Short note

Fatty acids profile and stability of Camelina (*Camelina sativa*) seed oil as affected by extraction method and thermal oxidation

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Camelina (*Camelina sativa*) seed oil was extracted using two different methods including hexane extraction to obtain hexane-extracted oil (HEO) and cold pressing extraction to obtain cold-pressed oil (CPO). The major fatty acid was linolenic acid (C18:3) that accounted for 34.5% and 33.9%, in CPO and HEO, respectively. Both extracted camelina oils showed high amounts of linoleic acid (C18:2), eicosanoic acid (C20:1) and oleic acid (C18:1). The oxidative stability was compared for both camelina oils as affected by thermal oxidation. High and moderate temperature experiments were used to determine the resistance of both oils to accelerated oxidation. Rancimat test was applied at 110°C, wherein the oxidation stability index (OSI) value for HEO (7.23 h) was higher than CPO (3.37 h). The increase rates in peroxide value (PV) and conjugated diene (CD) of HEO were higher than CPO during storage at moderate temperature (60°C). The initial PV of CPO and HEO was 3.57 and 4.32 meq O_2/kg, but after 10 days of storage at 60°C PV reached up to 107.30 and 11.26 meq O_2/kg, respectively. At the end of storage, CD values of CPO and HEO increased from 1.46, 1.73 to 10.08, 2.62, respectively. The volatile oxidation compounds including hexanal, 2,4-heptadienal, and (E,E)-2,4-heptadienal were identified in the headspace of CPO at the 10th day of storage at 60°C. It could be concluded that the extraction methods influenced significantly *Camelina sativa* seed oil oil stability and quality.

Keywords: Rancidity, oxidative stability, volatile oxidation compounds, solvent extraction, cold pressed oil.

1. INTRODUCTION

Camelina (*Camelina sativa*) is a member of the *Cruciferae* family and known as false flaxseed, German sesame, gold-of-pleasure, Siberian oilseed, linseed dodder or wild flax [1]. Camelina is an oilseed crop in vast areas of the world. The plant contains high amount of oil with a unique fatty acid composition [2, 3]. Camelina oil has been applied in different industries such as biofuels, jet fuel, feed, pharmaceutical, and cosmetics. This oil is also used in food applications such as salad, cooking oil, margarines, sauces, and dressings [4, 5].
Camelina oil is rich in α-linolenic (ALA, C18:3, 32.5%), linoleic (C18:2, 18.1%), gondoic (C20:1, 16.9%), and oleic (C18:1, 14.8%) acids as reported by Singh et al. [6]. Due to the high levels of ALA, camelina oil has potential health-promoting properties [7]. In addition, camelina oil contains about 15% gondoic acid (20:1 n-9) and about 3% erucic acid (22:1 n-9). These two fatty acids are typical of oils that are obtained from seeds of plants belonging to the *Cruciferae* family [3]. Because of its unique composition and beneficial health impacts, camelina oil has good potential to be used in the production of functional foods and nutraceuticals.
Oxidation is the main cause of loss of quality in fatty foods. The two compositional factors of lipids that determine their susceptibility to oxidation are their

fatty acid composition and the presence of antioxidants. Due to the high content of unsaturated fatty acids in camelina oil, its oxidative stability is an important factor [3]. Despite the health aspects of omega fatty acids, polyunsaturated fatty acids (PUFA) especially ALA in oil tends to oxidise with heat, oxygen, and light [8, 9]. Therefore, the assessment of lipid oxidation in camelina oil is important when formulating and producing foods with camelina oil [7]. Accelerated oxidation tests including Rancimat test (110°C) and Shaal oven test (60°C) were often used to detect lipid oxidation [10-12]. There have been several studies carried out on camelina oil, whereas some studies related to oxidative stability of the oil [5, 8, 13].

Cold pressed oils refer to oils that are extracted by cold pressing of plant seed with a screw press or hydraulic press. Cold pressing is used to extract oil from seeds instead of conventional solvent extraction method because cold pressing does not require the use of organic solvents or heat. Moreover, cold pressing is able to retain bioactive compounds like fatty acids, phenolics, flavonoids and tocols in the oils [14-16].

Despite *Camelina sativa* is an old oilseed crop, this plant is newly introduced to the semi-arid regions of Turkey. The adaptation trials of these seeds have begun in Field Crops Central Research Institute, Ministry of Food, Agriculture, and Livestock (Ankara, Turkey) since 2015 and the seeds are officially registered as 'Aslanbey'. Possible applications of seeds have been investigated. First technical application area of seeds is to produce oil and to study some physico-chemical properties of the oil. The aim of this study was to evaluate the fatty acid composition and oxidative stability of cold-pressed *Camelina sativa* seed oil (CPO) and hexane-extracted *Camelina sativa* seed oil (HEO). Oxidative stability of both oils was determined using two oxidation conditions including high temperature at 110°C (Rancimat test) and moderate temperature at 60°C (Schaal oven test). The oxidation at moderate temperature (60°C) was followed by the determination of peroxide value (PV), conjugated diene value (CD) and volatile compounds content of the analysed oils.

2. MATERIALS AND METHODS

2.1 MATERIALS

The camelina oil used in this study was extracted from seeds of *Camelina sativa* plants grown in 2015 in the Ankara region (Turkey).

2.2 METHODS

2.2.1 Extraction of hexane-extracted oil (HEO) and cold-pressed oil (CPO)

The oil was obtained by two methods including solvent extraction and cold pressing. In the solvent extraction, the seeds were extracted with *n*-hexane using the Soxhlet apparatus for 4 hours. In the cold-pressing extraction, the seeds were directly pressed with screw press at room temperature. The oils were placed in brown glass bottles, flushed with nitrogen, and stored in a refrigerator at 4°C for further analysis.

2.2.2 Fatty acids composition

Fatty acid methyl esters (FAME) were prepared according to IUPAC [17]. FAME were identified by Shimadzu (Kyoto, Japan) gas chromatography equipped with Rtx-2330 capillary column (60 m × 0.25 mm i.d., 0.20 µm film thickness) and FID (flame ionization detector). The temperature for the injector was 250°C and the temperature for the detector was 260°C. The oven temperature was held at 140°C for 5 min, then increased to 240°C at 4°C/min and held at 240°C for 20 min. Helium at a flow rate of 1.0 mL/min was used as a carrier gas. A sample of 1 µL was injected by the autosampler with a split mode (split ratio of 1:100). FAME were identified by comparison with standards and were quantified by the area percentage of each FAME. FAME standards were purchased from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany).

2.2.3 Thermal oxidation experiments

2.2.3.1 Rancimat test

Stability of tested oils was determined by the Rancimat test using a 743 Rancimat Metrohm apparatus (Switzerland) according to the official AOCS method Cd 12b-92 [18]. The test was carried out at a constant temperature (110°C) with air flow of 20 L/h, using 3 g oil sample and 0.06 L distilled water in a conductometric vessel.

2.2.3.2 Schaal oven test

Oil samples (20 g) were transferred to glass brown bottles (100 mL) and the bottles were closed. The oxidation was carried out in a forced-draft air oven at the temperature of 60°C for 10 days. The peroxide value (PV), conjugated diene (K_{232}) and volatile compounds released from both oils were analysed at the end of every twin-day up to the end of the tenth day of storage under thermal oxidation conditions (60°C).

2.2.3.3 Peroxide value (PV) and conjugated diene (CD) determination

Peroxide value (PV) of oils were iodometrically defined with respect to AOCS method Cd 8-53 [18]. The oils were analysed for the conjugated diene (K_{232}) according to AOCS method Cd 18-90 [18].

2.2.3.4 HS/SPME-GC/MS analysis of volatile oxidation compounds

Two grams of oil sample was placed in 20 mL headspace vial and subjected to balance for the duration of 15 min at the constant temperature of 35°C [19]. The headspace of samples was extracted for 45 min at 35°C with the aid of a CTC Combi PAL (CTC Analytics AG, Zwingen, Switzerland) autosampler with 75 µm carboxen/polydimethylsiloxane (CAR/PDMS) solid phase micro extraction (SPME) fibre. The volatile compounds were directly desorbed by inserting the fibre for 10 min into the injection port of the gas chromatography maintained at the constant temperature of 250°C.

An Agilent model 7890 Series (Agilent Technologies, Santa Clara, CA, U.S.A.) gas chromatograph in combination with a CTC Combi PAL autosampler and an Agilent 5975 N (Agilent Technologies, Santa Clara, CA, U.S.A.) mass selective detector was used to analyse volatile oxidation compounds. The compounds were separated in a capillary column of DB-624 (30 m length × 0.25 mm ID × 1.4 µm film thickness, Agilent Technologies, Santa Clara, CA, USA) with the following temperature program: hold for 5 min at 40°C; 3°C/min up to 110°C; 4°C/min up to 150°C; 10°C/min up to 210°C and hold for 12 min. The temperatures for the injection port, ion source, quadrupole, and interface were set to be 250°C, 230°C, 150°C, and 240°C, respectively. Mass spectra were recorded in full scan mode at the electron impact of 70 eV with the scan range from m/z 41 to 400.

The identification of compounds was detected by comparing mass spectra and Kovats index (KI) with the authentic standards and published data, as well as by comparing their mass spectra with the mass spectrometry library of Nist05 (National Institute of Standards and Technology, Gaithersburg MD, USA) and Wiley7.0 (Wiley, NY, USA). The parameters of KI were calculated using the series of n-hydrocarbons (C4 to C20).

3. RESULTS AND DISCUSSION

3.1 FATTY ACID COMPOSITION OF HEO AND CPO

Fatty acid composition of camelina oils is presented in Table I. Sixteen fatty acids were identified in camelina HEO and CPO. It could be noted form the results in Table I that the extraction method did not affect the fatty acids profile in camelina HEO and CPO. In both oils, the major fatty acid was linolenic acid (C18:3) that accounted for 34.56% and 33.92% in CPO and HEO, respectively. The levels of C18:3 were slightly lower than that reported by Raczyk et al. [20] who detected 35.35-37.64% of linolenic acid in camelina oil. The results were similar to those reported by Vollmann et al. [21] who detected 25.2-42.5% of linolenic acid, Angelini et al. [22] who detected 21.6-34.8% of linolenic acid, and Budin et al. [23] who detected 27.0-34.7% of linolenic acid in camelina oil. Both types of camelina oils showed high levels of linoleic acid (C18:2), eicosanoic acid (C20:1) and oleic acid (C18:1). The content of C18:2, C20:1 and C18:1 in CPO and HEO were generally close to the results reported by Vollmann et al. [21], Gugel and Falk [24], Szterk et al. [13], and Raczyk et al. [5]. Erucic acid (C22:1) content in the CPO and HEO was 2.82% and 2.80%, respectively and these values of erucic acid is considered within the limit values (< 5%). Both camelina oils could be suitable for human consumption as edible oils after performing nutritional studies.

3.2 RANCIMAT TEST

Figure 1 shows the oxidation stability index (OSI) of CPO and HEO of camelina. The OSI value in HEO (7.23 h) was two-fold higher than in CPO (3.37 h). The obtained results could be explained because solvent-extracted oils usually contain high levels of antioxidants including tocols, sterols and polar lipids (glycolipids and phospholipids) [9, 12]. The OSI value of oil samples were higher than the results of Fröhlich et al. [25] for unrefined camelina oil (2.4 h). These differences could be related to use of flow rate in 10 L/h in Rancimat method. Raczyk et al. [20] obtained higher values for OSI in cold pressed oils (4.58-6.18 h) than those found in our study. The dissimilarities in the OSI values of the samples were related to applied temperature (100°C) and sample amount (2.5 g) in the literature for Rancimat conditions.

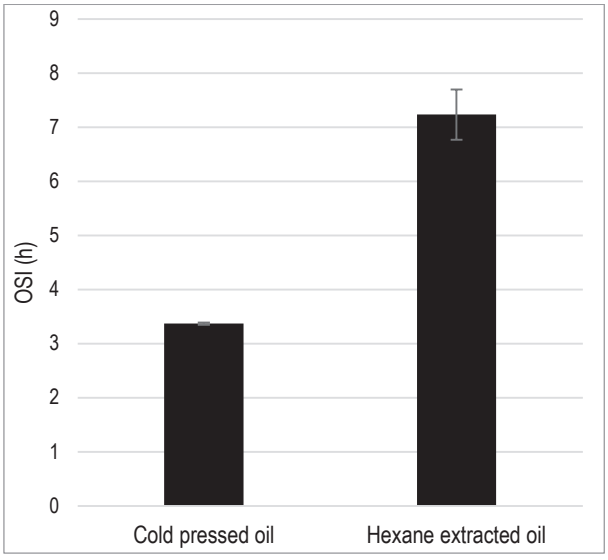

Figure 1 - OSI values of CPO and HEO stored at 110°C. Vaules are means of three determinations ± standard deviation.

Table I - Fatty acid composition % of HEO and CPO*

	RT	Fatty acid	CPO	HEO
1	13.531	Myristic acid (C14:0)	0.05 ± 0.01	0.05 ± 0.01
2	17.411	Palmitic acid (C16:0)	5.12 ± 0.01	5.24 ± 0.03
3	18.504	Palmitoleic acid (C16:1)	0.07 ± 0.01	0.07 ± 0.01
4	19.263	Heptadecanoic acid (C17:0)	0.04 ± 0.00	0.04 ± 0.01
5	20.297	*cis*- 10heptadecanoic acid (C17:1)	0.02 ± 0.00	0.02 ± 0.00
6	21.184	Stearic acid (C18:0)	2.66 ± 0.01	2.70 ± 0.01
7	22.172	Oleic acid (C18:1)	14.90 ± 0.02	14.92 ± 0.02
8	23.639	Linoleic acid (C18:2)	17.11 ± 0.02	17.66 ± 0.01
9	24.729	Arachidic acid (C20:0)	1.54 ± 0.00	1.58 ± 0.00
10	25.360	Linolenic acid (C18:3)	34.56 ± 0.05	33.92 ± 0.08
11	25.656	*cis*-11-eicosenoic acid (C20:1)	16.67 ± 0.03	16.53 ± 0.05
12	26.936	Heneicosanoic acid (C21:0)	2.07 ± 0.01	2.08 ± 0.00
13	27.876	Behenic acid (C22:0)	0.30 ± 0.00	0.30 ± 0.00
14	28.492	*cis*-8,11,14-eicosatrienoic acid (C20:3)	1.35 ± 0.02	1.33 ± 0.01
15	28.761	Erucic acid (C22:1)	2.82 ± 0.02	2.80 ± 0.01
16	30.025	*cis*-13,16-docosadienoic acid (C22:2)	0.11 ± 0.01	0.12 ± 0.01
17	30.883	Lignoceric acid (C24:0)	0.17 ± 0.01	0.17 ± 0.00
18	31.797	Nervonic aid (C24:1)	0.51 ± 0.00	0.52 ± 0.01

(*) Vaules are means of two determinations ± standard deviation.

3.3 SCHAAL OVEN TEST

Figure 2 shows the changes in PV in oils during storage at 60°C. The increase rates in PV of CPO were significantly higher than HEO. PV in CPO increased dramatically during storage, while the PV in HEO increased slowly and showed a rough trend for the increase rate in the oil during storage at 60°C. The PV of fresh CPO and HEO were 3.57 and 4.32 meq O_2/kg, respectively. At the end of 10 days of storage at 60°C, the PV of CPO and HEO were 107.30 and 11.26 meq O_2/kg, respectively. These results were in accordance with previously reported results for raw camelina oil [13], which mentioned that the changes in PV were in dynamic trend along thermal storage.

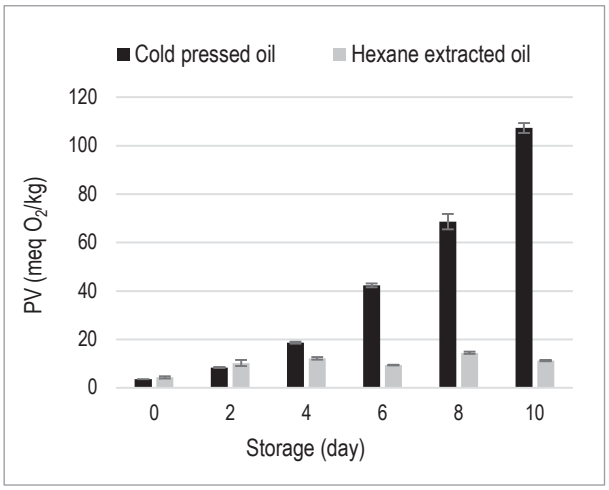

Figure 2 - Changes in PV of CPO and HEO during storage at 60°C. Values are means of two determinations ± standard deviation.

The other similarity with the literature was observed in the study by Ni Eidhin et al. [26] in which it is declared that the increase rate in PV of cold-pressed camelina oil occurred rapidly. The obtained results could be explained because extraction using organic solvent is more capable of extracting polar lipids (glycolipids and phospholipids) characterised by significant antioxidant properties under thermal oxidation [9].

Figure 3 shows the changes in K_{232} values of CPO and HEO during storage at 60°C. The increase trend of oils for K_{232} values was similar to PV along the storage period. Cold-pressed camelina oil had higher K_{232} values than hexane-extracted camelina oil. After 10 days of storage, the K_{232} value of CPO reached up to 10.08, while the K_{232} value of HEO was 2.62. The results for CPO agree with other findings previously reported by Ni Eidhin *et al.* [26] that mentioned that the K_{232} values of cold-pressed oils varied markedly during thermal storage.

According to the results of PV and K_{232} values during storage at 60°C, HEO had higher oxidative stability than CPO. These differences could be related to the extraction technique. Solvent extraction is more capable and more powerful in extracting lipid bioactive compounds including polar lipids, sterols, tocols, that contribute to the oxidative stability of the oil [27, 28]. Crude camelina oil had high a tocopherol amount (especially γ-isomers) and total phenolic content with potential antioxidative traits [3].

3.4 VOLATILE OXIDATION COMPOUNDS

During storage at 60°C, the volatile oxidation compounds were analyzed. Only on the 10th day of storage at 60°C, three volatile oxidation compounds including

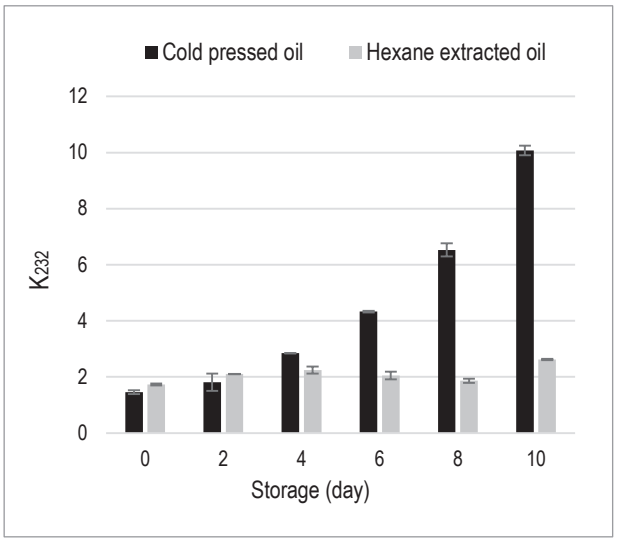

Figure 3 - Changes in K_{232} of CPO and HEO during storage at 60°C. Values are means of two determinations ± standard deviation.

hexanal, 2,4-heptadienal and (*E,E*)-2,4-heptadienal were detected in CPO. The value of these compounds was not shown on a separate table or figure due to lack of data. The average value of these compounds mentioned above were determined as 3.99, 0.51 and 0.45 × 10^6 AU, respectively. Hexanal and (*E,E*)-2,4-heptadienals levels were increased with oxidation of linseed oil that is rich in linolenic acid like camelina oil [29]. These volatile oxidation compounds have been identified in our study.

4. CONCLUSION

Interest in camelina oil was inspired by the recent search for natural antioxidants and for new vegetable sources of PUFA. The results of this study showed that the extraction methods and conditions influenced greatly the oil quality. Hexane-extracted oil had a significantly higher OSI value than CPO according to Rancimat test. The results of PV and CD values of HEO were lower than CPO during storage at moderate temperature (60°C). Results showed that HEO had a higher resistance to oxidation compared to CPO. Even though cold-pressed oils are assumed as healthy oils, oxidative stability of these oils was lower when compared with solvent-extracted oils.

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