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Biochemical, compositional, and spectral analyses of Isot (Urfa pepper) seed oil and evaluation of its functional characteristics

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SUMMARY: In this study, the physicochemical, functional, and antimicrobial properties of pepper seed oil (PSO) were determined. PSO was subjected to differential scanning calorimeter (DSC), fatty acid composition, carotenoid, capsaicin, and tocopherol analyses. LC-ESI-MS/MS and NMR were used to characterize and quantify phytochemicals. Resveratrol, luteolin, and 4-hydroxycinnamic acid were the principal phenolics in PSO. A high concentration of unsaturated fatty acids (85.3%), especially linoleic acid (73.7%) is present in PSO. Capsaicin, dihydrocapsaicin, α -tocopherol, δ -tocopherol, zeaxanthin, and capsanthin were determined in PSO at concentrations of 762.92, 725.73, 62.40, 643.23, 29.51, 16.83 ppm, respectively. PSO displayed inhibitory activity against α -glucosidase rather than α -amylase. The antimicrobial activity of PSO was tested against *Escherichia coli*, *Staphylococcus aureus subsp. aureus*, *Aspergillus brasiliensis* and *Candida albicans*. The antimicrobial potential of PSO was expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and inhibition zone (IZ) diameter. Polyunsaturated fatty acid, capsaicin, carotenoid, tocopherol, resveratrol contents; the antioxidant, α -glucosidase inhibitory and antimicrobial activities of PSO indicated its nutritional value and health promoting nature for the well-being of humans.

KEYWORDS: *Alpha-glucosidase; Antimicrobial activity; Capsaicin; Carotenoid; Pepper seed oil; Phenolics*

RESUMEN: *Análisis bioquímico, composicional y espectral del aceite de semilla de isot (pimiento Urfa) y evaluación de sus características funcionales.* En este estudio se determinaron las propiedades fisicoquímicas, funcionales y antimicrobianas del aceite de semilla de pimiento (ASP). El ASP se sometió a análisis de calorimetría diferencial de barrido (DSC), composición en ácidos grasos, carotenoides, capsaicina y tocoferoles. LC-ESI-MS / MS y RMN se usaron para caracterizar y cuantificar fitoquímicos. El resveratrol, la luteolina y el ácido 4-hidroxicinámico fueron los principales compuestos fenólicos en el ASP. Una alta concentración de ácidos grasos insaturados (85,3%), especialmente ácido linoleico (73,7%) están presentes en el ASP. Se determinaron capsaicina, dihidrocapsaicina, α -tocoferol, δ -tocoferol, zeaxantina y capsantina en el ASP en concentraciones de 762.92, 725.73, 62.40, 643.23, 29.51, 16.83 ppm, respectivamente. ASP mostró actividad inhibitoria contra la α -glucosidasa en lugar de la α -amilasa. La actividad antimicrobiana de ASP se probó contra *Escherichia coli*, *Staphylococcus aureus subsp. aureus*, *Aspergillus brasiliensis* y *Candida albicans*. El potencial antimicrobiano de ASP se expresó como concentración inhibitoria mínima (MIC), concentración bactericida mínima (MBC) y diámetro de la zona de inhibición (IZ). Los contenidos de ácidos grasos insaturados, capsaicina, carotenoide, tocoferol, resveratrol; las actividades antioxidantes, inhibidoras de la α -glucosidasa y antimicrobianas del ASP indicaron su valor nutritivo y la naturaleza promotora de la salud y el bienestar humano.

PALABRAS CLAVE: *Aceite de semilla de pimiento; Actividad antimicrobiana; Alfa-glucosidasa; Capsaicina; Carotenoide; Fenólicos*

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1. INTRODUCTION

The genus *Capsicum* belongs to the family *Solanaceae* and includes at least 27 species of annual and perennial herbs or shrubs that are native to Central and South America (Walsh and Hoot, 2001). *Capsicum annum* L., *Capsicum baccatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L. and *C. pubescens* Ruiz and Pav. are cultivated species in the *Capsicum* genus. Among these, *C. annum* – the common bell pepper – accounts for most commercial production worldwide (Jarret *et al.*, 2013). However, numerous other forms of these species are also grown on a commercial scale. These would include: Cayenne, Guindilla Verde, Scotch Bonnet Pepper, Basque Fryer, Jalapeño, Paprika, Anaheim, İso pepper and many other less commonly known locally grown ones. In the last decade, the worldwide pepper production continued to increase and reached 38.4×10^6 MT in 2014. Peppers are widely cultivated in China, and Turkey, as well as Mexico. Turkey is the third largest pepper producer in the world. The most famous pepper variety cultivated in the South East region of Anatolia is called ‘İso’. The İso pepper is generally consumed in dried powder form. After harvest, fresh peppers are sliced, dried and ground into a powder to produce İso pepper spice. Urfa cuisine is famous for using İso pepper flakes in dishes, salads, and sauces. It is often described as having a smoky, bitter-sweet taste due to its rich capsaicin and sugar content. During the processing of İso peppers into spices; seeds, stems and placenta are removed as by-products (Yılmaz *et al.*, 2015). Fruit and vegetable wastes are generally being used as livestock feed and sometimes as raw materials for the manufacturing of other added-value products. By 2050 the world is going to be feeding 2 billion more people and will possibly require additional sources for the production of foods or food ingredients. An ample amount of fruit and vegetable wastes and by-products from the food processing industry are available throughout the world. For most of the pepper varieties, seeds account for almost 20% of the dry-weight of the whole fruit (Bosland and Votava, 2000). During the industrial processing of peppers, the seeds are generally removed as waste, even though they contain a large amount of biologically active compounds with anti-inflammatory, and antioxidant properties (Di Sotto *et al.*, 2018; Sandoval-Castro *et al.*, 2017). Pepper seeds are composed of fibers, carbohydrates, proteins, lipids, and some micronutrients including phenolic acids, flavonoids, vitamins and pigments. Pepper seeds represent a rich source of nutritive macromolecules. Pepper seeds are widely used by small, landless farmers as livestock feed or adulterated into spices to make more profit. The oil content of pepper seeds is above 20% and the characteristic properties of seed oil should be

illuminated for processing into added-value products. Possible industrial uses of pepper (*Capsicum annum* L.) derived products and their technological benefits and biological advantages were recently reviewed (Baenas *et al.*, 2019). The evaluation of plant wastes to develop novel added-value products is important in both economic and environmental aspects. Nowadays, the demand for pepper seed oil is increasing and large quantities of pepper seed oil is transported to Europe and the USA. The use of fruit and vegetable waste extracts including oils has become a significant issue in order to develop novel added-value products. Therefore, it is necessary to characterize the oil extracted from İso pepper seeds grown in the southeastern region of Anatolia. In this study, İso pepper seeds were collected from the Şanlıurfa region and used for extracting oil by the cold-press method. The biochemical, thermal, compositional, and functional characteristics of İso (Urfa pepper) seed oil were determined; and its antimicrobial activity on pathogenic microorganisms was assessed in the context of the study.

2. MATERIALS AND METHODS

2.1. Plant materials

Seeds were obtained from a local pepper grower in Şanlıurfa, Turkey. The seeds were dried on trays refrained from sunlight at room temperature for 7 days and stored at 4 °C until used in the experiments. The chemicals and standards were obtained either from Sigma or Merck (Darmstadt, Germany) unless otherwise stated.

2.2. Pepper seed oil extraction

PSO extraction from İso pepper seeds was carried out using a laboratory scale cold pressing machine (12 kg seed/h capacity, single head, 750 W power). Centrifugation (1420 g, 10 °C) and filtration were applied to obtain pure oil. The resulting supernatant was collected and stored in amber-colored vials at -20 °C after flushing with nitrogen until analysis. 10 g of oil were mixed with 2 mL of methanol (X2) in a vial to extract PSO phenolics. After vortexing, the mixture was centrifuged at 1420 g for 30 min and then the resulting supernatant was used for total phenolic content, total flavonoid content, antioxidant activity, antimicrobial activity, enzyme inhibition activity, NMR, and LC-ESI-MS/MS analyses.

2.3. Physicochemical analyses

Physicochemical analyses (peroxide value, iodine number, free fatty acid (FFA), color (L^* , a^* and b^*), saponification, refractive index, acid value) (AOAC, 1984), total phenolic content (Chen and Young-Hwa, 2013), total flavonoid content (Zhishen *et al.*,

1999), four different antioxidant activity methods: DPPH and ABTS (Çam *et al.*, 2009), CUPRAC (Apak *et al.*, 2007), FRAP (Benzie and Strain, 1996) assays and enzyme (α -glucosidase and α -amylase) (McDougall *et al.*, 2005) inhibition activity were determined accordingly.

2.4. Antimicrobial activity

PSO antimicrobial activity was tested against two reference bacterial strains, one yeast and one mold including *Staphylococcus aureus subsp. aureus* (ATCC 6538P), *Escherichia coli* (ATCC 8739), *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans* (ATCC 29212). The agar-well diffusion method was employed for the evaluation of PSO antimicrobial activity. 9-mm holes were punched into the solidified agar and these holes were filled with 120 μ L of PSO extract at 200 mg/mL concentration. The microbial count was set at 0.5 McFarland standard equivalent to give a concentration of 1×10^7 bacteria, 1×10^5 yeast, 1×10^5 mold per milliliter. The bacteria, yeast and mold cultures were grown in the plates and incubated at 37 °C for 24 h, 25 °C for 24 h, and 25 °C for 5 days, respectively. For the determination of inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), the PSO extracts were diluted in the range of 6.25 mg/mL to 200 mg/mL.

2.5. HPLC analyses

2.5.1. Sample preparation for PSO tocopherol, carotenoid, and capsaicinoid contents

Prior to tocopherol and carotenoid analyses, the PSO was saponified to separate the triacylglycerides (Fromm *et al.*, 2012). Capsaicinoid extraction from PSO was evaluated by the method described previously (Collins *et al.*, 1995).

2.5.2. Tocopherol content

An HPLC system (Agilent 1100 series, Agilent, USA) was used to determine the tocopherol fraction. Chromatographic separations were carried out on a Lichrosorb SI60 column (250 x 4 mm, particle size 5 μ m) using hexan/2-propanol as the mobile phases, fluorescence detector (Ex 290 nm and Em 330 nm) at a flow rate of 1 mL/min and injection volume of 20 μ L (Pinheiro-Sant'Ana *et al.*, 2011).

2.5.3. Carotenoid content

The PSO carotenoid fractions were determined by a Purospher® STAR RP-18 column (250x4.6 mm particle size 5 μ m) at 31 °C with a Shimadzu prominence HPLC System (Shimadzu, Japan) using acetone (A) and water (B) as the mobile phases at a flow

rate of 1.25 mL/min and injection volume of 20 μ L. The gradient elution program was as follows: 25% B for 5 min, 20% B for 10 min, 5% B for 15 min, 100% A for 23 min and back to the initial conditions of 25% B. The UV-Vis detector was adjusted to 450 nm.

2.5.4. Capsaicinoid content

PSO extracts were injected to a HPLC system (Shimadzu, Japan) with an UV-Vis detector and a column ODS-3 (250 x 4.6 mm particle size 5 μ m) at 40 °C using solvent A (1% acetic acid) and solvent B (acetonitrile) as the mobile phases at a flow rate of 1 mL/min and injection volume of 20 μ L. The gradient conditions were: 100% A for 15 min, 100% B for 20 min, 100% A for 25 min and back to the initial conditions of 100% A. The absorbance was read at 280 nm.

2.6. Fatty acid profile

The fatty acid composition of PSO was analyzed by a gas chromatography (GC-FID, Shimadzu, GCMSQP2010, JAPAN) with a fused-silica capillary column (30 m 0.25 mm) coated with 0.25 mm dimethyl polysiloxane (RTX®-5MS, Restek). The column temperature was programmed from 40 °C (held for 2 min) to 240 °C at 3 °C/min increases. Injector, interface and detector temperatures were 250, 250 and 220 °C, respectively. Helium was used as carrier gas (1.0 mL/min.) at a split ratio of 1:20.

2.7. LC-ESI-MS/MS analysis of phenolic compounds

PSO extracts were filtered through a 0.45 μ m pore size membrane filter and then injected into Nexera Shimadzu UHPLC LC-ESI-MS/MS (Shimadzu, Japan) equipped with a binary gradient pump, an autosampler (SIL-20AC), a degasser (DGU-20A3R) and a column thermostat (CTO-10ASVP). The column was an Intersil ODS-4 analytical column with 3 mm i.d. x 100 mm and 2 μ m particle size. Experiments to elute phenolic compounds were conducted based on the following conditions: 0.3 mL min⁻¹ flow rate, 40 °C column temperature and 2 μ L injection volume, using a mixture of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol) as mobile phase; gradient conditions: from 0 to 4 min 5% B, from 4 to 7 min 95% B, from 7 to 7.01 min 95% B and at 7.01 min back to the initial conditions of 5% B. The concentrations of phenolic compounds are expressed in μ g/kg units.

2.8. Nuclear magnetic resonance spectroscopy (NMR)

NMR experiments were conducted on an Agilent Instrument NMR Spectrometer operating at 400 MHz. The PSO was dissolved in chloroform and

placed into 5 mm NMR tubes, and injected into the NMR spectrometer to obtain ^1H NMR spectra. The acquisition parameters were: spectral width, 6410.3 Hz; spin: 20; number of scans 32; relaxation delay 5s; pulse angle 90° ; observe pulse $8.50 \mu\text{s}$ for ^1H NMR spectra.

2.9. Thermal analysis by DSC

For the thermal parameters, a Shimadzu DSC-60 differential scanning calorimeter (DSC) (Shimadzu, Kyoto, Japan) was used. The DSC instrument was calibrated with indium (heat of fusion 28.45 J/g). Temperature calibration was carried out using hexane (m.p. 93.5°C), water (m.p. 0.0°C) and indium (m.p. 156.6°C). Samples were weighed into aluminum pans at 10–15 mg and covers were hermetically sealed into place. An empty pan was used for reference. Samples were heated to 60°C under nitrogen flow (0.5 mL/min), held for 10 min at constant temperature and cooled to -60°C , and then heated again to 60°C . The scanning rate was 2°C/min . The machine equipment program (TA-60WS) was used to plot and analyze the thermal data (Calligaris *et al.*, 2004).

2.10. Data processing

The PSO extracted from three different seeds of the same origin was used for each analysis and all analyses within each PSO were carried out at least in duplicate. Origin Pro 8 (Origin Lab Inc.) and Lab Solutions software (Shimadzu) was used to obtain DSC graphs. The differences among data were compared with independent samples according to the t-test and One-way analysis of variance (ANOVA) with Tukey's multiple comparisons test at the confidence level of 95% ($p \leq 0.05$) using SPSS 22.0.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of the İsoot seed oil

The physicochemical characteristics of İsoot seed oil are given in Table 1. All three color parameters for PSO were higher compared to capia pepper seed oil (Yılmaz *et al.*, 2015) and its color was a deeper reddish and yellowish than that extracted from seed of capia. There is no internationally accepted PSO standard defined in the codex alimentarius. Therefore, we compared the obtained data regarding PSO physicochemical properties with the virgin olive oil standards published in codex alimentarius. The FFA content of PSO (0.22%) was below 0.8%, which was required for an olive oil to be included in the extra virgin category according to the International Olive Oil Standards. The FFA of pepper seed oil for California Wonder

TABLE 1. Physicochemical properties of pepper seed oil

Physicochemical properties	Values
Iodide value	94.26 ± 1.00
Peroxide value (meq O_2/kg)	6.35 ± 0.63
Free fatty acid (%)	0.22 ± 0.01
Saponification value	181.45 ± 1.16
Color (L, a*, b*)	35.92 ± 0.02 ; 20.40 ± 0.05 ; 18.24 ± 0.14
Refractive index (24°C)	1.45 ± 0.01
Density (g/mL)	0.91 ± 0.02
Specific gravity	0.92 ± 0.02
Energy value (kcal/g)	9.00 ± 0.04

Each value is expressed as a mean \pm standard deviation of three replicates ($n = 3$).

(C. annum) and Aji Colorado were 0.71 and 4.74% (Jarret *et al.*, 2013), respectively, both higher compared to what we measured for İsoot PSO. The peroxide value of PSO was (6.35 meq O_2/kg), below 20 meq O_2/kg , which meets the criteria for high quality olive oils. Saponification value was slightly lower and Iodide value was slightly higher for PSO when compared to standards, as determined by 181.45 and 94.26, respectively (Table 1).

3.2. Capsaicin, tocopherol, and carotenoid contents of İsoot seed oil

Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin are members of the capsaicinoid family. Capsaicin and dihydrocapsaicin represent 80-90% of the total capsaicinoids present in the capsicum genus (Figueiredo *et al.*, 2013). The ratio of capsaicin/dihydrocapsaicin ranges from 1:1 to 2:1 (Hayman and Kam, 2008). The mean concentrations of capsaicin and dihydrocapsaicin in red chili pepper are 309.3 ppm and 238.2 ppm and 138.5 ppm and 146.4 ppm in green chili pepper (Othman *et al.*, 2011). The capsaicin and dihydrocapsaicin contents of PSO were 762.92 ppm and 725.73 ppm (Table 2), a higher concentration compared to those of red chili pepper and lower than hot chilies. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a unique alkaloid found primarily in pepper fruits with analgesic effects, which give them their hot taste and spicy flavor. Capsaicin exerts selective effects on the peripheral part of the sensory nervous system and relieves pain by depleting the neurotransmitter of painful impulses known as substance P from the sensory nerve terminals. The main source of capsaicinoids in nature is Capsicum fruits. The capsaicin content of peppers is closely linked to their commercial quality and pharmaceutical properties. Tocopherols are considered lipophilic antioxidants, which belong to the Vitamin E group of natural compounds. Tocopherols are found in distinct plant

TABLE 2. Some bioactive compounds in pepper seed oil

Capsaicinoids (mg/kg)	Tocopherols (mg/kg)	Caroteneoids (mg/kg)	Phenolics (mg GAE/kg-mg QE/kg)				
Capsaicin	762.92±1.51*	α Tocopherol	62.40±0.91	Violaxanthin	0.90±0.00	Total Phenolics	201.84±2.03*
Dihydrocapsaicin	725.73±1.42	δ Tocopherol	643.23±1.40*	Capsanthin	16.83±1.10	Flavonoids	164.80±1.61
				Zeaxanthin	29.51±2.00*	-	
				β -apo-8'-carotenal	0.31±0.00	-	
				Cryptoxanthin	12.74±0.73	-	
				β -Karoten	6.61±0.52	-	

Each value is expressed as a mean \pm standard deviation of three replicates (n = 3). Statistically significant differences among results in the same column at the confidence level of 95% for each parameter are marked with *. **GAE**: Gallic acid equivalents, **QE**: Quercetin equivalent.

tissues such as fruits, flowers and seeds. Peppers contain tocopherols in amounts comparable to those in spinach and asparagus; while higher than tomatoes (Bosland and Votava, 2000). The PSO evaluated in this study included both α (62.40 \pm 0.85 ppm) and δ tocopherols (643.23 \pm 1.40 ppm) (Table 2). A group of carotenoids including violaxanthin, capsanthin, cryptoxanthin, zeaxanthin, β -apo-8'-carotenal, cryptoxanthin, and β -carotene was detected in PSO. The unique keto carotenoids capsanthin and cryptoxanthin provide the brilliant red color; while β -carotene, zeaxanthin, and violaxanthin impart the yellow-orange color. β -apo-8'-carotenal is an aldehydic carotenoid which is widely distributed in peppers and has an orange-red color. Besides their use as natural colorants carotenoids promote human health due to their nutritional characteristics and preventive-protective roles against degenerative diseases (Arimboor *et al.*, 2014). Statistically significant differences ($p < 0.05$) were found among the carotenoid fractions of PSO and zeaxanthin was the principal carotenoid (29.51 \pm 2.00 mg/kg) in PSO. The phenolic and flavonoid contents of the PSO samples are shown in Table 2. Total phenolic content of PSO was higher than that of total flavonoids ($p < 0.05$). The concentration of total phenolic compounds was 201.84 \pm 2.03 (mg GAE/kg) and the flavonoid content was 164.80 \pm 1.56 (mg QE/kg). Total phenolic content of Ísot seed oil was higher compared to that of capia peppers (Yilmaz *et al.*, 2015). The capsaicin, tocopherol, phenolic and flavonoid contents together with the remarkable antioxidant activity of PSO reveal its rich nutraceutical value and importance for human nutrition.

3.3. Fatty acid composition of Ísot seed oil

The fatty acid content of PSO is shown in Table 3. The extracted PSO was mainly composed of unsaturated fatty acids (UFA), approximately 85.3%, including mono-unsaturated fatty acids (oleic) and the poly-unsaturated fatty acid (linoleic). The saturated fatty acids (SFA; palmitic and stearic

TABLE 3. Fatty acid contents in pepper seed oil

Fatty acids	Retention Time	Content (%)
Myristic acid (C14:0)	26.84	0.17±0.01
Pentadecanoic acid (C15:0)	28.73	0.02±0.00
Palmitic acid (C16:0)	30.59	10.86±0.68
Palmitoleic acid (C16:1)	31.79	0.29±0.01
Stearic acid (C18:0)	34.05	3.33±0.20
Oleic acid (C18:1n9c)	35.11	10.51±0.60
Linoleic acid (C18:2n6c)	36.82	73.77±1.50
Arachidic acid (C20:0)	37.27	0.20±0.02
<i>Cis</i> -11-eicosenoic acid (C20:1)	38.31	0.08±0.00
Linolenic acid (C18:3n6)	38.55	0.25±0.01
<i>Cis</i> -11,14-eicosadienoic acid (C20:2)	39.92	0.18±0.01
<i>Cis</i> -13,16-docosadienoic acid (C22:2)	43.99	0.12±0.02

Each value is expressed as a mean \pm standard deviation of three replicates (n = 3).

acids) were also present in PSO but at lower concentrations (14.7%). These results are in agreement with previously published reports (Yilmaz *et al.*, 2015). The ratio of UFA to SFA was 5.8, which was higher than previously reported (Chougui *et al.*, 2013). Linoleic acid was the dominating fatty acid (73.77%) followed by palmitic acid (10.86%) and oleic acid (10.51%). The percentage of linoleic acid (C18:2) found in Ísot pepper seed was higher than that found in *Capsicum galapagoense*, similar to *Capsicum flexuosum* and lower than *Capsicum tovarii* (Jarret *et al.*, 2013). The observed differences in the fatty acid composition and percentages of PSO could be variety-specific or due to environmental factors and agronomic practices. The essential fatty acids were named as f-vitamins and draw significant attention due to the health-promoting potential of polyunsaturated fatty acids. It has been reported that unsaturated fatty acids play a role in preventing cardiovascular diseases, diabetes, cancer, Alzheimer's disease and the alleviation of certain chronic defects (Shahidi and Ambigaipalan, 2018).

3.4. Phenolic composition of İso seed oil

There is limited study in the literature on the phenolic composition of *Capsicum annuum* L. seed oils. However, the phenolic compositions of peppers have been studied (Baenas *et al.*, 2019). Phenolic compounds belong to phenolic acid derivatives and flavonoids represent major secondary metabolites in peppers (Jayaprakasha *et al.*, 2012). Quercetin, luteolin, myricetin, kaempferol, and apigenin are present in pepper fruits (Asnin and Park, 2015; Baenas *et al.*, 2019; Jayaprakasha *et al.*, 2012). In addition to these compounds, phenolic acids such as caffeic, *p*-coumaric, sinapic, and ferulic acid are found in peppers (Materska and Perucka, 2005). In this study, the phenolic compounds in PSO were determined using a LC-ESI-MS/MS method. Fourteen phenolic compounds were detected in PSO and resveratrol (6269.10 μ g/kg) was the most abundant one, followed by luteolin (733.81 μ g/kg) and 4-hydroxycinnamic acid (517.90 μ g/kg) (Table 4). Resveratrol has curing effects on stress and disease in rodent models (Baur and Sinclair, 2006); luteolin significantly inhibits neuronal cell apoptosis, elevates cell viability, and attenuates diabetes-associated disorders (Liu *et al.*, 2017); and hydroxycinnamic acid and their derivatives are important components of medicinal herbs which are useful for treatment of chronic diseases (El-Seedi *et al.*, 2018). The synergistic activity of these compounds and vitamin C and vitamin E are responsible for the potent antioxidant capacity of peppers which is associated to the protection of the human body against arteriosclerosis, osteoporosis, diabetes, cancer, and coronary diseases (Pandey and Rizvi, 2009).

3.5. NMR spectral and DSC thermal analyses of İso seed oil

Gas chromatography equipped with a flame ionization detector (GC-FID) is one of the best known methods for detecting the presence of fatty acids in the samples or the fatty acid composition of vegetable oils. NMR is currently being used along with mass spectrometry for detecting a wide range of fatty acids and phytochemicals including phenolic compounds. Compared to other analytical analyses, ¹H-NMR is relatively fast and different chemical structures are accurately and simultaneously determined (Savorani *et al.*, 2013). The ¹H-NMR spectrum obtained in this study represented olefin protons at 5.28 ppm, bis-allylic protons at 2.71 ppm, allylic protons at 1.98 ppm and terminal methyl group protons at 0.83 ppm (Figure 1A). The unsaturated nature of PSO was also confirmed by NMR analysis. Linoleic acid was the most abundant fatty acid present in PSO and accounted for almost 74% of the total fatty acids found in PSO. Linoleic acid carbons, including the protons attached to olefinic carbons (–CH=CH–; C_{9,10,12,13}), bis-allylic protons (=CH–CH₂–CH=; C₁₁), the allylic/ α -C protons (=CH–CH₂–alkyl; C: 2,8,14), and alkyl protons (–CH₂/–CH₃; C: 3,4,5,6,7,15,16,17,18) were clearly visible on the ¹H-NMR spectrum (Figure 1A). The homonuclear 2D – ¹H NMR COSY experiment contained apparent cross-peaks between the –CH= protons at 5.2 ppm and neighboring –CH₂– groups at 2.01 ppm. The other cross-peak of the –CH= protons at 5.2 ppm was at 2.74 ppm and arose from the –CH₂– groups between two double bonds (Figure 1B). This peak is generally found in the samples containing polyunsaturated

TABLE 4. Phenolic composition of pepper seed oil

Phenolic compound	RT	MF	Concentration (μ g/kg)	Precursor Ion
Fumaric acid	3.674	C ₄ H ₄ O ₄	54.64 \pm 1.58	115.20
Gallic acid	4.134	C ₇ H ₆ O ₅	48.61 \pm 1.05	169.20
Catechin	4.958	C ₁₅ H ₁₄ O ₆	25.43 \pm 1.76	291.10
Caffeic acid	5.283	C ₉ H ₈ O ₄	10.12 \pm 0.76	179.20
Resveratrol	5.713	C ₁₄ H ₁₂ O ₃	6269.10 \pm 5.65	229.10
4-Hydroxycinnamic acid	5.738	C ₉ H ₈ O ₃	517.90 \pm 1.01	163.20
Vanillic acid	6.026	C ₈ H ₈ O ₄	108.32 \pm 2.24	168.80
Butein	6.084	C ₁₅ H ₁₂ O ₅	17.91 \pm 1.20	271.10
Quercetin	6.091	C ₁₅ H ₁₀ O ₇	62.71 \pm 0.29	301.10
Naringenin	6.104	C ₁₅ H ₁₂ O ₅	126.61 \pm 1.27	271.10
Salicylic acid	6.104	C ₇ H ₆ O ₃	52.62 \pm 1.02	137.20
4-Hydroxybenzoic acid	6.130	C ₇ H ₆ O ₃	68.42 \pm 0.77	137.20
Luteolin	6.190	C ₁₅ H ₁₀ O ₆	733.81 \pm 1.50	285.20
Curmin	6.516	C ₂₁ H ₁₉ O ₆	108.71 \pm 1.15	367.00

Each value is expressed as a mean \pm standard deviation of three replicates (n = 3). RT: Retention time, MF: Molecular formula.

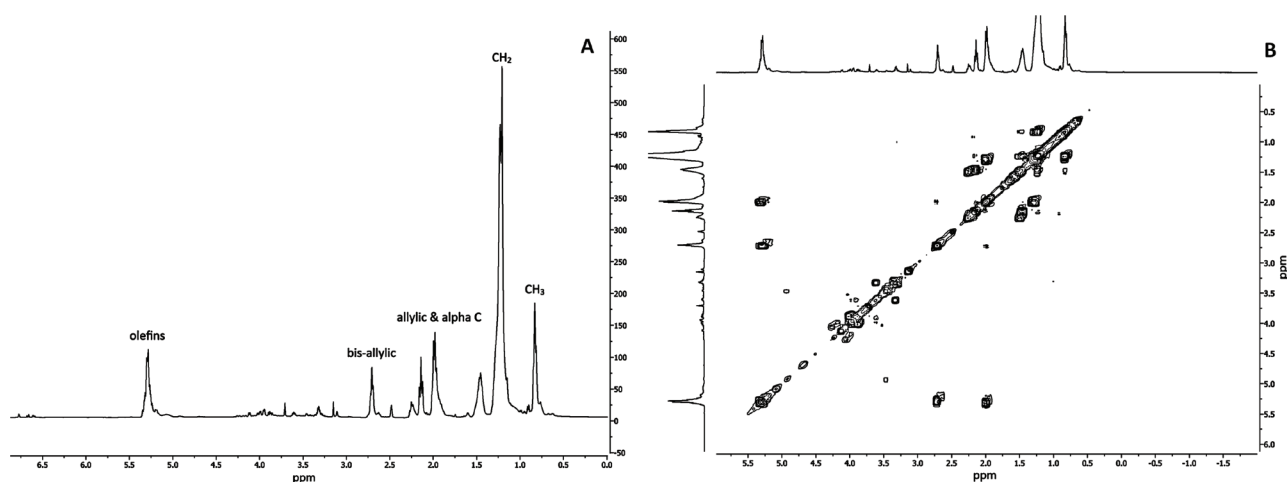


FIGURE 1. 400 Mhz ^1H NMR spectrum for pepper seed oil. Each point corresponds to the average value of three replicates. The relevant NMR spectral features are defined as olefinic protons ($-\text{CH}=\text{CH}-$), the bis-allylic protons ($=\text{CH}-\text{CH}_2-\text{CH}=\text{}$), the allylic/ α -C protons ($=\text{CH}-\text{CH}_2-\text{alkyl}$), the alkyl and terminal methyl protons ($-\text{CH}_2/-\text{CH}_3$; C) (A). Two-dimensional homonuclear NMR correlation spectroscopy (2D NMR COSY) of pepper seed oil (B).

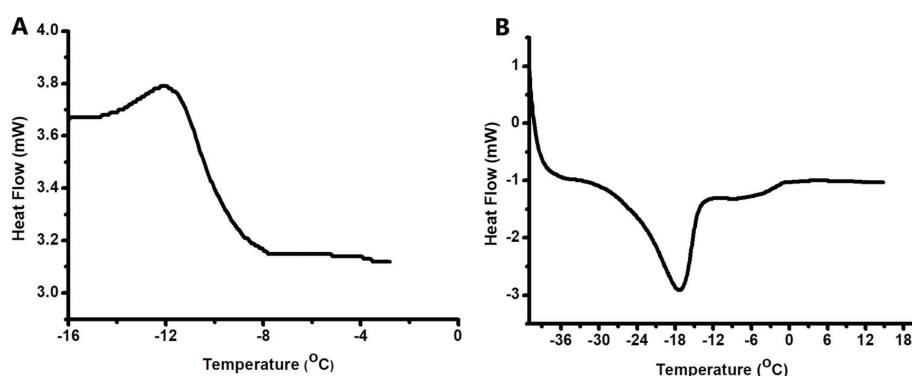


FIGURE 2. DSC thermogram for crystallization (A) and melting process (B). Each one of the two curves presented is representative of three different matching thermograms.

fats. The DSC heating and cooling curves from $-40\text{ }^\circ\text{C}$ to $40\text{ }^\circ\text{C}$ of PSO are given in Figure 2. The volume narrowing and exothermic effect resulting from crystallization started at $-11.51\text{ }^\circ\text{C}$ (T_{on}) and finished at $-14.14\text{ }^\circ\text{C}$ (T_{off}) with ΔH of 0.92 J/g . T_{on} , T_{off} and ΔH of the endothermic peak represented melting and were $-15.84\text{ }^\circ\text{C}$, $-17.38\text{ }^\circ\text{C}$ and 0.64 J/g , respectively. One endothermic peak and 1 exothermic peak were detected. Previous thermal behavior evaluated in sunflower oil indicated the presence of three endothermic peaks and one exothermic peak (Calligaris *et al.*, 2004). The differences in the number of the peaks could be due to the complexity of the Triacylglycerol (TAG) distribution (Tan and Che Man, 2002) because TAG could be present in different forms such as polymorphic and crystalline structures (Desmedt *et al.*, 1990). In addition, TAG with high saturated fatty acids melt at higher temperatures compare to highly unsaturated ones (Calligaris *et al.*, 2004). According to Myat *et al.*

(2009), crystallization and melting temperatures directly depend on SFA/PUFA and SFA/MUFA ratios.

3.6. Antimicrobial, anti-diabetic and antioxidant activities of İsoot seed oil

The antimicrobial properties of PSO were assessed against Gram-negative, Gram-positive bacteria, yeast and fungi, including *E. coli*, *S. aureus*, *C. albicans*, *A. brasiliensis*. The Agar well diffusion assay was used to test the antimicrobial activity of PSO and the diameters of the inhibition zones obtained are shown in Table 5. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PSO on pathogenic microorganisms were also determined. The aqueous extract of PSO showed bactericidal activity only against *S. aureus*, which is a Gram-Positive bacterium. There is no study in the literature describing

TABLE 5. Antimicrobial, antioxidant and anti-diabetic activities of pepper seed oil

Pepper Seed Oil	MIC (mg/mL)	MBC (mg/mL)	IZ (cm)	Anti-diabetic activity (mg/mL)	Antioxidant Activity (μmol TEAC/g)
<i>E. coli</i> (ATCC 8739)	nd	nd	nd	α-amylase nd	DPPH 11.38±0.31*
<i>S. aureus</i> (ATCC 6538)	50	100	1.3	α-glucosidase 22.29±0.20*	ABTS 7.81±0.01
<i>C. albicans</i> (ATCC 10231)	nd	nd	nd		CUPRAC 8.55±0.30
<i>A. brasiliensis</i> (ATCC 16404)	nd	nd	nd		FRAP 3.89±0.13

Each value is expressed as a mean ± standard deviation of three replicates (n = 3). Statistically significant differences among results in the same column at the confidence level of 95% for each parameter are marked with *. nd: not detected, MIC: Minimum inhibition concentration, MBC: Minimum bacterial concentration, IZ: Inhibition zone, DPPH: 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl, ABTS: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid, CUPRAC: Cupric reducing antioxidant capacity, FRAP: Ferric reducing antioxidant power, TEAC: Trolox equivalent antioxidant capacity.

the antimicrobial activity of PSO. However, some studies report the antimicrobial activity of aqueous extracts of olive oil. The antimicrobial activity of aqueous extracts of olive oil was tested against *Salmonella enteritidis*, *Listeria monocytogenes*, *S. aureus*, *E. coli* O157:H7, *Yersinia* sp. 5057655, and *S. sonnei* JCP, and showed bactericidal activity against the food borne pathogens tested (Medina *et al.*, 2007). The aqueous extract of virgin olive oil exerted strong antimicrobial activity. Neither *S. aureus* nor *Yersinia* sp. was detected after 5 min of contact with the aqueous extract of virgin olive oil, which represents as 5 log reduction in the count of pathogens (Medina *et al.*, 2007). In another study, extra virgin olive oil (EVOO) showed strong bactericidal activity, although refined oils were found to be ineffective. The percent inhibition results of the phenolic compounds in EVOO were found at up to 13.13% for *E. coli* O157:H7, up to 27.68% for *L. monocytogenes* and up to 11.64% for *S. enteritidis* (Karaosmanoğlu, 2010). There are few studies regarding the antimicrobial activity of edible oils when compared to essential oils. The essential oils obtained from various plant tissues including leaves, flowers, stems, fruits and seeds displayed antimicrobial activity against various bacterial strains (Semeniuc *et al.*, 2017). Much of the emphasis was devoted to the antimicrobial activity of essential oils rather than edible ones. It was generally shown that plant extracts are usually more potent inhibitors of Gram-positive bacteria than Gram-negative bacteria (Lin *et al.*, 1999). Similar findings were also presented in this study. The aqueous extract of PSO exerted antimicrobial activity against a Gram-positive bacterium while no inhibitory activity was observed against Gram-negative *E. coli*. We were not able to detect any inhibitory activity against mold (*A. brasiliensis*) or yeast (*C. albicans*) cultures either. The inhibitory effects of PSO extracts on α-amylase and α-glucosidase enzymes are shown in Table 5. The extracts were found incapable on preventing α-amylase activity but exhibited inhibitory activity against α-glucosidase with an IC₅₀ value of

22.29±0.20 mg/mL. Acarbose was used as a positive control. The PSO extracts possessed the same inhibitory effect ($p > 0.05$) against α-glucosidase as determined for acarbose (20.28±0.91 mg/mL), indicating that it was capable inhibiting the active center (Rubilar *et al.*, 2011). The enzyme activity of PSO could be explained by its bioactive compounds, including phenolic, carotenoid, capsaicinoid and tocopherol contents. Due to lack of studies on the enzymatic activities of oils, the results were compared to the results of other extracts, such as spinach (6.03 μg/mL) (Vyas, 2017). As expected, the plant extracts were more potent than the PSO aqueous extract in blocking the active center of the assayed enzymes. We tested the antioxidant activity of PSO by applying four different methods, including DPPH, ABTS, CUPRAC, and FRAP as a single method did not fully describe the antioxidant capacity of the samples (Table 5). DPPH-ABTS are radical scavenging and FRAP-CUPRAC are reducing power assays, and the results were expressed as μmol TEAC/g, as shown in Table 5. The radical scavenging ability of the assayed PSO was approximately 1.5 times higher in the DPPH assay (11.38±0.31 μmol TEAC/g) ($p < 0.05$) when compared to the ABTS assay (7.81 ± 0.01 μmol TEAC/g). The reducing power activities of PSO according to the CUPRAC and FRAP assays were also evaluated and varied significantly ($p < 0.05$), revealing 8.55 ± 0.30 and 3.89 ± 0.13 μmol TEAC/g, respectively.

4. CONCLUSIONS

Data on phytochemical content, phenolic composition, enzyme inhibitory and antimicrobial activity in PSO are scarce in the scientific literature. This study showed that the PSO obtained from İso pepper seeds had considerable amounts of unsaturated fatty acids, capsaicin, carotenoid and tocopherol, which are considered beneficial for human health. The LC-ESI-MS/MS indicated a broad complexity in the PSO phenolic composition and allowed for the identification of 14 distinct

compounds. Resveratrol, a potent antioxidant and health-promoting compound, represented at a high concentration in PSO. The inhibitory activity of PSO on the growth of pathogenic microorganisms, free radical scavenging activity, was thought to be related to its protective and antioxidant potential. Today, many people are not reaching micronutrient intake requirements from the diet alone as they consume energy-rich, micronutrient-poor foods. Phytochemicals, vitamins, antioxidants, and minerals are needed for an adequately balanced diet. Integrating PSO to foods represents a healthy way to supply micronutrient requirements and improve the quality of life through its antioxidant, antimicrobial, and nutritional powers.

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