

Cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils from the central Anatolia region of Turkey: Characterization of phytosterols, squalene, tocopherols, phenolic acids, carotenoids and fatty acid bioactive compounds

G. Akin^a, F.N. Arslan^{a,✉}, S.N. Karuk Elmas^a and I. Yilmaz^a

^aKaramanoglu Mehmetbey University, Faculty of Science, Department of Chemistry, 70100, Karaman, Turkey
✉Corresponding author: arslanfatmanur@gmail.com

Submitted: 26 June 2017; Accepted: 06 October 2017

SUMMARY: There is a growing interest in cold-pressed oils because they present high contents of bioactive compounds. These oils have the characteristic properties of seeds and are specific products of their regions. The aim of this study was to determine the compositions and contents of fatty acids, phytosterols, squalene, tocopherols, phenolic acids, carotenoids and phenolic bioactives, and the free radical scavenging as well as antioxidant activities of cold-pressed pumpkin (*Cucurbita pepo* L.) seed oils. Oil samples from raw pumpkin seeds cultivated in four different central Anatolia regions of Turkey were prepared using a laboratory screw-pressing machine. The results indicate that cold-pressed pumpkin seed oils (PSO) have excellent quality with high contents of polyunsaturated fatty acids (% Σ PUFAs) (53.60 ± 0.06 – 53.73 ± 0.05), total phytosterols (782.1 ± 9.7 – 805.2 ± 11.3 mg/100 g oil), squalene (591.3 ± 10.6 – 632.5 ± 11.4 mg/100 g oil), tocopherols (97.79 ± 0.76 – 94.29 ± 0.34 mg/100 g oil), phenolic acids (22.73 ± 0.41 – 23.98 ± 0.46 mg/100 g oil), carotenoids (6.95 ± 0.03 – 7.60 ± 0.03 mg/100 g oil), total phenolics (3.96 ± 0.13 – 5.82 ± 0.15 mg GAE/100 g), free radical-scavenging activity (5.70 ± 0.13 – 7.35 ± 0.15 mg GAE/100 g) and total antioxidant activity (26.67 ± 0.97 – 38.89 ± 1.41 mg GAE/100 g) values. Thus, this study demonstrates that the cold-pressed PSOs from the central Anatolia regions of Turkey are an excellent source of natural bioactive compounds, free of chemical contaminants and nutritious.

KEYWORDS: Antioxidant activity; Bioactive compound; Cold-pressed oil; *Cucurbita pepo* L.; Phenolic acid; Tocopherol profile

RESUMEN: Aceites de semillas de calabaza prensados en frío (*Cucurbita pepo* L.) de la región central de Anatolia en Turquía: Caracterización de fitosteroles, escualeno, tocoferoles, ácidos fenólicos, carotenoides y compuestos bioactivos de ácidos grasos. Existe un interés creciente en los aceites prensados en frío, ya que presentan altos contenidos de compuestos bioactivos. Estos aceites tienen las propiedades características de las semillas y son productos específicos de las regiones. El objetivo de este estudio fue determinar las composiciones y contenidos de ácidos grasos, fitoesteroles, escualeno, tocoferoles, ácidos fenólicos, carotenoides y bioactivos fenólicos, la actividad de eliminación de radicales libres, así como las actividades antioxidantes de aceites de semillas de calabaza prensados en frío (*Cucurbita pepo* L.). Las muestras de aceite de semillas de calabaza cultivadas en cuatro regiones diferentes de Anatolia central de Turquía se prepararon utilizando una máquina de laboratorio de prensado mediante tornillo. Los resultados indican que los aceites de semilla de calabaza prensados en frío (PSO) tienen una excelente calidad, con altos contenidos de ácidos grasos poliinsaturados (% Σ PUFA) ($53,60 \pm 0,06$ – $53,73 \pm 0,05$), fitoesteroles totales ($782,1 \pm 9,7$ – $805,2 \pm 11,3$ mg/100 g de aceite), escualeno ($591,3 \pm 10,6$ – $632,5 \pm 11,4$ mg/100 g de aceite), tocoferoles ($97,79 \pm 0,76$ – $94,29 \pm 0,34$ mg/100 g de aceite), ácidos fenólicos ($22,73 \pm 0,41$ – $23,98 \pm 0,46$ mg/100 g de aceite), carotenoides ($6,95 \pm 0,03$ – $7,60 \pm 0,03$ mg/100 g de aceite), fenólico total ($3,96 \pm 0,13$ – $5,82 \pm 0,15$ mg GAE / 100 g), actividad de eliminación de radicales libres ($5,70 \pm 0,13$ – $7,35 \pm 0,15$ mg GAE/100 g) y la actividad antioxidante total ($26,67 \pm 0,97$ – $38,89 \pm 1,41$ mg GAE/100 g) valores. Por lo tanto, el estudio demuestra que los PSO prensados en frío de las regiones centrales de Anatolia de Turquía son una excelente fuente de compuestos bioactivos naturales, químicamente libres de contaminantes y nutritivos.

PALABRAS CLAVE: Aceite prensado en frío; Ácidos fenólicos; Actividad antioxidante; Compuestos bioactivos; *Cucurbita pepo* L.; Perfil de Tocoferoles

ORCID ID: Akin G <https://orcid.org/0000-0001-7477-3186>, Arslan FN <https://orcid.org/0000-0002-5748-8268>, Karuk Elmas SN <https://orcid.org/0000-0002-1661-5902>, Yilmaz I <https://orcid.org/0000-0002-9447-3065>

Citation/Cómo citar este artículo: Akin G, Arslan FN, Karuk Elmas SN, Yilmaz I. 2107. Cold pressed pumpkin seed (*Cucurbita pepo* L.) oils from the central Anatolia region of Turkey: Characterization of phytosterols, squalene, tocopherols, phenolic acids, carotenoids and fatty acid bioactive compounds. *Grasas Aceites* 69 (1), e232. <https://doi.org/10.3989/gya.0668171>

Copyright: ©2018 CSIC. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License.

1. INTRODUCTION

Edible oils have been obtained by different technologies and utilized as food ingredients since ancient times. Recently, consumer interest in non-refined edible oils has been increasing, due to the growing evidence that among these oils, a special place belongs to so-called “cold pressed oils”, which have nutritional and health benefits (Nederal *et al.*, 2014). Cold pressing is one of the oldest oil extraction techniques in which no heat-treatment, refining, solvent extraction, or use of chemicals is included. This extraction technique does not provide as high an extraction yield as other technologies and a large amount of oil cannot be recovered from the oil source. However, it has been emphasized that cold pressing is a highly suitable technology for edible oil production enhanced with bioactive compounds. Cold pressing has a major advantage of minimizing the degradation of nutritive oil bioactives such as phytosterols, tocopherols, fatty acids, vitamins, carotenoids... etc. The produced oils are also more attractive to consumers in terms of their nutritional and functional properties. Therefore, in recent years cold pressing has become an increasingly popular technique for the production of different seed oils (Jafari *et al.*, 2012).

Nowadays, cold-pressed oil from the seeds of different pumpkin (*Cucurbita*) varieties is emerging as a commercial product in markets. Cold-pressed pumpkin seed oil (PSO) is obtained by continuous mechanical pressing of untreated, dried seeds at temperatures not exceeding 50 °C (Rabrenovic *et al.*, 2014; Nederal *et al.*, 2012). Cold-pressed PSOs as a rich source of bioactive components have been commonly used as edible oil or a potential nutraceutical product. It has been related to potential health benefits such as the prevention of prostate cancer, improvement of hypercholesterolemia, arthritis and bladder compliance, retardation of the hypertension, decreasing of bladder and urethral pressure, and alleviation of diabetes, lowering the levels of cancers, and possessing high-quality antioxidant potential (Fruhworth *et al.*, 2003; Jiao *et al.*, 2014; Rezig *et al.*, 2012). Due to their specific and positive health effects, there are several studies on pumpkin seed cultivars in different countries which assess the content of bioactive compounds. Vujasinovic *et al.*, (2010) reported the sensory properties, quality characteristics and oxidative stabilities of screw-pressed pumpkin (*Cucurbita pepo* L.) seed oils. The contents of essential fatty acids in the oil samples were determined as 30.35–42.07% for oleic and 43.68–52.15% for linoleic acid, and 0.16–0.26% for linolenic acid. Kulaitiene *et al.*, (2016) reported the characteristics of bioactive compounds and antioxidant properties of the PSOs obtained from different varieties. The study stated that oleic (22.10–36.20%) and linoleic (42.00–59.20%) unsaturated fatty acids are

dominant, and γ -tocopherol (6.14–40.70 mg/100 g oil) was identified as a major tocopherol isomer. The seeds of pumpkin varieties also exhibit high antioxidant activity. Andjelkovic *et al.*, (2010) reported the concentration of phenolic compounds and that phenolic content ranged from 2.471 to 5.093 mg GAE/100 g. Nederal *et al.*, (2014) concluded that PSOs contain an essential ω -6 fatty acid (18:2, linoleic acid) in the range of 41.51% to 51.55% of total polyunsaturated fatty acids (PUFA). Also, PSO has a high level of γ -tocopherols, phytosterols, and phospholipid bioactive compounds (Jiao *et al.*, 2014; Rezig *et al.*, 2012; Andjelkovic *et al.*, 2010).

The genetic factors, extraction technique, cultivation, climate, production and storage conditions of seed oils are critical factors for the content of bioactive compounds (Nawirska-Olszanska *et al.*, 2013). To the best of our knowledge, there is no research in the literature on the assessment of different pumpkin cultivars from the central Anatolia regions of Turkey to determine their potential for commercial and industrial applications. Therefore, the aim of this research was to determine the contents and profile of fatty acids, phytosterols, squalene, tocopherols, phenolic acids, carotenoids and total phenolic bioactives, and the free radical scavenging as well as antioxidant activities of the cold-pressed pumpkin (*Cucurbita pepo* L.) seed oils, cultivated in different central Anatolia regions of Turkey.

2. MATERIALS AND METHODS

2.1. Materials, reagents and raw pumpkin seed samples

A fatty acid methyl ester mix C14–C22 (wt. % varied conc., ampule of 100 mg), DL- α -tocopherol acetate ($\geq 96\%$), (+)- γ -tocopherol ($\geq 96\%$), rac- β -tocopherol, δ -tocopherol and tocotrienol (α -, β -, γ - and δ -) analytical standards were purchased from Sigma-Aldrich (St. Louis, USA). All chemicals, reagents and solvents used were of analytical or HPLC grade and obtained from VWR International (Poole, UK), Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany) companies. Deionized water (>18 M Ω .cm) was obtained from a Milli-Q system (Millipore, Brussels, Belgium). Standards for sterol (spinasterol, β -sitosterol, $\Delta 7,22,25$ -stigmastatrienol, $\Delta 7,25$ -stigmastadienol, $\Delta 7$ -stigmasterol, $\Delta 7$ -avenasterol), phenolic acid (arbutin, gallic acid, 5-hydroxymethyl furfural, protocatechuic acid, procyanidin, 4-hydroxybenzoic acid, catechin, chlorogenic acid, procyanidin B2, caffeic acid, syringic acid, vanillic acid, *p*-coumaric acid, ferulic acid, sinapic acid, quercetin-3-rutinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-xyloside, quercetin-3-arabinoside, quercetin-3-rhamnoside, isorhamnetin-3-glucoside, phloridzin, cinnamic acid, quercetin, phloretin)

and carotenoid bioactives, and silylating agents (pyridine, hexamethyldisilazane and chlorotrimethylsilane) were obtained from Sigma-Aldrich (St. Louis, USA). Four different cold-pressed pumpkin seed (*Cucurbita pepo* L.) oil samples were studied to determine their bioactive compound contents. Commercially available pumpkin seeds (*Cucurbita pepo* L.) harvested in the regions of central Anatolia of Turkey (Celtik-Konya, Cumra-Konya, Iceri Cumra-Konya and Polatli-Ankara), were purchased from local suppliers. Raw pumpkin seeds were of domestic origin and harvested in mid-autumn of 2015, and used for screw-pressing. The raw pumpkin seeds were stored in closed bags in the dark at 4 °C, until the extraction process.

2.2. Cold-press extraction procedure

Oils were extracted from sampled seeds according to the screw-pressing process. This pressing is performed by directly pressing raw/dried seeds onto a continuous screw press at low temperatures. A laboratory-scale screw press machine (15 kg seed.h⁻¹ capacity, single head, 2hp, 1.5 kw power) in our laboratory was used for the pressing of seed samples. 40 rpm screw rotation speeds and 40 °C ±0.05 temperatures were selected as process parameters. The temperature of the extracted oil was measured throughout the experiments with a digital thermometer. For screw-pressing, 10 kg of Celtik-Konya, 7 kg of Cumra-Konya, 10 kg of Iceri Cumra-Konya and 8 kg of Polatli-Ankara region raw pumpkin seed samples were used. At the end of the cold-pressing process, the oil and meal with oily cake were collected, and then the oil was filtered to remove suspended materials. After one day sedimentation in closed dark bottles at temperatures below 15 °C, the extracted oil was separated from the sediment by decantation, and then it was centrifuged in a centrifuge system (Sigma 2-16K, Germany) for 20 min at 10 °C. The cold-pressed oils were stored in dark green glass bottles. The bottles were kept in the refrigerator at 4 °C, until the analysis.

2.3. Determination of the fatty acid composition by GC-FID

The fatty acid composition analysis was performed in an Agilent 7890A gas chromatography system equipped with a flame ionization detector (FID) (Agilent Tech. Inc., USA). Fatty acid methyl esters (FAMES) of the oil samples were prepared according to the method described by Arslan *et al.*, (2017) with a 2N potassium hydroxide solution in methanol. Briefly, 0.1 g of the PSO was weighed into a centrifuge tube and dissolved in 10 mL of hexane. Then, 0.1 mL of 2N potassium hydroxide solution was added and the tube was vigorously shaken for 60 s. Then, the mixture was subjected to centrifugation for 5 min, at 2000 rpm. In

the last stage, the upper layer was moved to a vial for analysis. The operating conditions for FAME analysis were carried out according to Gu *et al.*, (2011) with a few modifications. Separations were performed on a HP-5MS (30m×0.25mm×0.25µm, Agilent) capillary column. The injection volume was 1 µL with a split ratio of 100:1. The inlet temperature was 230 °C. The oven temperature program was started at 60 °C, then linearly increased to 175 °C (15 °C/min) and then linearly increased to 240 °C (2 °C/min), and kept at this temperature for 10 min. Fatty acids were identified based on the retention times of a FAME mix C14:0–C22:0 standard injected under the proposed method conditions and expressed as g/100 g of totally fatty acids. The data were processed with the aid of Agilent ChemStation 2001–2010 data processor.

2.4. Determination of phytosterols and squalene by GC-MS

Phytosterol and squalene determination was performed according to the method described by Rabrenovic *et al.*, (2014). The phytosterol derivatives were analyzed using an Agilent 7890N gas chromatography system equipped with FID and 5975C MSD detector systems (Agilent Tech. Inc., USA). 7.5 mL of a cholesterol solution as an internal standard, with a concentration of 1.33 mg/mL prepared with methylene chloride, were put into 4.5 g of sample. After the evaporation of the solvent, the sample was saponified with 20 mL of a 6 M KOH aqueous solution. The blend was diluted with 30 mL of ethanol (with up to 50 mL/L diethyl ether), and then it was refluxed for 90 min at 70 °C. After saponification, 30 mL of water were added, and the unsaponifiables were extracted three times with 45 mL of petroleum ether and 45 mL of diethyl ether, respectively. The extracts were merged, washed three times with 20 mL of 0.5 M KOH and 20 mL of 50 mg/L NaCl until the neutral pH of washing water. The organic fraction was dried with Na₂SO₄ and filtrated. The phytosterol derivatives were prepared by adding 1.5 mL of drypyridine, 0.2 mL hexamethyldisilazane, and 0.1 mL chlorotrimethylsilane in a vial and heated to 80 °C for 30 min. The derivatized parts were transferred into a vial and after derivatization they were analyzed by GC-MS within 6 h. A mixture of derivized phytosterol standard solutions was also prepared by the same procedure.

GC-MS separations were performed on a HP-5MS (30m×0.25mm×0.25µm, Agilent) capillary GC column. The flow rate of carrier gas (helium) was 1.5 mL/min and the injected sample volume was of 1 µL in the splitless mode. The temperature program was as follows: a gradient of 3 °C/min from 60 °C to 300 °C, and held at this temperature for 10 min. MS data were obtained in the EI mode with a scan range of 30e550 m/z; the source temperature

was 230 °C, quadrupole temperature was 150 °C and hold-up time was 3 min.

2.5. Determination of the tocol profile by HPLC-FLD

The tocol (tocopherol and tocotrienol) profiles of the PSOs were identified using a normal-phase liquid chromatographic (NP-HPLC) method (Arslan *et al.*, 2016). An Agilent 1200 series HPLC system (Agilent Technologies Inc., USA) was equipped with a G1321C model fluorescence detector (FLD) and G1316A model column compartment set at 20 °C. Separation was achieved on a LiChrospher 100Å diol column (250×4.0 mm id., 5µm, Teknokroma, Barcelona) equipped with a guard column (75×4.0 mm i.d., 5µm, Teknokroma, Barcelona). The data were recorded using an Agilent ChemStation 2001–2010 data processor. Before HPLC analysis, one gram of oil was weighed, then dissolved in 10 mL hexane, centrifuged and filtered through a PTFE syringe filter (0.45mm). 5 µL of the sample were injected and a mixture of hexane:2-propanol (99.4:0.6, v/v) was used as a mobile phase under isocratic conditions with a flow rate of 0.7 mL·min⁻¹. Detection of tocol isomers was performed at λ_{exc} = 295 nm and λ_{em} = 330 nm by FLD. Commercial standards of all of the tocols were used for calibration, and curves ranging from 0.1 to 3.0 mg·mL⁻¹ were linear for all tocols ($R^2 > 0.99$). Contents of each tocol were expressed in mg·100g⁻¹ oil.

2.6. Determination of phenolic acid profile by HPLC-MS/MS

2.6.1. Phenolic acid extraction.

The phenolic acids were extracted according to the method of Mateos *et al.*, (2001) with some modifications. 2.500±0.001 g of PSO sample were weighed, and dissolved in 6 mL of hexane. A diol-bonded phase cartridge column (Grace; ps 50 mm, pore size 60 Å, not end-capped, 500 mg bed size and 4 mL column size) was conditioned by 6 mL of methanol and 6 mL of hexane, respectively. The oil solution was applied to the cartridge, and the solvent was pulled through by releasing the sample onto the column phase. The sample vessel was washed with 6 mL portions of hexane, followed by elution of the solvent which was then discarded. After that, the sample vessel was washed with 4 mL of hexane/ethyl acetate (90:10, v/v), which were removed from the cartridge and discarded. Finally, the phenolic compounds were eluted from the sorbent with 10 mL methanol, and the solvent was evaporated under vacuum. The residue was extracted with 500 µL of methanol: water (50:50, v/v) at 40 °C. An aliquot of the final extract (20 µL) was filtered through a

PTFE syringe filter (0.45mm) and was injected into the HPLC system.

2.6.2. Chromatographic analysis of phenolic acids.

A Chromatographic analysis was performed using an Agilent HPLC system equipped with a G4212B model photodiode array detector (DAD) and the column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. A Lichrospher 100RP-18 column (250 mm×4.0 mm, 5 µm), maintained at 40 °C was used. The injection was 10 µL of the extract (20 µL) obtained from the previous step. Elution was performed at a flow rate of 0.4 mL/min, using solvent A (1% HAc in ultra-pure water) and solvent B (1% HAc in methanol) as the mobile phase. The solvent gradient changed according to the following conditions: from 10% to 20% solvent B in 4 min, and a second step gradient from 20% to 100% solvent B in 20 min. Then, it was kept for 6 min with 100% solvent B, finally, the elution was obtained from 100% to 20% solvent B for 6 min (Rezig *et al.*, 2012). A stock solution of standards at a concentration 1 µg/µL each was prepared and the standards were injected into the system at various concentrations for calibration. Detection of the individual compounds was achieved by comparing retention time and SRM analyses of the sample peaks with that of the authorized pure individual standards. The parameters were used throughout the MS; the capillary voltage was set to 1.6 kV, the drying temperature to 350 °C, the nebulizer pressure to 40 psi, and the drying gas flow to 10 L/min. The maximum accumulation time was 50 ms, and the scan speed was 26,000 m Z⁻¹s⁻¹ even as the fragmentation moment was 30 ms. Phenolic compounds were identified using a combination of HPLC with a DAD and ESI-LC-MS detection on the basis of their ultraviolet spectra at 280 nm, mass spectra and by comparing the spectra with those of obtainable authentic standards (Rezig *et al.*, 2012).

2.7. Determination of of carotenoid composition by HPLC-DAD-ESI/MS/MS

The carotenoid composition analysis was performed according to a previously described method with some modifications (Parry *et al.*, 2006). One mL of PSO was dissolved in 160 mL of methanol:tetrahydrofuran (THF) (50:50, v/v), and the carotenoid composition was determined using a HPLC-DAD-ESI/MS/MS (high performance liquid chromatography-diode array detector/ electrospray ionization/ tandem mass spectrometry) system. An Agilent 1100 HPLC system (Agilent Tech. Inc., USA) and TSQ Quantumtandem mass spectrometry (Thermo-Finnigan, San Jose, CA) was equipped with

an ESI interface system and used for the analyses of β -carotene, lutein, cryptoxanthin, and zeaxanthin. The separations were performed using a Zorbax-SB C18 HPLC column (50×1.0 mm i.d., 3.5 μ m) (Agilent Tech. Inc., USA). A mobile phase of solvent A (ultra-pure water) and solvent B [methanol: acetonitrile:2-propanol (54:44:2, v/v/v)] was used with a gradient program as follows; the gradient was linear from 50% to 99% of solvent B, and the flow rate was increased from 0.2 to 0.27 mL/min in the first 10 min, and 99% of solvent B and flow rate of 0.27 mL/min for 10 min. Prior to the next injection, the column was re-equilibrated for 10 min with 50% of solvent B. The quantification of carotenoids was determined using the total ion counts compared to external standards of the individual carotenoids. The wavelength of DAD detection was set at 440 nm. The identification of carotenoids was achieved by comparing the retention time and SRM analysis of the sample peaks with that of the commercial carotenoid standards. The TSQ Quantum was operated in the positive-ion mode under the following conditions: nitrogen (>99.7 %) was used for sheath gas and auxiliary gas at pressures of 30 psi and 5 units, respectively. The temperature of the heated capillary was kept at 300 °C, and the spray voltage of ESI was set at 4.5 kV. A collision-induced dissociation (CID) was performed using argon as the collision gas at the pressure regulated to more than 1.0 mTorr, and the applied collision offset energy was set to - 45 eV.

2.8. Determination of the total phenolic compounds, free radical scavenging activity and antioxidant activity

Total phenolic compounds (TPCs) were extracted from the cold-pressed PSOs according to the method proposed by Feng-Lin *et al.*, (2010). Briefly, 200 μ L of the extract obtained from the oil sample and gallic acid (GA) standards (concentration range between 0.05 to 3 mg GAE), were transferred into centrifuge tubes. Then, 1 mL of the Folin-Ciocalteu (FC) reagent and 2 mL of sodium carbonate (7.5%, w/v) were added, diluted with ultra-pure water to 7 mL and mixed thoroughly. After shaking, the mixtures were then allowed to stand for 2 hours in the dark before measuring the absorbance in Lambda-25 UV-Vis spectrophotometer (PerkinElmer Inc., Waltham, Massachusetts, U.S.A.) at 765 nm. The measurements were performed in triplicate and the results were expressed as milligrams of gallic acid equivalents (GAEs) per 100 grams of sample. The contents of TPCs in the studied oils is calculated and reported by using calibration graph.

The radical scavenging activity of PSO extract was determined according to the method described by (Molyneux, 2004). In brief, 500 μ L of the extract obtained from the oil sample and GA standards (0.02–0.14 mg GAE) were transferred into

a centrifuge tubes. Then, 3 mL of 6×10^{-5} M solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) were added and mixed thoroughly. The absorbance at 517 nm was measured after reaction at room temperature in the dark for 30 min. A control sample, without oil, was prepared and measured daily. The DPPH concentration in the reaction medium was calculated from the following calibration curve, determined by the linear regression:

$$A_{517} = 0.1154 \cdot [\text{DPPH}] + 0.0231 \quad (R^2 = 0.9933)$$

The radical scavenging activity toward DPPH was expressed as the % reduction in DPPH concentration by the constituents of the oils:

$$\% [\text{DPPH}]_{\text{red}} = 100 \times (1 - [\text{DPPH}]_{30} / [\text{DPPH}]_0)$$

where $[\text{DPPH}]_0$ and $[\text{DPPH}]_{30}$ were the concentrations of DPPH in the control sample ($t=0$) and in the test mixture after 30 min reaction, respectively (Molyneux, 2004).

In last stage the antioxidant activities of the oil samples were determined according to the method of (Prieto *et al.*, 1999). 300 μ L of the extract obtained from the oil sample and GA standards (0.2–1.0 mg GAE), were transferred into centrifuge tubes. Three mL of reagent solution (mixture of 25 mL 0.6M H_2SO_4 , 25 mL 28 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 25 mL 4 mM ammonium molybdate) were added. The tubes were incubated at 95 °C for 90 min. After the mixtures had cooled to room temperature, the absorbance was measured at 695 nm. All analyses were performed in triplicate and the results were expressed as milligrams of GAEs per 100 grams of sample.

2.9. Statistical analysis

All analyses were carried out in triplicate and data were expressed as the mean \pm standard deviation. All statistical analyses were performed by means of Excel (Microsoft, 2007) and OriginPro8 (OriginLab, USA) databases.

3. RESULTS AND DISCUSSION

3.1. Fatty acid profiles

Table 1 presents an overview of the profile and content of fatty acids in the cold-pressed PSO samples. As can be seen in Table 1, the studied PSOs belong to the oleic-linoleic acid type of oil. A total of fourteen cis fatty acids and three trans fatty acids (TFA) in PSO was determined. Fourteen main components; seven saturated fatty acids (SFA), four monounsaturated fatty acids (MUFAs) and three polyunsaturated fatty acids (PUFAs) were identified. The main fatty acids in the studied oils were identified in the order of; linoleic (C18:2), oleic (C18:1), palmitic (C16:0),

and stearic acid (C18:0). The content of polyunsaturated linoleic acid ranged from $53.19 \pm 0.04\%$ to $53.27 \pm 0.04\%$ and was followed by oleic acid which was also present in high contents varying from $27.52 \pm 0.02\%$ up to $27.59 \pm 0.02\%$. As reported in previous studies, the high content of linoleic acid is an important nutritional aspect of oil because it is an essential fatty acid for humans and it is involved in the building of cellular membranes, vitamin D, and a variety of hormones (Jiao *et al.*, 2014). The presence of high amounts of linoleic acid also suggested that the analyzed PSOs could be used as a good source of essential fatty acids. High oleic acid contents, detected in all the analyzed oil samples, is also favorable from a cardio-protective and nutritional viewpoint (Fruhwrth *et al.*, 2007).

In all the pumpkin seed oils, palmitic and stearic acids were detected in relatively high amounts ranging from $11.90 \pm 0.01\%$ to $11.99 \pm 0.01\%$ and $5.26 \pm 0.01\%$ to $5.29 \pm 0.01\%$, respectively. Trace amount of the fatty acids were also identified and these data are similar to those reported by Kostadinovic Velickovska *et al.*, 2015. On the other hand, there are insignificant differences in fatty acid profiles between these results and

those reported by Nederal *et al.*, (2014). The differences in profile and amount of certain fatty acids depended on the origin of specific pumpkin (*Cucurbita pepo* L.) seed oil cultivars, climatic conditions, and post-harvest management (Rabrenovic *et al.*, 2014).

The nutritional values of the studied PSOs were also calculated by their total MUFA (Σ MUFA), total PUFA (Σ PUFAs) and total SFA (Σ SFA) contents (Jiao *et al.*, 2014; Rezig *et al.*, 2012). To the best of our knowledge, a higher intake of MUFAs may emerge as health-promoting food choices on the basis of both cardiovascular benefits and their capacity to decrease the risk of opportunistic diseases. For this reason, the vegetable oil industry has focused attention on vegetable oils with high oleic FAs or MUFAs (Jafari *et al.*, 2012). Herein, the detected amounts of Σ MUFA ranged from $27.77 \pm 0.04\%$ to $27.86 \pm 0.05\%$ (Table 1). PUFAs are more susceptible to oxidation reactions, while SFAs are more stable, so the ratio of Σ PUFA/ Σ SFA is generally defined as the best indicator for verifying the degree of oil deterioration and the high ratios signified that the samples were nutritionally valuable. Σ PUFA/ Σ SFA values have also favorable nutritional implications and a positive physiological effect in the

TABLE 1. Fatty acid profile of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils, g/100 g of totally fatty acids.

Fatty acids	Celtik PSO -Konya	Cumra PSO -Konya	I. Cumra PSO -Konya	Polath PSO -Ankara
14:0 (myristic acid)	0.08 ± 0.01	0.05 ± 0.001	0.07 ± 0.002	0.06 ± 0.002
16:0 (palmitic acid)	11.95 ± 0.01	11.99 ± 0.01	11.90 ± 0.01	11.97 ± 0.01
16:1 <i>trans</i>	0.01 ± 0.001	0.02 ± 0.001	0.01 ± 0.001	0.01 ± 0.001
16:1 (palmitoleic acid)	0.11 ± 0.01	0.10 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
17:0 (heptadecanoic acid)	0.04 ± 0.001	0.01 ± 0.001	0.02 ± 0.001	0.03 ± 0.001
17:1 (heptadecanoic acid)	0.03 ± 0.001	0.04 ± 0.002	0.01 ± 0.001	0.02 ± 0.001
18:0 (stearic acid)	5.26 ± 0.01	5.29 ± 0.01	5.27 ± 0.012	5.28 ± 0.01
18:1 (ω 9)(oleic acid)	27.55 ± 0.02	27.59 ± 0.02	27.52 ± 0.02	27.56 ± 0.02
18:2 <i>trans</i>	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.010 ± 0.001
18:2 (ω 6) (linoleic acid)	53.21 ± 0.03	53.25 ± 0.04	53.27 ± 0.04	53.19 ± 0.04
18:3 <i>trans</i>	0.35 ± 0.01	0.37 ± 0.01	0.31 ± 0.01	0.34 ± 0.01
18:3 (ω 3) (linolenic acid)	0.40 ± 0.01	0.42 ± 0.01	0.44 ± 0.01	0.39 ± 0.01
20:1 (eicosenoic acid)	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.14 ± 0.01
20:2 (eicosadienoic acid)	0.02 ± 0.001	0.01 ± 0.001	0.02 ± 0.001	0.02 ± 0.001
22:0 (behenic acid)	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.14 ± 0.01
23:0 (tricosylic acid)	0.06 ± 0.003	0.04 ± 0.001	0.05 ± 0.003	0.04 ± 0.002
24:0 (lignoceric acid)	0.08 ± 0.002	0.07 ± 0.002	0.07 ± 0.003	0.09 ± 0.004
Σ SFA	17.57 ± 0.04	17.58 ± 0.03	17.49 ± 0.02	17.61 ± 0.03
Σ MUFA	27.80 ± 0.03	27.83 ± 0.02	27.77 ± 0.04	27.86 ± 0.05
Σ PUFA	53.63 ± 0.05	53.68 ± 0.06	53.73 ± 0.05	53.60 ± 0.06
<i>trans</i> FAs	0.37 ± 0.01	0.40 ± 0.01	0.33 ± 0.01	0.36 ± 0.01

Results are reported as mean value \pm standard deviation of three replicate analyses ($n = 3$)

Abbreviations: nd; not determined, SFA; saturated fatty acids, MUFA; mono unsaturated fatty acids, PUFA; polyunsaturated fatty acids, ω ; omega, PSO; pumpkin seed oil.

prevention of both heart and cancer diseases (Arslan *et al.*, 2017; Omah *et al.*, 2000). Herein, the detected amounts of Σ PUFA ranged from $53.60 \pm 0.06\%$ to $53.73 \pm 0.05\%$, and Σ SFA ranged from $17.49 \pm 0.02\%$ to $17.61 \pm 0.03\%$ (Table 1). The detected ratios of Σ PUFA/ Σ SFA ranged from 3.05 ± 0.05 , 3.05 ± 0.05 , 3.07 ± 0.04 , and 3.04 ± 0.05 , respectively. Trans fatty acid contents were also detected and reported as $0.37 \pm 0.01\%$, $0.40 \pm 0.01\%$, $0.33 \pm 0.01\%$ and $0.36 \pm 0.01\%$ for the PSO samples of Celtik-Konya, Cumra-Konya, Iceri Cumra-Konya and Polatli-Ankara, respectively. As expected, cold-pressed PSOs contain much less TFAs on average than refined seed oils (Jafari *et al.*, 2012). Therefore, the fatty acid profiles of PSO can be an indicator of its stability and nutritional quality.

3.2. Phytosterols and squalene profiles

Phytosterols are of interest due to their cholesterol-lowering and physiological effects on human health. The quantities of phytosterols in vegetable oils have been used for the classification and assessment of their quality (Rabrenovic *et al.*, 2014; Kostadinovic Velickovska *et al.*, 2015). The cultivation and environmental conditions influence the concentration of phytosterols. Based on the position of the double bond in the ring, phytosterols could be classified as $\Delta 5$ - and $\Delta 7$ -sterols. Most species contain dominant $\Delta 5$ -sterols, while $\Delta 7$ -sterols are characteristic of only a few plant species and are specific bioactive compounds to pumpkin seed (*Cucurbitaceae*) oil (Rabrenovic *et al.*, 2014). The phytosterol profile of the PSOs is given in Table 2 and regression analyses were also performed. There was a good correlation between the concentration of phytosterol bioactive compounds and detector intensities. Regression analysis between $R^2=0.9740$ and 0.9990 values were indicated as satisfactory correlations for phytosterols. As can be seen in Table 2, $\Delta 7,22,25$ -stigmastatrienol was the major

phytosterol in the seed oils ranging from 28.3 ± 0.1 to 31.6 ± 0.3 mg/100 g of the total phytosterol contents. Spinasterol together with β -sitosterol were the second-most abundant phytosterol, showing 42.4 ± 2.5 - 47.20 ± 2.1 g/100 g of total phytosterols. They were followed by $\Delta 7$ -stigmasterol with 15.1 ± 0.1 - 17.1 ± 0.6 g/100 g, $\Delta 7,25$ -stigmastadienol with 4.9 ± 0.2 - 5.9 ± 0.4 g/100 g, and $\Delta 7$ -avenasterol with 3.20 ± 0.1 - 4.00 ± 0.3 g/100 g of total phytosterols. Significantly higher contents of total phytosterols were determined and total phytosterol contents for the PSO samples which ranged from 782.1 ± 9.7 to 805.2 ± 11.3 mg/100 g oil. Total phytosterol content in the studied samples is significantly higher than the data reported in the literature (Rabrenovic *et al.*, 2014; Kostadinovic Velickovska *et al.*, 2015). Thus, the high total phytosterol contents in the PSO samples from different regions of Turkey may also be related to the species of pumpkin, although the techniques used for oil extraction from seeds and the isolation procedure of phytosterols from oil may also considerably influence data.

Squalene is a natural acyclic compound produced by plant sources (mainly vegetable oils) and shark liver oils. This bioactive compound has been recommended to be an essential part of the human diet as it may be an inhibitive component that protects humans from several diseases (Ryan *et al.*, 2007). The obtained results for the content of squalene in cold-pressed PSO samples are given in Table 2. The squalene content in the samples ranged from 591.3 ± 10.6 to 632.5 ± 11.4 mg/100 g oil. These results are in good agreement with previously reported data for seed oils from different pumpkin cultivars (Rabrenovic *et al.*, 2014). Differences among the contents of squalene could be attributed to the applied temperature, oil extraction procedure, and seed origin. Thus, the high content of squalene compounds contributes to the significant nutritional and therapeutic value of the cold-pressed PSOs from different regions of Turkey.

TABLE 2. Composition of phytosterols (g/100 g of total phytosterols), total phytosterol contents (mg/100 g of oil) and squalene contents (mg/100 g of oil) of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils.

	Squalene	Spinasterol + β -sitosterol*	$\Delta 7,22,25$ - stigmastatrienol	$\Delta 7,25$ - stigmastadienol	$\Delta 7$ - stigmasterol	$\Delta 7$ - avenasterol	Total phytosterols
Celtik PSO-Konya	632.5 ± 11.4	42.4 ± 2.5	31.6 ± 0.3	5.7 ± 0.1	16.3 ± 0.4	4.0 ± 0.3	795.1 ± 10.1
Cumra PSO-Konya	597.2 ± 13.5	46.1 ± 2.8	28.7 ± 0.7	4.9 ± 0.2	17.1 ± 0.6	3.2 ± 0.1	805.2 ± 11.3
Iceri Cumra PSO-Konya	602.1 ± 16.1	44.9 ± 1.7	30.8 ± 0.5	5.3 ± 0.2	15.2 ± 0.5	3.8 ± 0.1	782.1 ± 9.7
Polatli PSO-Ankara	591.3 ± 10.6	47.20 ± 2.1	28.3 ± 0.1	5.9 ± 0.4	15.1 ± 0.1	3.5 ± 0.2	790.7 ± 12.4

* Predictable ratio spinasterol/ β -sitosterol 1:1.3 according to intensity of ion currents of molecular ions. Results are reported as mean value \pm standard deviation of three replicate analyses ($n = 3$)
Abbreviations: nd, not determined; PSO, pumpkin seed oil.

3.3. Tocol profiles

Tocols are very significant non-glyceride ingredients of vegetable oils. Tocols represent a family of tocopherols (α -, β -, γ -, and δ -T) and tocotrienols (α -, β -, γ -, and δ -TT) and natural lipophilic antioxidants mainly found in vegetable oils. They are believed to be primary chain-breaking antioxidants in free radical chain reactions or in converting lipid radicals to more stable products, therefore improving the antioxidant potentials of oils. Due to their role in the protection against the oxidative deterioration of PUFAs, the content and profile of tocol isomers in vegetable oil are exceptionally important. Tocols have also been associated with the reduction of heart disease, delay of Alzheimer and prevention of cancer diseases (Mariod *et al.*, 2009). Table 3 presents the tocol profile and content of cold pressed PSOs from central Anatolia region of Turkey. As can be seen in Table 3, significantly high amount of tocols were determined and total contents for the studied oil samples ranged from 94.29 ± 0.34 mg/100 g oil to 97.79 ± 0.76 mg/100 g oil. The amounts of tocols are higher than other reports and, in mentioned studies the maximum contents of tocols were detected as 70.90 mg/100 g oil, 35.10 mg/100 g oil, and 77.05 mg/100 g oil in pumpkin (*Cucurbita pepo* L.) seed oils, respectively (Stevenson *et al.*, 2007; Nederal *et al.*, 2012; Siger *et al.*, 2008). The contents of these natural antioxidants in vegetable oils depends on a number of factors, such as variety characteristics, the kind of oil, extraction process, and they are possibly managed by the content of unsaturated FAs. The content of tocols in oils is also significantly affected by the processing conditions of the oil production (Rabrenovic *et al.*, 2014).

In all the analyzed PSO samples, α -T, β -T, γ -T, β -TT and γ -TT isomers were identified, and α -TT isomers were detected only in Celtik-Konya and Polatlı-Ankara PSO samples (Figure 1). As is well known, the γ - and δ -tocol isomers possess a much higher antioxidant activity than the α - and β - isomers; however, α -tocol is considered to have a higher vitamin effectiveness than other tocol isomers

(Nederal *et al.*, 2012). As can be seen in Figure 1, β -T was identified as the major tocol isomer in all the varieties, and its contents ranged from 83.00 ± 0.38 to 86.23 ± 0.87 mg/100 g oil, while the other tocol isomers were present in relatively lower quantities. The amounts of α -tocopherol detected in the PSOs ranged from 3.66 ± 0.03 to 4.39 ± 0.04 mg/100 g oil. Thus, the obtained results demonstrated that the studied PSO samples were good sources of α -, β - and γ -tocols.

3.4. Phenolic acid and carotenoid profiles

Phenolic acids are known as minor antioxidant constituents, and play a determinant role due to their characteristics such as flavor, quality and resistance against the oxidation process (Nyam *et al.*, 2009). Common vegetable oils contain different kinds and contents of phenolic acids, and data about the phenolics in cold-pressed oils are very rare in the literature. In this research, six phenolic acids; protocatechuic acid, caffeic acid, syringic acid, vanillic acid, *p*-coumaric acid, and ferulic acid were identified. The chromatogram obtained from the analysis of a standard mixture of 27 phenolic acids at 280 nm, is illustrated in Figure 2. The concentration of the detected phenolic acids in the cold-pressed PSOs is given in Table 4 and regression analyses were also performed. There was a good correlation between the concentration of phenolic bioactive compounds and detector intensities. Values for the regression analysis between $R^2=0.9510$ and 0.9950 are indicated as satisfactory correlations for phenolics.

As can be seen in Figure 2, the proposed method permits the baseline separation for all components including quercetin glycosides, hydroxybenzoic acids, hydroxycinnamic acids, flavanol monomers, dimers, and phloridzin. The obtained results showed that syringic acid (7.62 ± 0.13 - 8.03 ± 0.16 mg/100 g oil) was the predominant acid in all the samples, followed by ferulic acid (4.72 ± 0.22 - 5.17 ± 0.38 mg/100 g oil). Cumra-Konya cold-pressed PSO sample had the highest amount of phenolic acids (23.98 ± 0.46 mg/100 g

TABLE 3. Tocol profile of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils, mg/ 100 g oil.

	α -T	β -T	γ -T	δ -T	α -TT	β -TT	γ -TT	δ -TT	total tocol
Celtik PSO-Konya	3.96 ± 0.12	85.88 ± 0.71	3.23 ± 0.11	nd	0.35 ± 0.10	0.21 ± 0.02	2.82 ± 0.07	nd	96.45 ± 0.53
Cumra PSO-Konya	4.39 ± 0.04	86.23 ± 0.87	4.27 ± 0.11	nd	nd	0.21 ± 0.05	2.69 ± 0.03	nd	97.79 ± 0.76
Iceri Cumra PSO-Konya	3.66 ± 0.03	83.00 ± 0.38	3.43 ± 0.02	nd	nd	0.78 ± 0.02	3.42 ± 0.05	nd	94.29 ± 0.34
Polatlı PSO-Ankara	4.07 ± 0.02	85.35 ± 0.22	3.44 ± 0.01	nd	0.43 ± 0.04	0.27 ± 0.01	2.88 ± 0.01	nd	96.44 ± 0.16

Results are reported as mean value \pm standard deviation of three replicate analyses ($n = 3$)

Abbreviations: nd, not determined; T, tocopherol; TT, tocotrienol; α , alpha; β , beta; γ , gamma; δ , sigma; PSO, pumpkin seed oil.

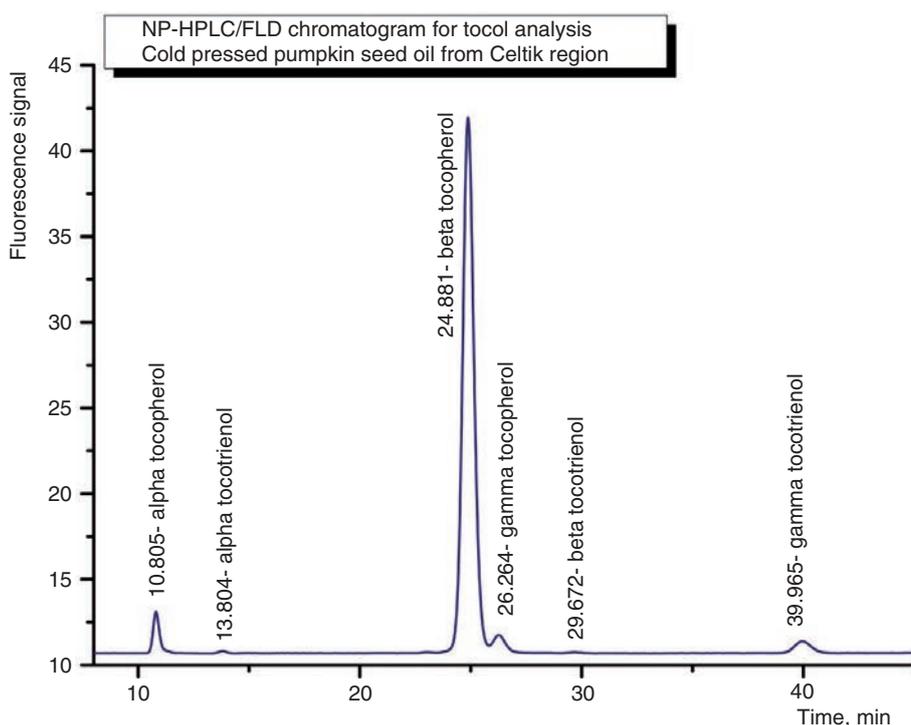


FIGURE 1. NP-HPLC/FLD chromatogram of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oil from the Cumra region for tocol analysis.

oil) followed by Iceri Cumra-Konya cold-pressed PSO sample (23.66 ± 0.34 mg/100 g oil). Minor amounts of phenolic acids such as protocatechuic acid, caffeic acid, vanillic acid and *p*-coumaric acids were also detected. The differences in the amounts of phenolic compounds may occur due to differences in the extraction methods, origin of seeds, environmental conditions and the treatments applied to the seeds before extraction (Allothman *et al.*, 2009).

The carotenoid composition (β -carotene, lutein, zeaxanthin and cryptoxanthin) of the cold-pressed PSOs is also given in Table 4. Zeaxanthin was the major carotenoid pigment present in all the studied PSOs with a range of 2.65 ± 0.05 – 2.91 ± 0.03 mg/100 g oil. The Cumra-Konya PSO sample contained the highest amount of total carotenoids (7.60 ± 0.03 mg/100 g oil), followed by the Iceri cumra-Konya (7.21 ± 0.05 mg/100 g oil) PSO samples. This PSOs also had the highest β -carotene content of 0.54 ± 0.02 to 0.60 ± 0.02 mg/100 g oil, among all the studied oil samples which was much higher than that observed in cold-pressed seed oils (0.01–0.24 mg/100 g), and also much higher than that of refined oils (0.01–0.12 mg/100 g) (Parry *et al.*, 2006). The obtained results demonstrated that different contents and profiles of carotenoids are influenced by the genetics and phase of seed maturation, and also by oil extraction and storage conditions. Thus, these data suggest that the studied cold-pressed PSOs may serve as dietary sources of carotenoids, especially zeaxanthin (Parry *et al.*, 2006).

3.5. Antioxidant activity data

Phenolic compounds which have unique chemical characteristics posing low-energy radicals through stable hybrids are thought to be good antioxidants for foodstuffs. They have also proven to possess important roles in their quality and nutritional values. Natural oils contain more polar compounds that have mainly phenolic characteristics. These phenolics are secondary components and represent only a minor fraction of the oil. Phenolic compounds may also exert high biological activity far beyond their antioxidant properties, so the determination of phenolic compounds in vegetable oil is of great interest (Latif *et al.*, 2011). As can be seen in Table 5, the content of total phenolic compounds (TPCs) in cold-pressed PSOs is in the range of 3.96 ± 0.13 – 5.82 ± 0.15 mg GAE/100 g. The content of TPCs are in the following order: 4.32 ± 0.09 mg GAE/100 g, 5.82 ± 0.15 mg GAE/100 g, 3.96 ± 0.13 mg GAE/100 g, 4.32 ± 0.12 mg GAE/100 g for PSOs from Celtik-Konya, Cumra-Konya, Iceri Cumra-Konya and Polatli-Ankara, respectively. Values from the regression analyses with $R^2=0.9944$, 0.9933 and 0.9972 are indicated as satisfactory correlations for total phenolic compounds, free radical-scavenging and antioxidant activity analyses, respectively. There was also a directly relative correlation between TPCs and total tocol content. It is clear that Cumra-Konya PSO is the richest cold-pressed oil in terms of both the TPCs and tocol content (Tables 3

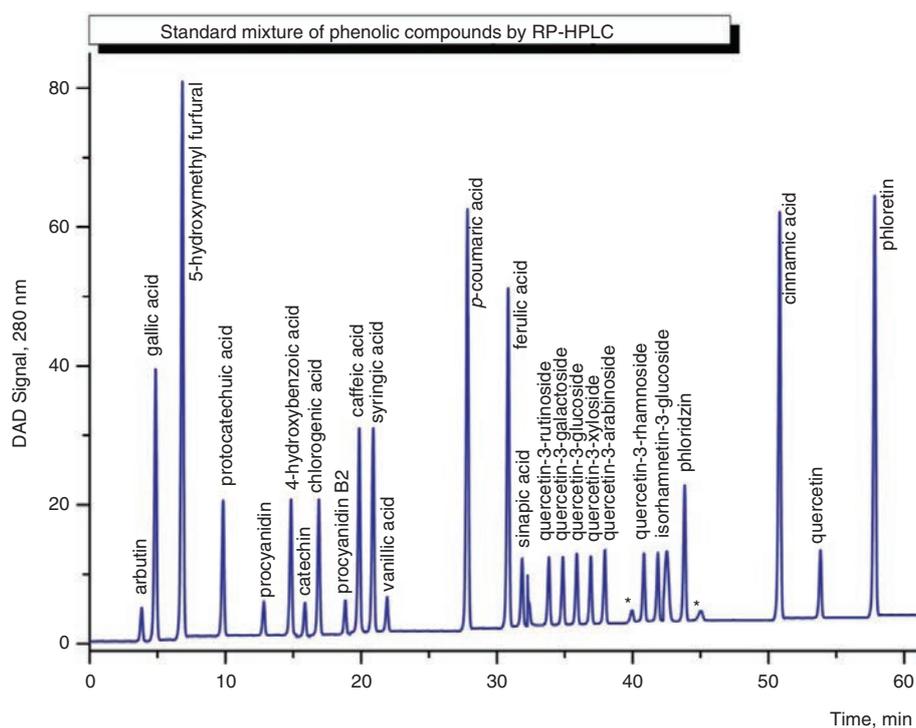


FIGURE 2. RP-HPLC/DAD chromatogram of the standard mixture of phenolic acids.

TABLE 4. Phenolic acid and carotenoid compositions of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils.

	Celtik PSO-Konya	Cumra PSO-Konya	Iceri Cumra PSO-Konya	Polath PSO-Ankara
Protocatechuic acid	1.78 ±0.25	2.07 ±0.18	1.84 ±0.22	1.56 ±0.24
Caffeic acid	3.75 ±0.31	3.68 ±0.60	3.83 ±0.44	3.41 ±0.33
Syringic acid	8.03 ±0.16	7.92 ±0.21	7.62 ±0.13	7.85 ±0.11
Vanillic acid	2.44 ±0.37	2.75 ±0.41	2.54 ±0.19	2.61 ±0.23
p-coumaric acid	2.48 ±0.87	2.59 ±0.31	2.66 ±0.53	2.58 ±0.27
Ferulic acid	5.04 ±0.26	4.97 ±0.18	5.17 ±0.38	4.72 ±0.22
β-Carotene	0.59 ±0.01	0.60 ±0.02	0.55 ±0.01	0.54 ±0.02
Lutein	0.03 ±0.001	0.03 ±0.001	0.02 ±0.001	0.02 ±0.001
Zeaxanthin	2.74 ±0.06	2.91 ±0.03	2.73 ±0.02	2.65 ±0.05
Cryptoxanthin	0.49 ±0.01	0.46 ±0.01	0.47 ±0.01	0.43 ±0.01
Total carotenoids, μmol/kg	7.06 ±0.02	7.60 ±0.03	7.21 ±0.05	6.95 ±0.03

Results are reported as mean value ± standard deviation of three replicate analyses ($n = 3$)
Abbreviations: PSO, pumpkin seed oil.

and 5). According to the literature, the content of total phenolic compounds in oil is mainly related to the oil from roasted pumpkin seeds, and is reported to range from 0.28 to 2.46 mg GAE/100 g (Tuberoso *et al.*, 2007; Siger *et al.*, 2008). Andjelkovic *et al.*, (2010) reported the contents of phenolics in PSO samples in a range from 2.47 to 5.09 mg GAE/100 g. Also, higher contents of phenolic compounds in a range from 7.33 to 128.84 mg GAE/100 g were reported by Jiao *et al.*, (2014).

In order to identify the antioxidant activity of the studied pumpkin seed samples, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of oil extracts were determined. The values of free radical-scavenging activity were detected in the following order; 5.70 ± 0.13 mg GAE/100 g, 7.35 ± 0.15 mg GAE/100 g, 6.32 ± 0.11 mg GAE/100 g, 6.67 ± 0.14 mg GAE/100 g for the seed oils from Celtik-Konya, Cumra-Konya, Iceri Cumra-Konya and Polath-Ankara, respectively (Table 5). As

TABLE 5. Total phenolic compounds, free radical scavenging activity and antioxidant activity of the cold-pressed pumpkin seed (*Cucurbita pepo* L.) oils, mg GAE/ 100 g.

	Total phenolic compound content	Free radical scavenging activity	Total antioxidant activity
Celtik PSO-Konya	4.32 ± 0.09	5.70 ± 0.13	27.78 ± 1.12
Cumra PSO-Konya	5.82 ± 0.15	7.35 ± 0.15	38.89 ± 1.41
Iceri Cumra PSO-Konya	3.96 ± 0.13	6.32 ± 0.11	26.67 ± 0.97
Polath PSO-Ankara	4.32 ± 0.12	6.67 ± 0.14	31.11 ± 1.03

Results are reported as mean value ± standard deviation of three replicate analyses ($n = 3$)
Abbreviations: GAE, gallic acid equivalents; PSO, pumpkin seed oil.

shown in Table 5, the extract of Cumra-Konya PSO exhibited superior antioxidant activity (7.35 ± 0.15 mg GAE/100 g) compared with other oil extracts. As expected, Cumra-Konya PSO exhibited a higher antioxidant activity than others, which can be substantially explained by the higher tocol, carotenoid and phenolic contents of this oil than that in other studied oils. In fact, the antioxidant potential of vegetable oils is directly related to their tocol, carotenoid and phenolic compound contents (Fruhworth *et al.*, 2003; Latif, 2011).

The total antioxidant activities of the studied PSOs were determined at 695 nm. In the studied PSOs, the total antioxidant activity values were in the range of 26.67 ± 0.97 – 38.89 ± 1.41 mg GAE/100 g. The lowest value for total antioxidant activity was determined for the Iceri Cumra-Konya PSO sample (26.67 ± 0.97 mg GAE/100 g), whereas, the Cumra-Konya sample had the highest value (38.89 ± 1.41 mg GAE/100 g). In view of the oxidative stability of vegetable oils, the presence of natural bioactive compounds with anti-oxidative activity is a vital factor. Tuberoso *et al.*, (2007) deduced that the changeability of the relationship between the antioxidant activity and profile of the seed oils could be characteristic of the differences in the contents of bioactive compound of the oils and their interactions. Frushworth *et al.*, (2003) also reported that PSO samples have 59% of their antioxidant activity due to the phenolic compounds, and 41% to the tocols. Similar to the other antioxidant activity data, Cumra-Konya seed oil exhibited higher antioxidant activity than others. As known from previous studies; the cultivar, genetic factors, extraction system, production and storage conditions of seed oils are quite critical factors for the contents of antioxidant compounds (Nawirska-Olszanska *et al.*, 2013). Thus, the differences in antioxidant activity data of the PSOs in this study are predicted to be mainly derived from their cultivar variations and genetic factors.

4. CONCLUSIONS

In the present study, the cold-pressed oils from pumpkin (*Cucurbita pepo* L.) seeds cultivated in different central Anatolia regions of Turkey were

analyzed and they have shown high contents of bioactive compounds. High contents of phytosterols, squalene, tocols, phenolic acids, carotenoids and phenolic bioactives, and high values of free radical scavenging as well as antioxidant activity make these oils nutritionally and medicinally valuable. The presence of essential fatty acids and well-balanced fatty acid profiles (ratios of SFAs, MUFAs and PUFAs) of PSOs can be an indicator of their stability and nutritional quality. High levels of bioactives detected in these oils could contribute to stability toward oxidation reactions and provide high quality oil used as edible oil. Therefore, the content of bioactive compound for the studied PSOs can be an indicator of its stability and nutritional quality. Also, the screw-pressing process used in this study involves no organic solvent, which results in high quality cold-pressed PSO that is chemically-contaminant free.

ACKNOWLEDGMENT

The present study was supported by Karamanoglu Mehmetbey University (Karaman, Turkey) Scientific Research Project Center with 05-YL-15 and 18-M-17 project numbers. The authors wish to thank K.M.U. Scientific Research Project Center for the facilities provided.

ABBREVIATIONS

ANOVA: analysis of variance; AOCS: American oil chemical society; FA: fatty acids; GAE: gallic acid equivalent; GC: gas chromatography; GC-FID: gas chromatography-flame ionization detector; GC-MS: gas chromatography-mass spectroscopy; HPLC: high performance liquid chromatography; HPLC-FLD: high performance liquid chromatography-fluorescence detector; HPLC-DAD: high performance liquid chromatography-diode array detector; MS: mass spectroscopy; MUFA: monounsaturated fatty acids; NP-HPLC: normal phase-high performance liquid chromatograph; RP-HPLC: reversed phase-high performance liquid chromatograph; PSO: pumpkin seed oil; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; T: tocopherol; TT: tocotrienol; UV-Vis: ultraviolet-visible

REFERENCES

- Allothman M, Bhat R, Karim AA. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem.* **115**, 785–788. <https://doi.org/10.1016/j.foodchem.2008.12.005>
- Andjelkovic M, Camp JV, Trawka A, Verhe R. 2010. Phenolic compounds and some quality parameters of pumpkin seed oil. *Eur. J. Lipid Sci. Technol.* **112**, 208–217. <https://doi.org/10.1002/ejlt.200900021>
- Arslan FN, Kara H. 2016. Fully Automated Three-Dimensional Column-Switching SPE-FIA-HPLC System for the Characterization of Lipids by a Single Injection: Part I. Instrumental Design and Chemometric Approach to Assess the Effect of Experimental Settings on the Response of ELSD. *J. Am. Oil Chem. Soc.* **93**, 11–26. <https://doi.org/10.1007/s11746-015-2750-0>
- Arslan FN, Sapcı AN, Duru F, Kara H. 2017. A study on monitoring of frying performance and oxidative stability of cottonseed and palm oil blends in comparison with original oils. *Int. J. Food Prop.* **20**, 704–717. <https://doi.org/10.1080/10942912.2016.1177544>
- Feng-Lin S, Ren-You G, Yuan Z, Qin X, Lei K, Hua-Bin L. 2010. Total Phenolic Contents and Antioxidant Capacities of Selected Chinese Medicinal Plants. *Int. J. Mol. Sci.* **11**, 2362–2372. <https://doi.org/10.3390/ijms11062362>
- Fruhwith GO, Hermetter A. 2007. Seeds and oil of the Styrian oil pumpkin: Components and biological activities. *Eur. J. Lipid Sci. Technol.* **109**, 1128–1140. <https://doi.org/10.1002/ejlt.200700105>
- Fruhwith GO, Wenzl T, El-Toukhy R, Wagner FS, Hermetter A. 2003. Fluorescence screening of antioxidant capacity in pumpkin seed oils and other natural oils. *Eur. J. Lipid Sci. Technol.* **105**, 266–274. <https://doi.org/10.1002/ejlt.200390055>
- Gu Q, David F, Lynen F, Vanormelingen P, Vyverman W, Rumpel K, Xu G, Pat S. 2011. Evaluation of ionic liquid stationary phases for one dimensional gas chromatography–mass spectrometry and comprehensive two dimensional gas chromatographic analyses of fatty acids in marine biota. *J. Chromatogr. A* **1218**, 3056–3063. <https://doi.org/10.1016/j.chroma.2011.03.011>
- Jafari M, Goli SAH, Rahimmalek M. 2012. The chemical composition of the seeds of Iranian pumpkin cultivars and physicochemical characteristics of the oil extract. *Eur. J. Lipid Sci. Technol.* **114**, 161–167. <https://doi.org/10.1002/ejlt.201100102>
- Jiao J, Li ZG, Gai QY, Li XJ, Wei FY, Fu YJ, Wei M. 2014. Microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. *Food Chem.* **147**, 17–24. <https://doi.org/10.1016/j.foodchem.2013.09.079>
- Kostadinovic Velickovska S, Bruhl L, Mitrev S, Mirhosseini H, Matthaüs M. 2015. Quality evaluation of cold-pressed edible oils from Macedonia. *Eur. J. Lipid Sci. Technol.* **117**, 2023–2035. <https://doi.org/10.1002/ejlt.201400623>
- Kulaitiene J, Jariene E, Danilcenko H, Kita A, Venskutoniene E. 2016. Oil pumpkins seeds and their quality. *Pol. J. Food Nutr. Sci.* **4**, 349–352.
- Latif S, Anwar S. 2011. Aqueous enzymatic sesame oil and protein extraction. *Food Chem.* **125**, 679–684. <https://doi.org/10.1016/j.foodchem.2010.09.064>
- Mateos R, Espartero JL, Trujillo M, Ríos JJ, León-Camacho M, Alcudia F, Arturo C. 2001. Determination of Phenols, Flavones, and Lignans in Virgin Olive Oils by Solid-Phase Extraction and High-Performance Liquid Chromatography with Diode Array Ultraviolet Detection. *J. Agric. Food Chem.* **49**, 2185–2192. <https://doi.org/10.1021/jf0013205>
- Mariod AA, Yousif MA, Matthaüs B, Khaleel G, Siddig A, Gabra AM, Siddig IA. 2009. A Comparative Study of the Properties of Six Sudanese Cucurbit Seeds and Seed Oils. *J. Am. Oil Chem. Soc.* **86**, 1181–1188. <https://doi.org/10.1007/s11746-009-1459-3>
- Molyneux P. 2004. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarın J. Sci. Technol.* **26**, 211–219.
- Nederal S, Skevin D, Kraljic K, Obranic M, Papesa SI, Bataljaku A. 2012. Chemical composition and oxidative stability of roasted and cold pressed pumpkin seed oils. *J. Am. Oil Chem. Soc.* **89**, 1763–1770. <https://doi.org/10.1007/s11746-012-2076-0>
- Nederal S, Petrovic M, Vincek D, Pukec D, Skevin D, Kraljic K, Obranic M. 2014. Variance of quality parameters and fatty acid composition in pumpkin seed oil during three crop seasons. *Ind. Crops Prod.* **60**, 15–21. <https://doi.org/10.1016/j.indcrop.2014.05.044>
- Nyam KL, Tan V, Lai V, Long K, Che Man YB. 2009. Physicochemical properties and bioactive compounds of selected seed oils. *LWT-Food Sci. Technol.* **42**, 1396–1403. <https://doi.org/10.1016/j.lwt.2009.03.006>
- Nawirska-Olszanska A, Kita A, Biesiada A, Sokół-Letowska A, Kucharska AZ. 2013. Characteristics of antioxidant activity and composition of pumpkin seed oils in 12 cultivars. *Food Chem.* **139**, 155–161. <https://doi.org/10.1016/j.foodchem.2013.02.009>
- Omah BD, Ladet S, Godfrey DV, Liang J, Girard B. 2000. Characteristics of raspberry (*Rubus idaeus* L.) seed oil. *Food Chem.* **69**, 187–193. [https://doi.org/10.1016/S0308-8146\(99\)00260-5](https://doi.org/10.1016/S0308-8146(99)00260-5)
- Parry J, Zhigang H, Luthera M, Lan S, Kequan Z, Liangli Y. 2006. Characterization of Cold-Pressed Onion, Roasted Pumpkin, and Milk Thistle Seed Oils. *J. Am. Oil Chem. Soc.* **83**, 847–854. <https://doi.org/10.1007/s11746-006-5036-8>
- Prieto P, Pineda M, Aguilar M. 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.* **269**, 337–341. <https://doi.org/10.1006/abio.1999.4019>
- Rabrenovic BB, Dimic EB, Novakovic MM, Tesevic VV, Basic ZN. 2014. The most important bioactive components of cold pressed oil from different pumpkin (*Cucurbita pepo* L.) seeds. *LWT - Food Sci. Technol.* **55**, 521–527. <https://doi.org/10.1016/j.lwt.2013.10.019>
- Rezig L, Chouaibi M, Msaada K, Hamdi S. 2012. Chemical composition and profile characterisation of pumpkin (*Cucurbita maxima*) seed oil. *Ind. Crop. Prod.* **37**, 82–87. <https://doi.org/10.1016/j.indcrop.2011.12.004>
- Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM. 2007. Phytosterol, Squalene, Tocopherol Content and Fatty Acid Profile of Selected Seeds, Grains, and Legumes. *Plant Foods Hum. Nutr.* **62**, 85–91. <https://doi.org/10.1007/s11130-007-0046-8>
- Siger A, Nogala-Kalucka M, Lampart-Szczapa E. 2008. The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. *J. Food Lipids* **15**, 137–149. <https://doi.org/10.1111/j.1745-4522.2007.00107.x>
- Stevenson DG, Eller FJ, Wang L, Jay-Lin J, Wang T, Inglett GE. 2007. Oil and Tocopherol Content and Composition of Pumpkin Seed Oil in 12 Cultivars. *J. Agric. Food Chem.* **55**, 4005–4013. <https://doi.org/10.1021/jf0706979>
- Tuberoso CIG, Kowalczyk A, Sarritzu E, Cabras P. 2007. Determination of antioxidant compounds and antioxidant activity in commercial oil seeds for food use. *Food Chem.* **103**, 1494–1501. <https://doi.org/10.1016/j.foodchem.2006.08.014>
- Vujanovic V, Djilas S, Dimic E, Romanic R, Takaci A. 2010. Shelf life of cold-pressed pumpkin (*Cucurbita pepo* L.) seed oil obtained with a screw press. *J. Am. Oil Chem. Soc.* **87**, 1497–1505. <https://doi.org/10.1007/s11746-010-1630-x>