chromatograph model 7890A manufactured by Agilent Technologies (Santa Clara, CA, USA), with a capillary column Omegawax (30 m × 320 m × 0.25 m) (Supelco, Bellefonte, USA). The carrier gas was helium (the flow rate was 1.4 mL/min). The temperature program was: 5 min at 70 °C, then increase with a rate of 20 °C/min up to 160 °C, ramp up with a rate of 4 °C/min up to 200 °C, and finally, with a rate of 5 °C/min up to 240 °C. The temperatures for the injector and FID were set at 240 and 250 °C, respectively. The makeup flow of helium is 50 mL/min, the flow rate of hydrogen is 50 mL/min, and the flow rate for air is 450 mL/min. Sample injection was carried out by injecting 1 μL of the sample with a split ratio of 1:100. The Supelco 37-Component FAME Mix (Supelco, Bellefonte, USA) reference standards were used for the identification of the compounds. The normalization approach (without correction factors) was used to calculate the percentage composition of the samples from the GC peak regions. From a triplicate GC-FID analysis, the component percentages were calculated as mean values.

2.6.2. Untargeted profiling by LC-MS/MS

A micro-LC liquid chromatography system of the Eksigent company (Darmstadt, Germany) was used with an automatic sampler thermostated at 5 °C and a thermostated Halo C18 column (2.7 μm, 90 Å, 0.5 × 50 mm^2) of the Eksigent company. The liquid chromatography system was connected to the Triple TOF 4600 mass spectrometer from ABSciex (Darmstadt, Germany). Experiments were performed by electrospray ionization (ESI) in negative ionization. The method for data acquisition consisted of TOF-MS full scan *m*/*z* 50–850 and some scans (IDA)-TOF-MS/MS with 40 V collision energy (CE) and 15 V collision energy spread (CES) for each candidate ion in each cycle of data acquisition. The mobile phase was made up of a gradient elution system (A: ACN/0.01% formic acid/i-pro 80/20 *v*/*v*; B: H_2O/0.01% formic acid). The elution system used started with 95% phase B for 0.5 min before progressively decreasing to 2% over the course of the following 7.5 min. The column was then re-equilibrated for 1.5 min before the second injection (flow: 55 L/min) by restoring the initial conditions (95% solvent B, 5% solvent A) within 0.1 min. For 0.5 min, these conditions persisted.

2.6.4. Total Polyphenol Content (TPC)

An eppendorf tube was used to mix 100 μL of Folin-Ciocalteu reagent with 100 μL of a sample. After 2 min, 800 μL of a 5% *w*/*v* Na_2CO_3 solution was added. After vortexing, the solutions were heated for 20 min at 40 °C. The absorbance at 740 nm was measured using a Shimadzu UV-1700 PharmaSpec Spectrophotometer from Kyoto, Japan. Using a calibration curve with standard gallic acid solutions (10–100 mg/L in methanol), results (C_{TP}) were reported as mg gallic acid equivalents (GAE) per L. Using the following equation (S1), the extraction of total polyphenol content (TPC) was expressed as mg GAE per kg dry weight (dw):

$$\text{TPC (mg GAE/kg dw)} = \frac{C_{TP} \times V}{w} \quad \text{(S1)}$$

where *V* is the volume of the extraction medium (in L) and *w* is the dry weight of the sample (in kg).

2.6.5. Total Flavonoid Content (TFC)

After diluting the sample tenfold, 100 μL of the sample was combined with 860 μL of aqueous ethanol (35% *v*/*v*), 40 μL of a reagent containing 0.5 M sodium acetate, and 5% (*w*/*v*) aluminum chloride. The mixture was left to react for 30 minutes at room temperature, followed by a measurement of the absorbance at 415 nm. Using a rutin (quercetin 3-*O*-rutinoside) calibration curve (30–300 mg/L in methanol), the concentration of flavonoid compounds (C_{TF}) was estimated. Equation (S2) was used to convert the total flavonoid content (TFC) into mg rutin equivalents (RtE) per Kg of dry weight:

$$\text{TFC (mg RtE/kg dw)} = \frac{C_{TF} \times V}{w} \quad \text{(S2)}$$

where *V* is the volume of the extraction medium (in L) and *w* is the dry weight of the sample (in kg).

2.6.6. Reducing Power (P_R, FRAP assay)

For the investigation of FRAP, 50 μL of the extract was combined with 50 μL of $FeCl_3$ solution (4 mM in 0.05 M HCl) in an Eppendorf tube, and the mixture was then incubated for 30 min at 37 °C. After adding 900 μL of TPTZ solution (1 mM in 0.05 M HCl), the absorbance at 620 nm was measured after 5 min. Equation (S3) was used to convert the results (P_R) into μmoL ascorbic acid equivalents (AAE) per kg of dry weight using an ascorbic acid calibration curve (C_{AA}, 50–500 moL/L in 0.05 M HCl):

$$P_R \text{ (μmoL AAE/kg dw)} = \frac{C_{AA} \times V}{w} \quad \text{(S3)}$$

where *V* is the volume of the extraction medium (in L) and *w* is the dry weight of the sample (in kg).

2.6.7. Antiradical Activity (A_{AR}, DPPH assay)

The absorbance at 515 nm was measured immediately after mixing ($A_{515(i)}$) and after 30 minutes ($A_{515(f)}$) of incubation of a mixture comprised of 25 µL sample and 975 µL of DPPH solution (100 µmoL/L in methanol). Equation (S4) was utilized to calculate the A_{AR} as µmoL DPPH per kg of dw:

$$A_{AR}\ (\mu\text{moL DPPH/g dw}) = \frac{\Delta A}{\varepsilon \times l \times C} \times \text{TPC} \quad \text{(S4)}$$

where $\Delta A = A_{515(i)} - A_{515(f)}$; ε (DPPH) = 11,126 × 10^{-6} μM^{-1} cm^{-1}; $C = C_{TP} \times 0.025 \times$ dilution (1/10); TPC is the total polyphenol content of the extract (mg/kg), and l is the path length (1 cm).

Supplementary Figures

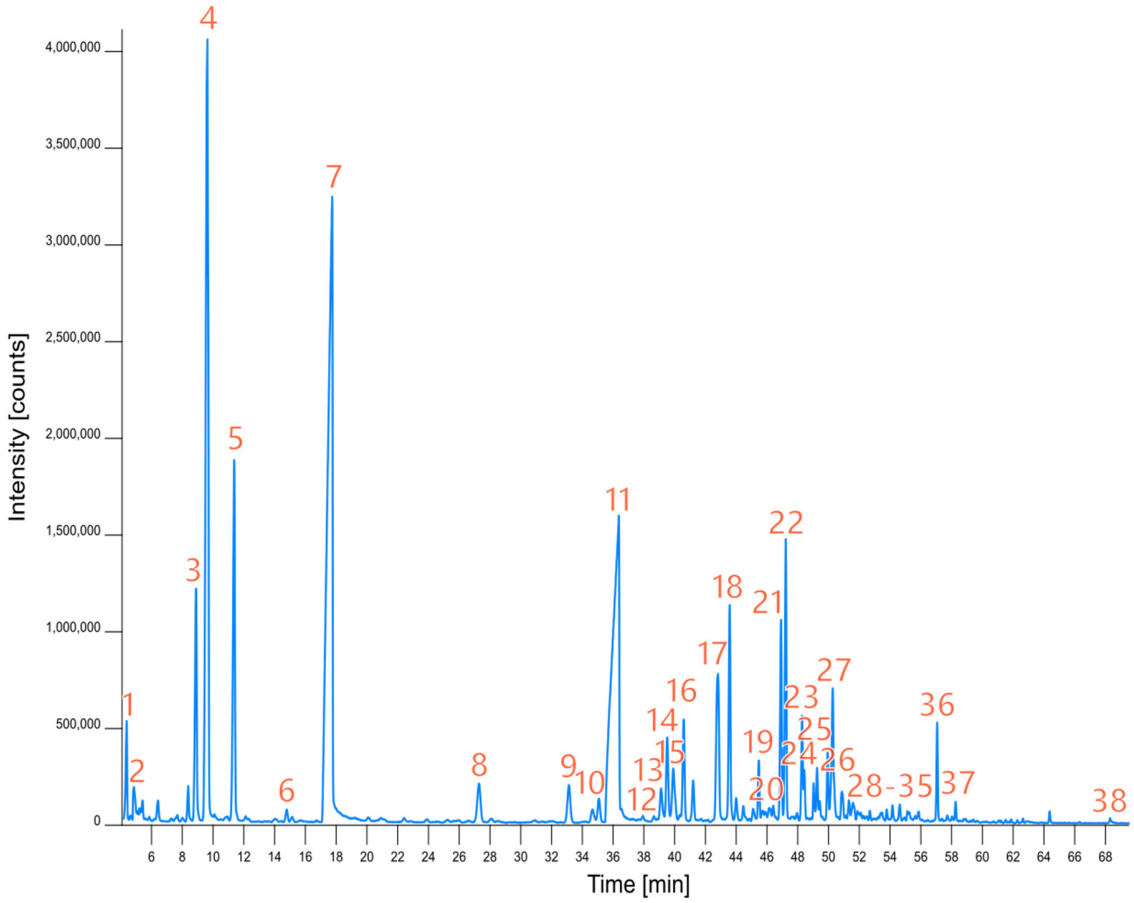

Figure S1. A TIC (total ion chromatogram) of the volatile compounds by HS-SPME/GC-MS. Peak 1: Toluene; Peak 2: 2,3-Butanediol; Peak 3: Ethylbenzene; Peak 4: 2-methyl-propanal; Peak 5: 1,3-Dimethyl-benzene; Peak 6: Nonane; Peak 7: Benzaldehyde; Peak 8: 1,2,4-Trimethyl-benzene; Peak 9: 1,2,3-Trimethyl-Benzene; Peak 10: Decane; Peak 11: Benzyl alcohol; Peak 12: Butyl-cyclohexane; Peak 13: 1,2-Diethyl-benzene; Peak 14: 1-Methyl-3-propyl-benzene; Peak 15: 1-Methyl-2-propyl-benzene; Peak 16: 1-Ethyl-3,5-dimethyl-benzene; Peak 17: 2-Ethyl-1,3-dimethyl-benzene; Peak 18: *o*-Cymene; Peak 19: 2-Ethyl-1,4-dimethyl-benzene; Peak 20: Decahydro-2-methyl-naphthalene; Peak 21: 1,2,3,5-tetramethyl-benzene; Peak 22: 1,2,4,5-tetramethyl-benzene; Peak 23: Undecane; Peak 24: 2,3-Dihydro-4-methyl-1H-indene; Peak 25: 1-Phenyl-1-butene; Peak 26: 1,2,3,4-Tetramethyl-5-methylene-1,3-cyclopentadiene; Peak 27: 1-Phenyl-1,2-propanedione; Peak 28: Benzyl acetate; Peak 29: Azulene; Peak 30: Ethyl benzoate; Peak 31: 2,4-Diethyl-1-methyl-benzene; Peak 32: 1-Methyl-4-(1-methylpropyl)-benzene; Peak 33: 6-Methyl-undecane; Peak 34: 2-Methyl-undecane; Peak 35: Benzoin; Peak 36: Dodecane; Peak 37: 2,6-Dimethyl-undecane; Peak 38: Tridecane.

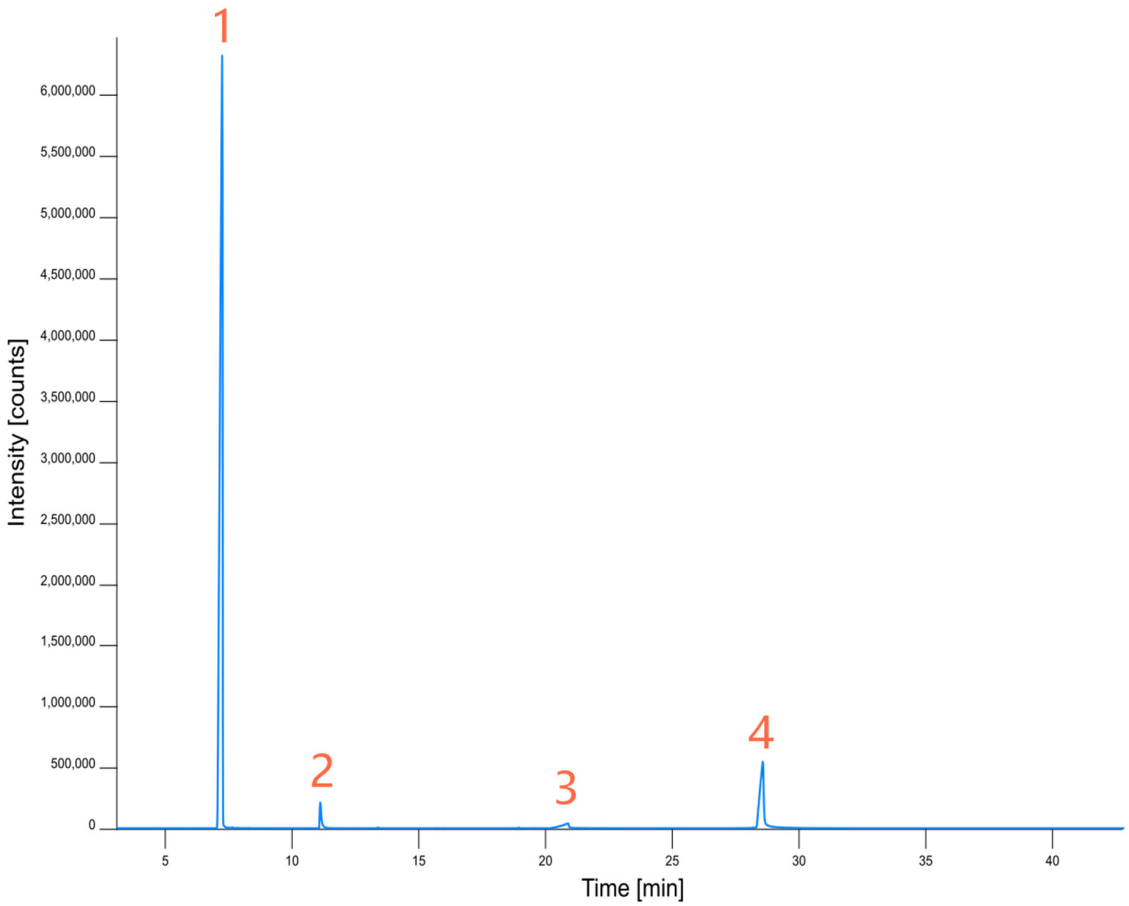

Figure S2. A TIC of the essential oil of AK by GC-MS. Peak 1: Benzaldehyde; Peak 2: Benzyl alcohol; Peak 3: Benzoic acid; Peak 4: Mandelonitrile.

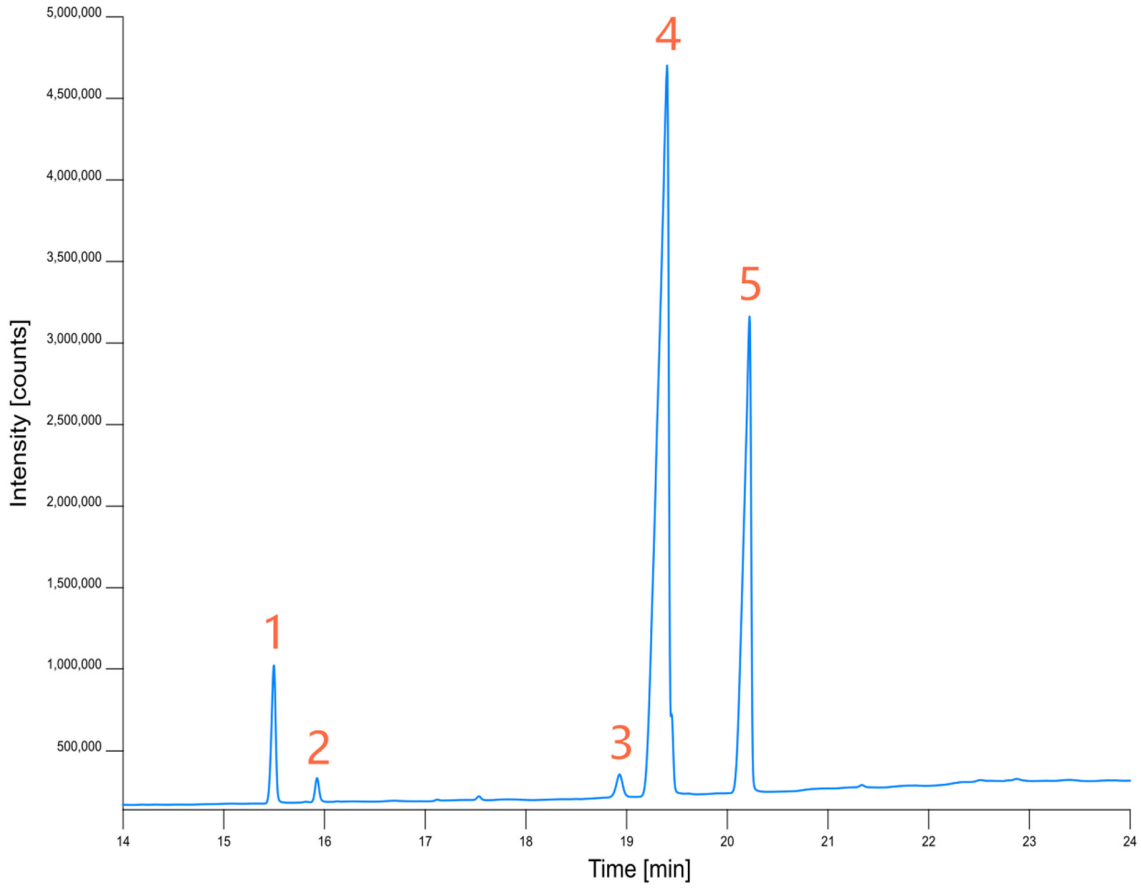

Figure S3. A GC-FID chromatogram of the fatty acid profile. Peak 1: Palmitic acid (C16:0); Peak 2: Palmitoleic acid (C16:1); Peak 3: Stearic acid (C18:0); Peak 4: Oleic acid (C18:1, ω-9); Peak 5: Linoleic acid (C18:2, ω-6).

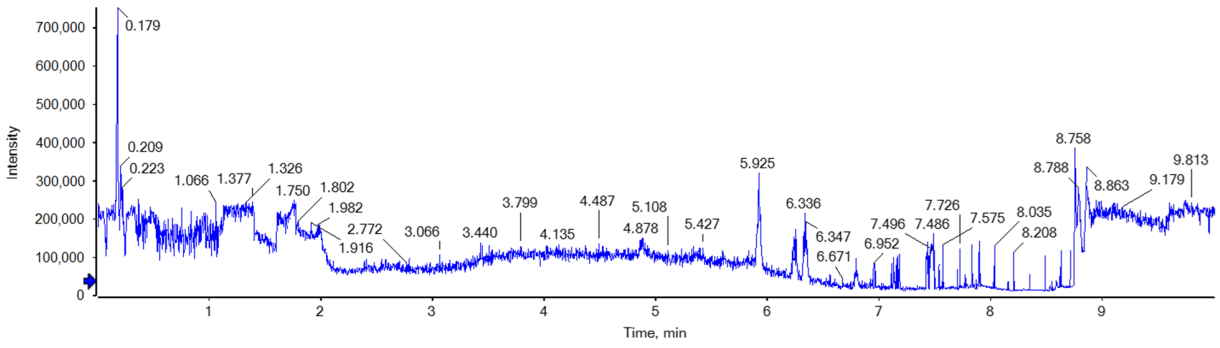

Figure S4. A TIC of the fatty acid content by LC-MS/MS.