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ORIGINAL PAPER

Antioxidant Activities and Oxidative Stabilities of Some Unconventional Oilseeds

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Abstract The oils of some unconventional oilseeds (hemp, radish, terebinth, stinging nettle, laurel) were obtained by a cold-press method in which the total oil content, fatty acids, tocopherol isomers, some metal contents (Ca, Mg, Fe, Cu), antioxidant activity and oxidative stability were determined. The total oil content was determined ranging between 30.68 and 43.12%, and the oil samples had large amounts of unsaturated fatty acids, with oleic acid and linoleic acid. Of all the oils, terebinth seed oil had the highest α-tocopherol content (102.21 ± 1.01 mg/kg oil). Laurel oilseed had the highest antiradical activity in both the DPPH and ABTS assays. The peroxide value of the non-oxidized oils ranged between 0.51 and 3.73 mequiv O_2/kg oil. The TBARS value of the non-oxidized oils ranged between 0.68 ± 0.02 and 6.43 ± 0.48 mmol MA equiv/g oil. At 110 °C, the Rancimat induction period of the oils ranged between 1.32 and 43.44 h. The infrared spectra of the samples were recorded by FTIR spectroscopy. The absorbance values of the spectrum bands were observed and it was determined that some of the chemical groups of oxidized oils caused changes in absorbance. As a result of the present research, the analyzed oils could be evaluated as an alternative to traditionally consumed vegetable oils or as additives to them.

Keywords Oilseed · Tocopherols · Antioxidant · Antioxidant capacity · Oxidative stability

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Introduction

Vegetable oils are widely used in cooking and alimentary, as well as the cosmetic, pharmaceutical and chemical industries [1]. Due to the fact that the constituents of non-traditional vegetable oils have unique chemical properties, they are important and may augment other edible oil sources. Some species of the newer sources of edible oils are important because they can be used for both health benefits and the production of formulations and they contain phytochemicals with significant antioxidative properties [2]. Terebinth (*Pistacia terebinthus*) is used in eczema treatment, asthma and is known for its antibacterial and anti-inflammatory properties [3]. Hempseed (*Cannabis sativa*), in addition to its nutritional value, has demonstrated positive health benefits, including the lowering of cholesterol and high blood pressure [4]. Radish (*Raphanus sativus*) is not only a vegetable crop but also an important source of medicinal compounds. Radish is used by people suffering from various gastrointestinal, biliary, hepatic, urinary and respiratory disorders, and in cardiovascular diseases such as hypertension [5]. Stinging nettle (*Urtica dioica* L.) has been used in the diet and, as a pharmaceutical for a long time [6]. Laurel (*Laurus nobilis*) is an important plant for many industries. It is widely used in the food, cosmetic, and pharmaceutical industries [7].

On the other hand, oxidation is one of the most common causes of flavor quality deterioration for oils and oil products. Deterioration occurs through rancidity resulting from oxidation which takes place at the double bond sites in the triacyleglycerol molecules. Oxidation causes great economic loses to the food industry [8]. Protection against the oxidation reaction is provided by the tocopherols, phenolic compounds and carotenoids present in the vegetable oils [5]. The addition of antioxidants is method of

increasing shelf life of oils and oils products. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have a restricted use in foods as they are suspected as being carcinogenic [9]. Natural antioxidants are important for human health because of decreasing heart disease risks and possessing anticarcinogenic properties and also they are safer than synthetic antioxidants [10].

The aim of the present study is to determine the fatty acids, tocopherol isomers, some metal contents, the oxidative stability and the antioxidant capacity of selected seeds. These seeds are unconventional seeds and their importance is increased because of their chemical properties. Therefore, they may to be utilized for the food and medical industries due to their antioxidative properties and health benefits.

Materials and Methods

Material

A tocopherol standard (50 mg of α, β, γ, and δ-tocopherol mixture) was purchased from Calbiochem (La Jolla, CA). A fatty acid methyl ester (FAME) mixture (37 component FAME mix) was purchased from Supelco (Bellefonte, PA). All the other chemicals and reagents for analysis were purchased from Sigma (St. Louis, MO).

Sample Preparation

Stinging nettle (*Urtica dioica* L.), laurel (*Laurus nobilis*), terebinth (*Pistacia terebinthus*), hemp (*Cannabis sativa*), and radish (*Raphanus sativus*) seeds were purchased from local shops in Malatya, Istanbul, Mersin and Muğla and were blended. The seeds were cleaned and ground. The oils were extracted by using a laboratory type oil-press (Cesalsan, Giresun). Until use, the oils were kept in glass-containers having a −20 °C nitrogen atmosphere.

Total Oil Content

The total oil content of the samples was determined by the AOAC standard methods [11]. A 10-g oil sample was taken from the seeds ground in the coffee mill and the extraction was made with 200 mL *n*-hexane in a Soxhlet apparatus for 8 h. The solvent was then evaporated at 40 °C by using a rotary evaporator (Bibby Sterilin Ltd, Staffordshire, UK). The above mentioned process was implemented three times for each sample. The average value was obtained and the oil content was expressed as a percentage.

Fatty Acid Analysis

Fifty milligrams of oil was methylated with 3 mL HCl in methanol at 95 °C for 1 h. The fatty acid methyl esters (FAME) were extracted with 2 mL of hexane and dried over sodium sulfate [12]. One microliter of the FAME was analyzed with a Shimadzu GC-17A gas chromatograph (Shimadzu Company, Japan) equipped with a flame ionization detector, and an AOC-20i automatic injector. A SP-2560 capillary column (100 m × 0.25 mm i.d. × 0.2 μm; Cat No. 2-4056) was used. The oven temperature was programmed as follows: 120 °C for 5 min, increased to 240 °C at 4 °C/min, and kept at 240 °C for 25 min. The injector and detector temperatures were each kept at 260 °C. The carrier gas helium, the flow rate 30 mL/min, and the split ratio was 1/50. FAME identification was based on the retention times as compared with those of the standard FAME mixture. Results were expressed as percentage of the peak area without any corrections. Fatty acid analysis was performed in triplicate for each sample, and the average values were reported.

Tocopherol Analysis

Tocopherol composition of the samples was determined as described by Turan et al. [13]. Tocopherols were analyzed by a HPLC system (Shimadzu Prominence, Kyoto, Japan). The normal phase column in the system was an Inertsil NH_2 column (250 mm × 4.6 mm, 5 μm) and the column temperature was maintained at 30 °C. Separation of tocopherols was based on isocratic elution with *n*-hexane (96%) and isopropanol (4%) at 1 mL/min. The eluate was monitored at 292 nm by using a photodiode-array detector (SPD-M20A). The compounds were identified by comparing their retention times and the UV spectra with the authentic standards. Tocopherols were quantified based on the peak areas compared with the external standards. Tocopherol analysis was performed in triplicate for the single samples of each variety, and the average values were reported.

Antioxidant Tests

DPPH Test

The radical scavenging power was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) [14] method. For the DPPH test, DPPH solution was dissolved in a small volume of ethyl acetate and diluted with ethyl acetate by adjusting the absorbance to 0.700 ± 0.020 at 520 nm. A 20-mg oil sample was weighed in a test tube, and 80 μL ethyl acetate as well as 2.9 ml $DPPH^{\bullet}$ free radical solution

were added. Next, the sample was agitated with a vortex mixer for 20 s. After 30 min of incubation in darkness, absorbance was measured at 520 nm against ethyl acetate. The results of the DPPH test were expressed as microgram trolox equivalent/g oil. (equivalent to 10 mL methanolic extract).

ABTS Test

The radical scavenging power was determined by the 2,20-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) [15] method. ABTS radical cation ($ABTS^{+}$) stock solution was produced by reacting 7.0 mM ABTS stock solution with 2.45 mM (final concentration) potassium persulfate in the dark for 16 h. The resulting solution was diluted with ethanol by adjusting the absorbance to 0.700 ± 0.020 at 765 nm. 100 µL diluted oil samples in ethanol and 2.9 mL diluted $ABTS^{+}$ solution were added. The solution was agitated with a vortex mixer for 20 s. The absorbance was measured after 6 min at 765 nm. The results of the ABTS test were expressed as microgram trolox equivalent/g oil.

Oven Test

Ten grams of oils were weighed in glass Petri dishes (15 mm height and 80 mm diameter) and placed in a forced-draft air oven set at 60 ± 1 °C. Hemp oil (0.5 g) was removed from the oven after 1, 3, 6 days, stinging nettle oil (0.5 g) was removed from the oven after 1, 3, 6, 8 and the oil samples of radish, terebinth (0.5 g) were removed from the oven after 1, 3, 6, 7, 8, 10 days and laurel oil (0.5 g) was removed from the oven after 1, 3, 6, 7, 8, 10, 14, 20, 24 days and flushed with nitrogen, covered with parafilm and kept at −20 °C for the oxidative stability test.

Oxidative Stability

The peroxide value (PV) was determined by the ferric thiocyanate method [9]. A 0.1-g oil sample was weighed and 9.7 ml ethanol added. Thus, the oil was dissolved. Next, 0.1 mL NH_4SCN and 0.1 mL $FeCl_2$ were added to the above-mentioned solution, and kept at room temperature for 5 min. Absorbance of the sample was measured at 500 nm. The results were expressed in mequiv/kg oil.

TBARS (2-thiobarbituric acid-reactive substances) was determined as described by Abuzaytoun et al. [16]. A 0.05–0.20-g oil sample was weighed into 25-mL volumetric flasks, dissolved in a small volume of 1-butanol, and made up to the mark with the same solvent. A 5.0-mL portion of this mixture was transferred into a dry test tube, and then a fresh 2-TBA reagent (5 mL of a solution of 200 mg 2-TBA in 100 mL 1-butanol) was added to it. The contents were mixed and heated in a water bath at 95 °C for 2 h. The absorbance of the resultant colored complex was measured at 532 nm. The TBARS values were calculated by multiplying the absorbance reading by a factor of 0.347. This factor was determined from a standard line prepared using 1,1,3,3-tetramethoxypropane as a precursor of malonaldehyde (MA). The results were expressed in mmol MA equiv/g oil.

The induction period of the oil samples were determined by the Metrohm Rancimat apparatus model 743 (Metrohm, Switzerland) [17]. Then, 4.0 g of each oil sample was weighed in the reaction vessel glassware. The conductimetry cells were filled with deionized water up to 90 mL. Samples were heated at 110 °C and air was passed through the heated oil at a rate of 20 L/h. The induction period was determined automatically by the device and expressed in hours.

The infrared spectra of the samples were recorded on a FTIR (Varian 1000 Model) system with a horizontal attenuated total reflectance (ATR) apparatus [18]. The spectrometer was equipped with a deuterated triglycine sulfate detector and purged with dry nitrogen (DuraDry, Haverhill, MA). The ATR crystal was cleaned with pure chloroform before each measurement. A 40-µL oil sample was spread as a thin layer in the ATR crystal and periodical scans (18 scans, 4 cm^{-1} resolution) were obtained in the spectral range of 400–4,000 cm^{-1} at 20-min intervals for 360 min. The induction times, i.e. the time needed for a dramatic increase in absorbance, were determined algebraically [18].

Metal Analysis

Some metal contents of these samples were determined by methods, as described by Cindric et al. [19]. A 0.5-g sample of oil was digested using a mixture of 6 mL nitric acid in a microwave digestion system. All the glassware was cleaned with nitric acid prior to use. The oils were digested according to the following optimized procedure [program power(W)/time(min)]: 250/1, 0/2, 250/10, 650/5, 600/5, ventilation 7 min and measured by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP–OES). The instrument-operating parameters are summarized in Table 1. For ICP–OES (Perkin Elmer Optima 2100

Table 1 ICP–OES plasma condition

	Emission wavelength (nm)	
Power 1,300 W	Zn	206.000
Plasma velocity of gas flow 15 L/min	Fe	238.204
Auxiliary velocity of gas flow 0.2 L/min	Ca	317.933
Nebulizer velocity of gas flow 0.80 L/min	Mg	285.213

DV, USA), the measurements were accomplished by calibration using aqueous mixed standards prepared in HNO_3 (1 M). The standard stock solutions (1 g/L) were used diluted by fivefold standard dilutions.

Statistical Analyses

Experimental data were evaluated by using analysis of variance (ANOVA) and the significant differences amongst the means of the three replicates ($P < 0.05$) were determined by Duncan's multiple range test, using the ''SPSS 9.0 for Windows".

Results and Discussion

Total Oil Content

Total oil content of seeds is shown in Table 2. The oil contents of these seeds were found ranging from 30.68 to 43.12%. Terebinth and radish seed had the highest oil content (43.12 ± 0.34%, 42.64 ± 1.36%, respectively), followed by laurel seed (36.82 ± 0.36%), hemp seed (31.48 ± 1.19%) and stinging nettle seed (30.68 ± 1.78%) ($P < 0.05$). In a previous study concerning the hemp seed, the oil content identified and obtained ranged between 25 and 35% [4]. In the present study, the total oil content was identified as 31.48%. In another study concerning the terebinth seed, the total oil content identified and obtained ranged from 35.8 to 43.1% [3]. In the present study, the total oil content was identified as 43.12%. These results agreed with the literature data.

Fatty Acids

The fatty acids of the oilseeds are shown in Table 2. It was determined that these analyzed oils which are very essential for health, are rich in oleic and linoleic acids [20]. The oleic acid content of terebinth seed oil contributed 50.58 ± 0.82% to the total fatty acids, followed by laurel seed oil (36.41 ± 0.38%), stinging nettle (19.09 ± 1.01%), and radish seed oil (15.63 ± 0.04%) ($P < 0.05$). The linoleic acid content of stinging nettle seed oil contributed 66.37 ± 0.10% to the total fatty acids, followed by hemp seed oil (55.48 ± 0.12%), terebinth seed oil (19.88 ± 0.02%), laurel seed oil (19.06 ± 0.05%), and radish seed oil (10.09 ± 0.76%) ($P < 0.05$). The linolenic acid content of hemp seed oil contributed 21.5 ± 0.14% to the total fatty acids ($P < 0.05$). The erucic acid content of radish seed oil contributed 40.83 ± 1.48% to the total fatty acids. In a research aiming to define the components of fatty acids of terebinth seed oil taken from different regions, oleic acid mostly ranged between 43 and 51.3% [3]. In the present research, the oleic acid content of terebinth seed oil is 50.58 ± 0.82%. When the values were compared, it was observed that the present study agreed with the those found in the literature. Likewise, the components of stinging nettle and hemp seed oil fatty acids also complied with the literature [4, 6]. Total saturated fatty acids (SFA) were found ranging from11.92 ± 0.07 to 41.00 ± 0.17%, total monounsaturated fatty acid (MUFA) were found ranging from 10.55 ± 0.19 to 62.28 ± 0.09% and total polyunsaturated fatty acid (PUFA) were found ranging from 17.11 ± 0.83 to 77.53 ± 0.09% (Table 2). For all the oils, total unsaturated fatty acids value (monounsaturated and polyunsaturated

Table 2 Fatty acid composition and total oil content of samples

Fatty acid	Hemp	Terebinth	Radish	Stinging nettle	Laurel
Lauric (C12:0)	nd	nd	nd	nd	17.31 ± 0.66
Palmitic (C16:0)	7.06 ± 0.11c	23.34 ± 1.03a	4.08 ± 0,33d	7.57 ± 0.04c	17.53 ± 0.02b
Stearic (C18:0)	2.77 ± 0.09b	1.51 ± 0.02d	1.81 ± 0.04c	4.11 ± 0.01a	1.80 ± 0.13c
Oleic (C18:1)	10.55 ± 0.19d	50.58 ± 0.82a	19.08 ± 1.01c	19.88 ± 0.02c	36.41 ± 0.38b
Linoleic (C18:2)	55.48 ± 0.12b	19.88 ± 0.20c	10.09 ± 0.76d	66.37 ± 0.10a	19.06 ± 0.05c
Linolenic (C18:3)	21.51 ± 0.14a	1.13 ± 0.01d	7.02 ± 0.91b	0.82 ± 0.01e	1.99 ± 0.01c
Heneicosanoic (C21:0)	0.42 ± 0.14b	0.19 ± 0.01b	10.31 ± 1.18 a	0.34 ± 0.01b	0.74 ± 0.19b
Erucic (C22:1)	nd	nd	40.83 ± 1.48	nd	nd
ΣSFA	11.92 ± 0.07	25.69 ± 0.25	20.61 ± 0.32	12.87 ± 0.01	41.00 ± 0.17
ΣMUFA	10.55 ± 0.19	53.30 ± 0.30	62.28 ± 0.09	19.94 ± 0,02	37.95 ± 0,16
ΣPUFA	77.53 ± 0.09	21.01 ± 0,11	17.11 ± 0.83	67.19 ± 0,06	21.05 ± 0.03
Total oil content (%)	31.48 ± 1.19c	43.12 ± 0.34a	42.64 ± 1.36a	30.68 ± 1.78c	36.82 ± 0.36b

Fatty acid contents are given as % peak area, For all oils; some fatty acids which have peak areas below 1% are not shown in the table. Each value is the mean ± SD of triplicate determinations

Means with different letters in the rows are significantly different ($P < 0.05$)

nd not detected, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

Table 3 Tocopherol isomers of samples, parameters of oxidative stability and antioxidant tests

	Hemp	Terebinth	Radish	Stinging nettle	Laurel
α-Tocopherol	25.58 ± 0.58c	102.21 ± 1.01a	28.66 ± 0.23c	34.96 ± 0.29b	28.96 ± 3.23c
β-Tocopherol	5.96 ± 0.05c	3.24 ± 0.23d	12.41 ± 0.72b	nd	313.96 ± 0.05a
γ-Tocopherol	597.91 ± 12.14a	130.54 ± 8.65d	545.67 ± 15.55b	372.29 ± 1.17c	45.33 ± 0.94e
δ-Tocopherol	39.71 ± 1.47a	13.79 ± 1.94b	12.41 ± 0.12b	3.80 ± 0.17c	3.29 ± 0.41c
PV	0.90 ± 0.64c	2.13 ± 0.29b	3.73 ± 0.51a	0.85 ± 0.17d	0.51 ± 0.12e
TBARS	6.43 ± 0.48a	0.68 ± 0.02e	4.43 ± 0.01b	3.88 ± 0.68c	1.34 ± 0.13d
DPPH	62.37 ± 0.32b	52.13 ± 0.75c	52.62 ± 1.16c	46.01 ± 0.22d	85.79 ± 4.81a
ABTS	39.69 ± 0.46c	46.53 ± 1.15b	35.57 ± 0.13d	33.18 ± 0.69e	85.28 ± 1.16a

Tocopherol isomers are expressed as mg/kg oil. TBARS are expressed mmol MA equiv/g oil. DPPH and ABTS values are expressed as mg trolox/100 g oil. Each value is the mean ± SD of triplicate determinations

Means with different letters in the rows are significantly different ($P < 0.05$)

nd not detected

fatty acid) was higher than the saturated fatty acids (SFA) value. More saturated fatty acid was determined in laurel seed oil in comparison with the other oils (lauric, palmitic, stearic acids). These oils are basically used for human diets as all the oil samples contain unsaturated fatty acids, such as oleic, linoleic and linolenic acids. Furthermore, linoleic and linolenic acids are known to reduce the incidence of cancer and heart diseases [21]. Thus, terebinth, hemp, stinging nettle, laurel oil seeds may be used in edible oil production.

Tocopherol Content

Tocopherol isomers are shown in Table 3. Terebinth seed oil had the highest α-tocopherol isomer value (102.21 ± 1.01 mg/kg oil) ($P < 0.05$). Hemp and radish seed oil had the highest γ-tocopherol value (597.91 ± 12.14 and 545.67 ± 15.55 mg/kg oil, respectively). Hemp oilseed had the highest δ-tocopherol value (39.71 mg/kg oil). Laurel seed oil had the highest β-tocopherol isomer value (313.96 ± 0.05 mg/kg oil). Tocopherols are important antioxidant compounds for oils. The oxidative stability of the oils is mostly based on these compounds [22]. Antioxidants are used as lipid stabilizers in the food industry [23]. Moreover, antioxidants have positive effects on oxidation, a destructive force which causes cancer and ageing [2]. Recently natural antioxidants have been preferred rather than synthetic antioxidants, due to health concerns. Therefore, cold-pressed seed oils may be used as food additives for improving food quality and stability.

Antioxidant Tests

The DPPH and ABTS radical scavenging capacities are shown in Table 3. The radical-scavenging activity of oils may be influenced by the radical system and other testing conditions. For estimating the radical capacities of antioxidants, stable radicals such as DPPH and ABTS [24] were used. Laurel seed oil had the strongest DPPH-scavenging capacity amongst the five tested oil extracts (85.79 mg trolox/100 g oil), followed by hemp seed oil (62.37 mg trolox/100 g oil), radish seed oil (52.62 mg trolox/100 g oil), terebinth seed oil (52.13 mg trolox/100 g oil), and stinging nettle seed oil (46.01 mg trolox/100 g oil) ($P < 0.05$). Laurel seed oil had the strongest ABTS scavenging capacity (85.28 mg trolox/100 g oil), followed by terebinth seed oil (46.53 mg trolox/100 g oil), hemp seed oil (39.69 mg trolox/100 g oil), radish seed oil (35.57 mg trolox/100 g oil), and stinging nettle oil (33.18 mg trolox/100 g oil). It was determined that laurel seed oil had the highest activity in both the tests ($P < 0.05$). It might be due to contents of tocopherol and phenolic compounds Fig. 1.

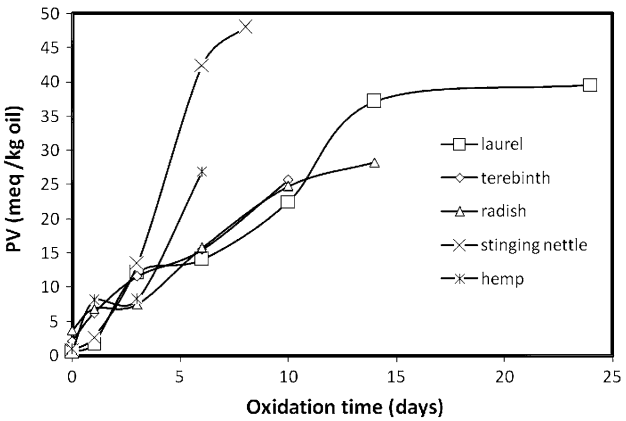

Fig. 1 PV values of the oil samples versus the time in days of their oxidation process

Oxidative Stability Test

The PV is commonly used to estimate the level of oxidative deterioration in heated oils [25]. The PV of the oils tested

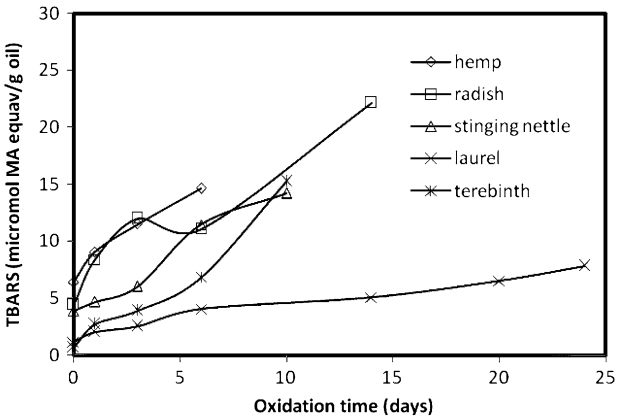

Fig. 2 TBARS values of the oil samples versus the time in days of their oxidation process

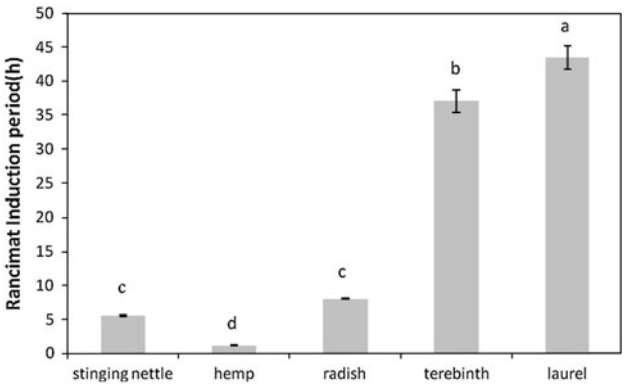

Fig. 3 Rancimat induction period of oil samples. Different letters on the bars indicate statistically significant differences between the means ($P < 0.05$)

in the present study is given in Table 3. The PV of non-oxidized oils ranged between 0.51 and 3.73 mequiv O_2/kg oil. The Codex Alimentarius Commission (1982) stipulated a permitted maximum peroxide level of not more than 10 mequiv/kg oil [25]. As a result, the peroxide value of these edible oils was below the acceptable level. The variations amongst the peroxide values in the oils kept in 60 °C oven for oxidation are shown in Fig. 2. The PV values of the oils increased at various ratios. The rise in the PV value of laurel oil continued from day 1 to day 14 and reached its maximum value on the 24th day. In the other oils, the rise of PV was very fast. In hemp oil, the pronounced rise in PV was produced between day 3 and day 5, in stinging nettle oil between day 1 and day 3, and in radish and terebinth oil between day 3 and day 7. Hemp oil is also known to be highly unstable, despite the presence of different minor antioxidant components such as tocopherol that play a dramatic role in oxidative stability [15]. Hemp seed oil has high content of unsaturated fatty acids which are highly susceptible to oxidation. So, we can say that hemp seed oil is more prone to oxidation than others. In contrast, laurel seed oil was more stable than the others. It may be due to a higher content of saturated fatty acids and higher antioxidant activity.

Secondary oxidation products are indicators of oil rancidity. We used the TBARS method that is commonly used to estimate the secondary oxidative products in heated oils [15]. TBARS of the oils tested in the present study is given in Table 3. TBARS values of hemp, stinging nettle, terebinth and laurel seed oil increased over 5, 10, 10 and 24 days, respectively. A sharp increase in TBARS values was noted for radish oil for the first 3 days followed by a decrease and then they increased again. This may be due to volatilization of secondary oxidation products or their break down.

The Rancimat induction periods of oils are shown on Fig. 3. According to the result of the Rancimat analysis, the induction periods of the oils ranged between 1.32 and 43.44 h. Laurel had the highest induction period (43.44 h), followed by, terebinth seed oil (37.55 h), radish seed oil (8.02 h), stinging nettle seed oil (5.57 h), and hemp seed oil (1.32 h) ($P < 0.05$). The Rancimat method is a powerful and fast technique for estimating the oxidative stability of oils [17, 26]. Laurel and terebinth had higher induction periods than the other oils ($P < 0.05$). The tocopherol content and unsaturation degree of oil have a significant impact on its oxidative stability [16]. Lower Rancimat induction times were observed for oils containing highly unsaturated fatty acids, such as hemp seed and stinging nettle. The seed oils which have high antioxidant activity and high content of fatty acid were observed to be stable during the oxidation process.

FTIR spectroscopy, used for detecting and quantifying functional groups arising during the oxidative degradation of lipids is a new technique. At present, the characteristic wavenumber regions of the oxidation products are used for observing their formation. Non-oxidative fats have a very low level of hydroperoxide shown as R–O–O–H. During oxidation, the hydroperoxide level increases due to the binding of oxygen to double bonds [27]. The infrared spectrum of non-oxidized stinging nettle oilseed is shown Fig. 4. The overall appearance of the FTIR spectra of non-oxidized edible oils is very similar, but they show differences in absorbance of the bands because of their different composition. Figure 5 illustrates the important spectral changes under the oxidative conditions produced in stinging nettle oil on different days. An increase and decrease in some of the wavenumber regions was observed. However only the regions that were known to be due to certain oil oxidation products were evaluated. The wavenumber region of 700–725 cm^{-1} belongs to the *cis* double bonds in unsaturated fatty acids [28]. During oxidation owing to the loss of *cis* double bonds, the weak band near 1,650–1,700 cm^{-1} is associated with the stretching vibration of the carbon–carbon

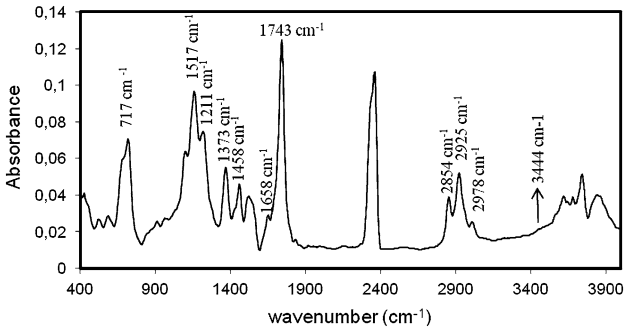

Fig. 4 FTIR spectrum of non-oxidized stinging nettle oilseed

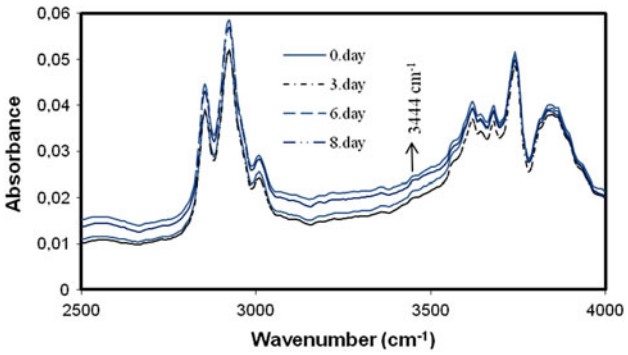

Fig. 5 Changes produced in the region between 2,500 and 4,000 cm^{-1} the FTIR spectrum of stinging nettle oil on different days of the process

double bonds of *cis*-olefins. The non-oxidized oil samples show the band of the triglyceride ester groups at 1,746 cm^{-1} [29]. The absorbance increase near the 3,400–3,600 cm^{-1} region was reported to be caused by the formation of hydroperoxides and alcohols [28]. Generally it is accepted that 3,444 cm^{-1} is a characteristic band for hydroperoxides [30]. Due to the increase in the hydroperoxide ratio, the bands observed in oxidized oils had higher levels than those observed in unoxidized oils. A summary of various spectral changes and their possible oxidative significance is given in Table 4.

Metal Analysis

It is important to know the metal contents of the oils to define their nutritional values and shelf-life. Some metals are required for the normal functioning of all the biochemical processes in the body [34]. In the present study, the contents such as Ca, Mg, Zn and Fe were analyzed by ICP–OES. The metal contents of samples are shown in Table 5. The Ca content of the oils ranged between 338.21 and 680.25 mg/kg oil. The Fe and Zn contents of the oils ranged between 9.83 and 16.58 mg/kg oil, 1.74 and 22.20 mg/kg oil, respectively. The study of metal analysis of the oils is scarce. The presence of metals is important for oil quality and human health. Some metals (Fe, Ni, Cu) are known to accelerate autoxidation and, thus have a

Table 4 Evaluation of the FTIR spectrum for all sample

Spectral region	Responsible groups
3,444 cm^{-1}	Overtone of the glyceride ester carbonyl absorption [30]
3,006 cm^{-1}	C–H stretching vibration of *cis*-double bond (=CH) [31, 32]
2,925 cm^{-1}, 2,854 cm^{-1}	Asymmetric and symmetric stretching vibration of the aliphatic CH_2 functional group [33]
2,962 cm^{-1}, 2,872 cm^{-1}	Symmetric and asymmetric stretching vibration shoulder of the aliphatic CH_3 group [32]
1,743 cm^{-1}	Ester carbonyl functional group of the glycerides [31, 32]
1,654 cm^{-1}	C=C stretching vibration *cis*-olefins [32]
1,458 cm^{-1}	Bending vibrations of the CH_2 and CH_3 aliphatic groups [31]
1,397 cm^{-1}	Bending in plane vibration of CH *cis*-olefin groups[31, 32]
1,373 cm^{-1}	Bending vibration of CH_2 groups [31, 32]
1,238 cm^{-1}, 1,163 cm^{-1}	Stretching vibration of C–O ester groups[32]
717 cm^{-1}	Overlapping of CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted olefins [31]

Table 5 Metal contents of samples

Metals	Hemp	Terebinth	Radish	Stinging nettle	Laurel
Zn	1.74 ± 0.04c	9.95 ± 3.01b	4.56 ± 0.98c	4.63 ± 0.60c	22.20 ± 2.69a
Ca	338.21 ± 28.50c	436.33 ± 7.48b	340.84 ± 10.68c	407.47 ± 47.66b	680.25 ± 6.72a
Mg	74.47 ± 6.07b	69.48 ± 11.52b	57.17 ± 6.26b	79.37 ± 9.12b	104.68 ± 9.93a
Fe	10.94 ± 0.30b	11.78 ± 0.07b	9.83 ± 0.73b	13.06 ± 1.88a	16.58 ± 2.86a

Metal contents are expressed as mg/kg oil. Each value is the mean ± SD of triplicate determinations

Means with different letters in the rows are significantly different ($P < 0.05$)

significant impact on shelf life of the oil products [35]. In the present study, Fe content of oils was at lower level than the other metals.

Conclusion

Cold-pressed hemp, terebinth, laurel, and stinging nettle seed oils contain significant levels of polyunsaturated fatty acids which are important to health. Particularly, hemp seed oil has a high level of linolenic acid. Due to the high content of erucic acid, radish seed oil is not used for the food industry, but it can be used in other industries. These oils, except radish seed oils, are utilized as an important source of antioxidants for food. Natural antioxidants are important for health and dietary conditions. Laurel seed oil has a higher antioxidant activity than the other oils. Therefore, it has the longest induction period.

These oils can be blended with common oils, during storage and heating. These oils can also be used to improve the quality, stability and safety of food products. To investigate the other chemical compounds of these cold-pressed oils and their potential biological activities, further research is required.

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