Short note

Fatty acids, triglycerides, tocol, and sterol contents of oils of some Moringa seed varieties

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Three moringa oliefera varieties (MOMAX 3, ODC and PKM-1) seeds were used in this study. The oil was extracted from the seeds of ODC, PKM1 and MOMAX 3 moringa varieties by cold press (CP) and the solvent extraction (SE) method.
Fatty acids, triglycerides, tocols (tocopherols and tocotrienols) and sterol contents of moringa seed oils obtained by two different methods were
determined and compared with one another. The crude oil yield was between 26.46 - 28.19% in solvent extraction and 24.20 - 26.30% in the cold press method. It was determined that the main fatty acid in moringa seed oils (ODC, PKM1 and MOMAX 3) was Oleic acid (63.31 - 70.04%). Other dominant fatty acids were determined to be palmitic acid (16:0) (5.59-7.26%), stearic acid (18:0) (5.37-5.89%), oleic acid (18:1n9) (%63.31-70.04), arachidic acid (20:0) (2.67 - 3.71%) and behenic acid (22:0) (3.82 - 5.73%). It was determined that the main triglyceride in moringa seed oils was triolein (OOO; 35.63 - 36.50%), the main sterol was β-Sitosterol (39.04 - 42.11%) and the main tocopherol was α-tocopherol (15.88 - 18.91 µg/g).
Keywords: moringa, seed oil, fatty acid, triglyceride, sterol, tocopherol

1. INTRODUCTION

It is known that medicinal and aromatic plants have been used in many sectors such as food, medicine, cosmetics and spices since the onset of human history. One of the most important features of medicinal and aromatic plants is their use for therapeutic purposes [1]. Herbal treatment has many advantages over chemical treatment, such as less side effects, lower cost and higher availability [2].
moringa is a versatile plant used for medicinal, aromatic, and therapeutic purposes. Moringa (*Moringa oleifera*-MO), a member of the *Moringaceae* family, is native to India. Moringa grows best in dry, sandy, or slightly alkaline soil. Various parts of the Moringa plant (leaf, seed, etc.) can also be consumed directly by eating them [3]. According to a report from the Bureau of the Plant Industry, moringa is reported to be an excellent food source. It has been stated that only leaves (according to the amount of dry matter) contain four times more calcium than milk, seven times more vitamin C than oranges, three times more potassium than bananas, three times more iron than spinach, four times more vitamin A than carrots and twice more protein than milk. In addition, the bark, and leaves of moringa contain high amounts of Ca, Mg, K, Mn, P, Zn, Na, Cu and Fe [4]. Moringa is a great source of protein, and its protein content ranges from 7.12% to 39.17%.
In addition, it contains very low fat and carbohydrates. At the same time, moringa delays aging because it contains effective antioxidants and high levels of vitamins A, C and D. Antioxidants reduce the onset of wrinkles on the skin and fine lines on the face and help prevent and cure various chronic diseases such as arthritis, cancer, heart, and kidney diseases. The leaves can be con-

sumed directly by being dried and powdered. It has also been stated that moringa leaves have a prebiotic effect and regulate the intestinal flora [5].
Depending on the variety, moringa seeds contain about 30-60% oil. Moringa seed oil is commercially known as Behen oil or Ben oil [6, 7]. Moringa oil is classified as a nutraceutical food because of its chemical composition (tocopherols, triglycerides, fatty acids, sterols, and minor components), which includes high nutritional qualities linked to potential health advantages such as decreasing blood sugar levels and reducing the risk of cardiovascular disease. In addition to this, it can be used as an adjunct in the treatment of hyperthyroidism, rheumatism, gout, cramps, epilepsy and with these properties, it can function as antimicrobial and anti-inflammatory agents. Moringa esters might also be used in lubricants or as environmentally friendly additives [8]. *Moringa oleifera* oil is known to contain very high amounts of oleic acid (70%), palmitic acid, stearic acid, behenic acid, quercetin, luteolin and tocopherol [9].
The oxidative stability of *Moringa oleifera* oil is higher than that of canola oil, palm oil and soybean oil for use as frying oil [10, 11].
The fatty acid composition of moringa seed oil is equivalent to olive oil. This oil is also used in medical and mechanical industries [10]. Moringa oil has the potential to become a new source of oleic acid-type vegetable oil. Moringa oil, surprisingly, has a lower linoleic acid level (4.2%) than other vegetable oils (such as soybean, palm, and canola oils). Cold press (CP), solvent extraction (SE), enzyme-assisted aqueous extraction, and supercritical carbon dioxide extraction are some methods used to extract oil from *Moringa oleifera* seeds (SC-CO_2) [12].
In this study, oil was extracted from the seeds of ODC, PKM1 and MOMAX 3 moringa varieties by cold pressing and solvent extraction method. Fatty acids, triglycerides, tocols (tocopherol and tocotrienols) and sterol contents of moringa seed oils obtained by two different methods (cold pressing and solvent extraction) were determined and compared with one another.

2. EXPERIMENTAL PROCEDURES

Three *Moringa oliefera* varieties (MOMAX 3, ODC and PKM-1) seeds were used in this study. Seeds of MOMAX 3, ODC and PKM1 moringa varieties were supplied by "MORINGANTEP Entrepreneurial Women Production and Development Cooperative", approximately 2 kg each from seeds imported directly from SVM Exports (No: 2F/1049 P&T Colony 6th Street West Tuticorin-628 008-Tamil Nadu, India). These seeds were packaged under vacuum in polyethylene bags and stored at room conditions until oil extraction. Moringa oils obtained by cold pressing and solvent extraction method were stored at -18°C until analysis.
Naturefuel 500 (NF 500) model cold press oil machine manufactured by Karaerler Machine (Ankara, Turkey) was used for the extraction of oil by cold pressing method.
Extraction of crude oil from moringa seeds with the Soxhlet method was carried out using the "Gerhardt Soxtherm brand SE-414" model oil extraction device.

2.1. EXTRACTION OF OILFROM MORINGA SEEDS

Seed oil was obtained from the seeds of MOMAX 3, ODC and PKM1 moringa varieties by cold pressing and solvent extraction methods in this study.

2.1.1. Extraction of Crude Oil from Moringa Seeds by Cold Pressing

Oil extraction from moringa seeds by cold pressing method was carried out with a cold press oil machine (Karaerler brand NF500 model, Turkey). Moringa seeds was pressed at a frequency of 15 Hz at 40 - 45°C in a cold press oil machine preheated to 100°C. It was ensured that the oil obtained was below 45°C. For this purpose, 300 g of seeds of MOMAX 3, ODC and PKM1 moringa varieties were weighed and fed through a cold-pressed oil machine to obtain moringa oil. The crude oil yield (%) was obtained by weighing the amount of cold pressed oil samples.

2.1.2. Extraction of Crude Oil from Moringa Seeds by Soxhlet Method

Extraction of crude oil from moringa seeds by Soxhlet method was carried out using a Gerhardt Soxtherm brand SE-414 model oil extraction device. A 10 g sample of each of the three moringa seeds was ground in a Waring brand blender, dried at 105°C for 2 hours and placed in a Soxhlet cartridge. Then, 150 mL of petroleum ether was added to the soxhlet flask which was placed in the extractor. At the end of the process, by reweighing the extraction container, the tare of which was determined before, the amount of crude oil in it was determined as a percentage based on dry substance with the help of the following formula:
Crude Oil (%, w/w) = $[100 \times (M_2 - M_1) / M_0]$
M_0 : Mass of the test sample (w)
M_1: Tare of extraction flask (w)
M_2: Mass of oil and balloon together after extraction (w)

2.2. DETERMINATION OF FATTY ACID COMPOSITION IN MORINGASEED OILS

For esterification in the fatty acids of oils of moringa varieties, 0.1 g of oil was taken into test tubes and 10 ml of n-heptane was added. Then 0.5 ml of methanolic KOH solution was added and the tubes were shaken vigorously for 30 seconds and centrifuged at 4000 rpm for 10 minutes. Then, the supernatant was placed into 2 ml vials and prepared for injection. Fatty acids methyl ester analyses were performed on a gas chromatography (Agilent 7820A GC) equipped with

flame ionization detector (FID). The fatty acid methyl esters (FAMEs) analysis was performed on an HP-88 Column (100 m × 250 µm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 15mL/min. Detector temperature was set at 260°C and the column oven temperature at 230°C. The initial oven temperature was gradually increased starting from 50°C. It was increased at a ratio of 10°C/min to 175°C and preserved at 175°C for 10 min. Then the temperature was raised to a ratio of 2°C/min to 210°C and preserved at 210°C for 5 min. It was raised to a ratio of 10°C/min to 230°C and kept at 230°C for 2 min. A 1 µL of each of the diluted samples [n-heptane 1/100(v/v)] was injected automatically in split mode (1/100). The composition of fatty acids (%) was determined by defining individual fatty acids by checking with retention time of known standards and denoted as a percentage of the total fatty acids.

2.3. ANALYSIS OF TRIACYLGLYCEROLS BY HPLC

Triacylglycerol analysis was carried out by modifying the COI method (2017) and the method used by Essid et al. (2014) [13, 14]. Agilent Infinity II 1260 HPLC device with a reversed phase column and refractive index detector (RID) was used. Triacylglycerols were separated from other oil components by column chromatography. The oil sample dissolved in petroleum ether was placed on a previously conditioned chromatography column containing a silica gel absorber.

HPLC conditions: mobile phase acetonitrile/acetone (36.5:63.5), an ACE 5 C18 column (250 mm × 4.6 mm × 5 µm), column temperature 35°C, flow rate 1.0 mL/min, and injection volume of samples 20 µL. Triacylglycerols were identified by comparison with a reference chromatogram [14].

2.4. DETERMINATION OF TOCOLS COMPOSITION IN MORINGASEED OILS

Analysis of tocols (tocopherols and tocotrienols) in oils of moringa varieties was carried out using the Shimadzu Prominence-I LC 2030C 3D Plus model HPLC according to "TS ISO 9936 (2004)" method. Depending on the tocols concentration, approximately 1 g of oil sample was weighed into a 20 mL test tube. A 10 mL of hexane was added and the tube was vortexed for 2 minutes to dissolve the sample. It was then filtered through a 0.45 µm nylon filter and added to a 1.5 mL vials [15, 16].

The chromatographic column was an Inertsil NH2 5 µm 250 × 4.6 mm with 5 µm particle size. The mobile phase consisted of n-hexane/acetic acid/iso-propanol (IPA) (98.9:0.5:0.6 mL) in isocratic conditions at a flow rate of 1 mL/min. The tocopherols (α, β, γ, δ) and tocotrienols (α, β, γ, δ) were detected by UV where the wavelengths were set up at 296 nm. The injection volume was set at 10 µL. The column temperature was set at room temperature (~ 20°C). Peak identification was carried out by comparing retention times of authentic standards of tocopherols and tocotrienols.

2.5. DETERMINATION OF STEROLCOMPOSITION IN MORINGASEED OILS

The sterol compositions of moringa oils were carried out according to TS EN ISO 12228-1. Moringa seed oils were saponified by boiling with ethanolic potassium hydroxide solution. The unsaponifiable matter was removed by solid phase extraction in an aluminium oxide column. The sterol was separated from the unsaponifiable matter by thin layer chromatography. Quantitative and qualitative compositions of the sterol, using betulin as internal standard, were determined with the help of gas chromatography device [17]. The sterols recovered from the plate were transformed into a mixture of ethanol and diethyl ether, and the mixture was analysed by GC using an Agilent 6850 GC equipped with FID detector and a HP-5 (30 m × 320 µm × 0.25 µm) column. The chromatographic conditions: injector at 280°C at 7.9 psi, HP-5 column at 260°C, and detector at 290°C. Injection volume was 1.0 µL and the split ratio was 10:1. Hydrogen as a carrier gas was used at a flow rate of 35 mL/min.

3. RESULTS AND DISCUSSION

3.1. CRUDE OIL YIELD OF MORINGA SEED OILS

It was determined that the total amount of crude oil of moringa varieties varied between 26.46 - 28.19% in solvent extraction and between 24.20 - 26.30% in cold pressing method. It was determined that the highest total crude oil amount was obtained from the seeds of PKM1 moringa variety by solvent extraction (28.19%), while the lowest total crude oil amount was obtained from the seeds of ODC moringa variety by cold pressing method (24.20%). According to these data, it was seen that the total crude oil amount of the seeds of moringa varieties was the highest in PKM1, MOMAX 3 and ODC, respectively.

Anwar and Bhanger (2003) determined in their study that the total amount of crude oil obtained by solvent extraction from moringa oleifera seeds using hexane varied between 38 - 42.00%. This result differs from the results of our study. It is thought that this difference is largely due to the differences between the moringa seed varieties used [18].

3.2. FATTYACID COMPOSITION OF MORINGA SEED OILS

The fatty acid composition of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods are presented in Table I and Figure 1. Major fatty acids in moringa seed oils are Palmitic acid (16:0), Stearic acid (18:0), Oleic acid (18:1n9), Arachidic acid (20:0) and Behenic acid (22:0). It was determined that these fatty acids varied between 5.59-7.26%, 5.37-5.89%, 63.31-70.04%, 2.67-3.71% and 3.82-5.73%, respectively, and the main

fatty acid was oleic acid (63.31 - 70.04%). The highest oleic acid content was obtained from the seeds of the MOMAX 3 moringa variety by solvent extraction (70.04%), while the lowest oleic acid content was obtained from the seeds of the PKM1 moringa variety by solvent extraction method (63.31%). In ODC and MOMAX3, the Behenic acid content is higher in CP than in SE, but in PKM1, the Behenic acid content is higher in SE than in CP.

It was determined that the seed oils of moringa varieties were rich in unsaturated fatty acids and the total unsaturated fatty acid (UFA) content varied between 67.86 - 74.66%. It was determined that the total saturated fatty acid (SFA) content was 10.90 - 16.82% and the main saturated fatty acid was palmitic acid (5.59 - 7.26%).

In a study by Lalas and Tsaknis (2002), similar to like our study, it was determined that the main unsaturated fatty acid was oleic acid (71.60%) [19]. Likewise, in another studies by Leone et al. (2016) and Özcan et al. (2019) it was determined that the main unsaturated fatty acid was oleic acid, and the ratio of these fatty acids were 73.59 and 75.49, respectively [20, 21]. These data were seen to be compatible with our study.

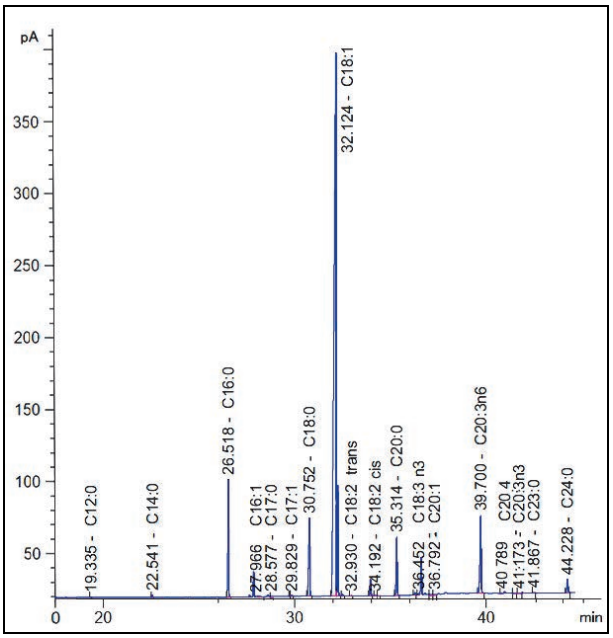

Figure 1 - The fatty acid composition of Moringa seed oils

3.3. GLYCERIDE COMPOSITION OF MORINGA SEED OILS

The glyceride compositions of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods were presented in Table II and Figure 2. It was observed that the main triglyceride structures in moringa seed oils were POL+SLL, OOO, SOL+POO, POP, SOO, and SOP. It was determined that these triglycerides varied between 0.80-2.48%,

Table I - Fatty acid composition and distribution of oils obtained from seeds of different Moringa varieties (%)

Fatty Acid (%)		ODC		PKM1		MOMAX 3	
		SE	CP	SE	CP	SE	CP
Lauric acid	C12:0	0.01	0.02	0.01	0.02	0.01	0.02
Myristic acid	C14:0	0.10	0.13	0.11	0.13	0.11	0.13
Palmitic acid	C16:0	7.26	6.01	6.46	5.98	5.59	6.12
Palmitoleic acid	C16:1	0.03	0.08	0.07	0.02	0.06	0.08
Margaric acid	C17:0	0.08	0.11	0.09	0.10	0.07	0.09
Heptadecenoic acid	C17:1	0.04	0.05	0.04	0.05	0.04	0.04
Stearic acid	C18:0	5.70	5.89	5.58	5.71	5.37	5.79
Cis Oleic acid	C18:1	63.88	66.55	63.31	66.42	70.04	66.65
Trans Linoleic acid	C18:2	0.02	0.02	0.01	0.02	0.01	0.02
Cis Linoleic acid	C18:2	0.04	0.05	0.04	0.05	0.03	0.03
Linolenic acid	C18:3	0.06	0.08	0.07	0.08	0.06	0.08
Arachidic acid	C20:0	2.69	3.71	2.88	3.61	2.67	3.63
Eicosenoic acid	C20:1	0.07	0.10	0.08	0.10	0.07	0.10
Behenic acid	C20:3	3.82	5.73	4.24	4.11	4.36	5.72
Tricosylic acid	C23:0	0.05	0.06	0.05	0.06	0.05	0.06
Lignoceric acid	C24:0	0.67	0.98	0.76	0.98	0.76	0.98
Σ SFA		16.57	10.90	15.94	16.58	14.64	16.82
Σ MUFA		64.02	66.78	63.50	66.59	70.20	66.86
Σ PUFA		3.93	5.87	4.36	4.26	4.46	5.85
Σ UFA		7.96	72.66	67.86	70.85	74.66	72.71

SE: Solvent extraction, CP: Cold pressing

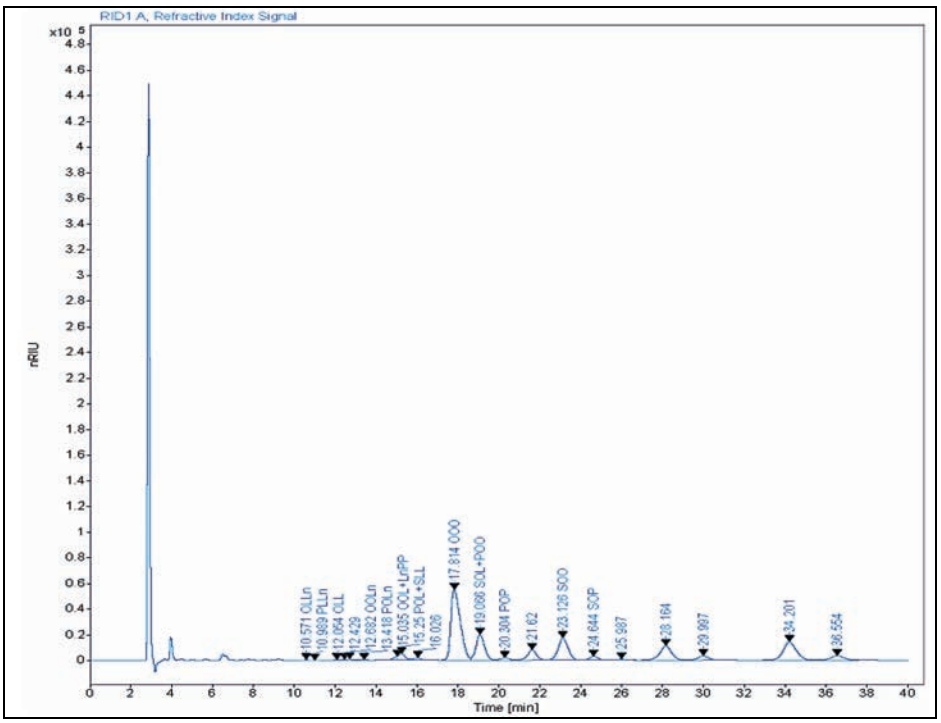

Figure 2 - The glyceride composition of Moringa seed oils

35.63%-36.50%, 11.49-12.06%, 1.08%-1.15%, 11.57-11.81% and 1.71-1.83%, respectively. In addition, it was determined that the main triglyceride structure was OOO (35.63 - 36.50%), followed by SOL + POO and SOO.

In a study, the triglyceride composition of the seed oil of moringa olifera was examined and the results showed that OOO, POO and SOO were the dominant triglycerides in the seed oil [22]. This data shows that our study agrees with previous studies.

3.4. TOCOLS COMPOSITION OF MORINGA SEED OILS

The tocols composition (tocopherols and tocotrienols) of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods are presented in Table III and Figure 3. It was observed that the main tocopherols and tocotrienols were α-tocopherol, α-tocotrienol and γ-tocopherol, varying between 15.88 - 18.91 µg/g, 1.27 - 1.51 µg/g and 5.93 - 7.38 µg/g, respectively. It was determined that the main

Table II - Glyceride composition and distribution of oils obtained from seeds of different Moringa varieties (%)

Triglycerides Content (%)	ODC		PKM1		MOMAX 3	
	SE	CP	SE	SE	CP	SE
LLL	0.03	0.16	-	-	0.02	0.03
OLLn	0.10	0.12	0.07	0.10	0.09	0.11
PLLn	0.05	0.07	0.04	0.07	0.06	0.05
OLL	-	0.45	0.05	0.04	-	0.05
OOLn	0.37	0.34	0.39	0.36	0.62	0.57
POLn	0.16	0.08	0.14	0.12	0.17	0.15
OOL+LnPP	-	-	0.81	0.82	0.78	0.80
POL+SLL	2.25	2.32	2.11	2.16	2.48	0.80
PPL	0.20	0.13	-	-	0.20	0.20
OOO	35.69	35.63	36.25	36.50	36.37	36.10
SOL+POO	11.49	11.77	11.86	12.06	11.65	11.59
POP	1.14	1.09	1.09	1.08	1.15	1.13
SOO	11.79	11.57	11.80	11.81	11.62	11.72
SOP	1.83	1.76	1.71	1.80	1.78	1.80

SE: Solvent extraction, CP: Cold pressing

Table III - Tocols (tocopherols and tocotrienols) composition and distribution of oils obtained from seeds of different Moringa varieties (µg/g)

Tocols (µg/g)	ODC		PKM1		MOMAX 3	
	SE	CP	SE	CP	SE	CP
α-tocopherol	17.57	18.42	15.88	18.91	16.29	18.86
α -tocotrienol	1.30	1.34	1.27	1.30	1.51	1.50
β-tocopherol	0.00	0.00	0.00	0.00	0.00	0.00
β -tocotrienol	0.00	0.00	0.00	0.00	0.00	0.00
γ-tocopherol	7.23	7.38	7.05	7.24	5.93	6.72
γ -tocotrienol	0.00	0.00	0.00	0.00	0.00	0.00
δ-tocopherol	0.00	0.00	0.00	0.00	0.00	0.00
δ-tocotrienol	0.00	0.00	0.00	0.00	0.00	0.00

SE: Solvent extraction, CP: Cold pressing

Table IV - Sterol composition and distribution of oils obtained from seeds of different Moringa varieties (%)

Sterols (%)	ODC		MOMAX 3		PKM1	
	SE	CP	SE	CP	SE	CP
Kolesterol	0.20	0.20	0.21	0.23	0.88	0.90
Brassikasterol	0.10	0.09	0.13	0.14	0.46	0.44
24-Metilenkolesterol	1.12	1.14	1.17	1.16	1.18	1.19
Kampesterol	12.27	12.35	12.51	12.58	11.69	11.67
Kampestanol	0.13	0.11	0.12	0.10	0.15	0.13
Stigmasterol	21.87	21.93	21.84	21.89	21.03	20.98
Delta-7-Kampesterol	0.62	0.55	0.70	0.66	1.07	1.10
Delta-5-23-Stigmastadienol	0.33	0.32	0.22	0.23	0.50	0.48
Klerosterol	1.01	1.00	0.95	0.92	0.98	0.96
Beta-Sitosterol	40.91	40.86	42.07	42.11	39.08	39.04
Sitostanol	0.95	0.92	1.04	0.99	1.82	1.92
Delta-5-Avenasterol	12.93	13.00	12.23	12.27	12.53	12.61
Delta-5-24-Stigmastenol	2.99	3.03	2.74	2.72	4.37	4.41
Delta-7-Stigmastenol	1.74	1.71	1.66	1.61	1.52	1.47
Delta-7-Avenasterol	2.75	2.78	2.41	2.40	2.74	2.71

SE: Solvent extraction, CP: Cold pressing

tocopherol was α-tocopherol (15.88 - 18.91 µg/g), followed by γ-tocopherol and α-tocotrienol.
In previous research carried out by Leone et al. (2016) and Özcan et al. (2019), it was determined that the main tocopherols are α-, γ- and δ-tocopherols [20, 21]. This result is very close to the results of our study.

3.5. STEROL COMPOSITION OF MORINGA SEED OILS

The sterols composition of oils of PKM1, MOMAX 3 and ODC moringa varieties obtained by two different methods are presented in Table IV. It was determined that the main sterols in were campesterol, stigmasterol, β-Sitosterol and delta-5-Avenasterol and the sterol contents varied between 11.67-12.58%, 20.98%-21.93%, 39.04%-42.11% and 12.23-13.00%, respectively. It was determined that the main sterol was β-Sitosterol (39.04 - 42.11%), followed by stigmasterol, delta-5-Avenasterol and campesterol. In general, the sterol contents of oils obtained by cold pressing were higher than those obtained by solvent extraction.
In a previous study conducted by Lalas and Tsaknis (2002), it was determined that moringa oil contains high levels of β-sitosterol (up to 45.58%), stigmasterol (up to 23.10%) and campesterol (up to 15.81%) [19]. In another study, conducted by Leone et al. (2016), it was determined that the main sterols in the oils of moringa seeds were β-sitosterol, stigmasterol, delta-5-Avenasterol and campesterol [20]. These data were found to be compatible with our study.

CONCLUSION

In the light of the findings above, it was observed that there was no significant difference between the values of fatty acid composition, triglyceride composition, tocols (tocopherols and tocotrienols) and sterol contents of the oils obtained from the seeds of ODC, PKM1 and MOMAX 3 moringa varieties, either by sol-

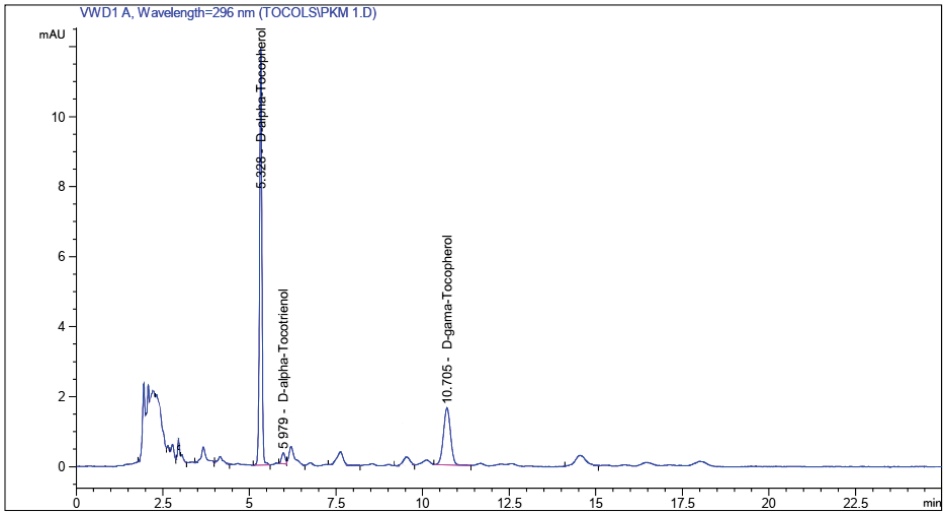

Figure 3 - The tocols composition (tocopherols and tocotrienols) of Moringa seed oils

vent extraction or by cold press method. It has been determined that the oils obtained from the seeds of moringa varieties are rich in oleic acid and have similar properties with olive oil (55 - 83%) and hazelnut oil (71 - 91%). Therefore, it seems that oils obtained from moringa seed can be considered as vegetable cooking oil. Moringa seeds and the oils obtained from the seeds can be easily used as a food supplement due to their nutritional properties.

The findings in our study show that moringa seed oil contains many components that are important in terms of both nutritional value and health.

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