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Determination of fatty acid compositions of total lipid, phospholipid and triacylglycerol fractions of aboveground parts of four species of the genus *Hyoscyamus*

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Abstract Nine different FAs were identified in TL, TG and PL fractions. The major FAs of TL, TG, PL in all *Hyoscyamus* species were C16:0 (palmitic acid, PA), C18:1 n−9 (oleic acid, OLA), C18:2 n−6 (linoleic acid, LA) and C18:3 n−3 (linolenic acid, ALA). Fatty acids composition range was as follows: saturated fatty acids (SFAs) 12.45–33.91%, 31.51–41.67%, 10.32–39.03% monounsaturated fatty acids (MUFAs) 7.66–16.57%, 6.24–14.67%, 11.40–26.78% and polyunsaturated fatty acids (PUFAs) 58.42–70.97%, 51.04–55.53%, 49.55–72.90% in TL, PL and TG fractions, respectively. In all *Hyoscyamus* species, total PUFA amounts were found to be higher than total MUFA and total SFA in TL, TG and PL fractions. The present study is a guide for biochemical and nutritional values of the *Hyoscyamus* species and can be useful for further investigation on industrial applications.

Keywords *Hyoscyamus* species, GC, fatty acid, phospholipid, triacylglycerol.

Introduction

The genus *Hyoscyamus*, belongs to the tribe Hyoscyameae Miers of Solanaceae family, is represented by six species in Turkey as *Hyoscyamus reticulatus* L., *Hyoscyamus pusillus* L., *Hyoscyamus niger* L., *Hyoscyamus aureus* L., *Hyoscyamus albus* L., and *Hyoscyamus leptocalyx* Stapf [1-2]. *Hyoscyamus* species especially contain tropane alkaloids (hyoscyamine and scopolamine), which are of anaesthetic, analgesic, anticholinergic, antispasmodic, mydriatic and sedative effects as well as being used as a hallucinogenic drug in the east of Turkey [2-7].

The qualities of the oils are closely dependent on their obtained source and the fatty acids (FAs) composition which has acquired much attention owing to its beneficial implications for human nutrition and health [8]. Recently, clinical researchers have reported that the intake of a higher proportion of monounsaturated fats in the diet reduce the risk of cardiovascular diseases by decreasing the low-density lipoprotein (LDL) cholesterol levels in blood [8-10]. Oils with a high proportion of oleic acid (OLA, the main fatty acid from olive oil) are more stable than other edible oils, and contribute to reduction of cholesterol level and the risk of developing a cardiovascular disease in humans [11]. Essential fatty acids, linoleic acid (LA) and linolenic acid (ALA) are precursors of the biosynthesis of longer chain polyunsaturated fatty acid (PUFA) as docosahexaenoic and eicosapentaenoic acids that are the main components of the neural cell membrane [12]. Researchers reported that these FAs may have different dietary effects depending on whether or not they are present in oils as TG form or diacylglycerol (DAG) form [13]. The high content of LA, which is an essential fatty acid, makes oil very important to the industry such as stabilizers in plastic formulations, protective coatings, varieties of synthetic intermediates, urethane derivatives, plastics, dispersants, surfactants, cosmetics, lubricants and in the preparations of other long chain polymeric materials [14].

TGs are a major type of neutral lipids and they are the major storage molecules of metabolic energy in most living organisms. Phospholipids (PLs) are ubiquitous in nature, and are key components of the lipid bilayer of cells in plants. In addition, TG is used in the food industry [15]. PLs are of positive effects on human health [16]. Moreover, they have a wide array of non-food and food applications, mainly as nutrition supplements, industrial lubricants and nontoxic biodegradable emulsifiers [17].

There are few reports on the FA profile of *Hyoscyamus* species [2,18-19]. However, no reports have been published about the FA compositions of TL, TG and PL fractions of *Hyoscyamus* species. From this point of view, TL, TG and PL fatty acid compositions of *Hyoscyamus* species were investigated in detail. The results obtained from the present work may be important in understanding the suitability of these plants in food industry and pharmacology.

Materials and Methods

Sampling

Plant materials of *Hyoscyamus* species were collected from different locations of Mardin (Southeastern of Turkey) at the flowering stage. The plant species, locations, herbarium numbers and coordinates are listed in Table 1. Voucher specimens were deposited at the Mardin Artuklu University Herbarium, Mardin/Turkey. They were identified by Dr. A. Selçuk Ertekin from the Dicle University, Faculty of Science, Department of Biology, Diyarbakir/Turkey and Dr. Cumali Keskin from the Mardin Artuklu University. The aerial parts (the flower, leaf and stem) of the plants were used in the study. The plant materials were dried at room temperature and ground in a grinder with a 2 mm diameter mesh before experimental studies.

Table 1: Details of vouchers specimens of *Hyoscyamus* species

Species	Locality	Herbarium number of the vouchers	Altitude (m)
Hyoscyamus albus L.	C8 Mardin, Mardin Castle, old walls, ruins	C.KESKİN 2012-10 (MAU)	1200
Hyoscyamus aureus L.	C8 Mardin, Mardin Castle, old walls, ruins	C.KESKİN 2012-11 (MAU)	1200
Hyoscyamus leptocalyx Stapf.	C8 Mardin, Mardin to Diyarbakır, Karasu, rock crevices	C.KESKİN 2012-12 (MAU)	788
Hyoscyamus reticulatus L.	C8 Midyat, Vicinitiy of Mor Gabriel Monestry	C.KESKİN 2012-13 (MAU)	965

Lipid extraction and transmethylation of fatty acids

5.0 g of dried materials were added to 15 mL mixture of methanol:chloroform (1:2, v/v) as an extraction solvent. Then, solids were filtrated and clear filtrate was evaporated to obtain lipid fraction. TG and PL were separated by Camag twin trough chambers (20 × 20 cm) previously saturated for 20 min with the diethyl ether/petroleum ether/acetic acid (20:80:1; v/v) as the mobile phase. The mixtures of lipids were spotted on thin layer chromatography aluminium plates (TLC, 0.25 mm silica gel 60 F_{254}, 20 × 20 cm Merck) by using 500 µL syringe. After chamber saturation the plates were developed to a distance of 16 cm with the development time of 40 min. Subsequent to the development, plates were dried in a drying over at 60ºC. Revelation of lipids fractions was carried out with 2',7'-dichlorofluorescein (0.2% w/v in methanol, obtained from Supelco), and the appropriate bands of TG and PL fractions were visualized under CAMAG UV cabinet (254 and 366 nm). After scraping, TG and PL fractions were transmethylated using acidified methanol. It was refluxed for a 2 h at 85ºC. Methylated fatty acids (FAMEs) were extracted twice into hexane [20].

Gas chromatography analysis

Nine different fatty acids (FAs) include C14:0 (myristic acid, MA), C15:0 (pentadecylic acid, APA), C16:0 (palmitic acid, PA), C17:0 (margaric acid, AMA), C18:0 (stearic acid, SA), C16:1 n−7 (palmitoleic acid, PLA),

C18:1 n−9 (oleic acid, OLA), C18:2 n−6 (linoleic acid, LA), C18:3 n−3 (linolenic acid, ALA) were identified in TL, TG and PL fractions by GC. FAMEs were analysed by capillary gas chromatography (GC, Model 6890; Hewlett Packard Inc, Wilmington, DE, USA) with a flame ionization detector (FID), and Hewlett Packard ChemStation software. All the samples were analysed according to the procedure described by Kayhan et al. [20] without major changes. Samples were identified solely by a code number and ordered randomly within a batch. FAME composition was determined by capillary GC. A BPX70 capillary column (70% cyanopropyl 30% dimethyl polysilphenylene-siloxane bonded phase, 30 m × 0.32 mm (i.d.) × 0.250 μm film thickness; SGE, Milton Keynes, UK) was used. The flow rates of compressed air and hydrogen were 300 mL/min and 30 mL/min, respectively. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The injection port temperature was 260ºC, the detector temperature 280ºC. One microliter was injected through the cold on-column injector with a split ratio of 1:20. The oven temperature was programmed at initial temperature 130ºC and kept constant for a min, and then to rise from 130–170ºC at a rate of 6.5ºC/min, from 170–215ºC at a rate of 2.75ºC/min and kept constant for 12 min, and from 215–230ºC at a rate of 40ºC/min and kept constant for 3 min. Total analysis time was 38.89 min. The Hewlett Packard 3365 ChemStation software operated the sampling, analysis and integration of the chromatographic sample. The chemical structures of the FAs were identified as their methyl esters by comparison of their relative retention times of authentic standards (Sigma-Aldrich Chemicals), and the relative amount of each FA was quantified by integrating the peak at baseline and dividing the results by the total area for all FAs [21]. The integrations of observed peak areas were performed by the same laboratory worker.

Statistical analysis

Mean and standard deviation (SD) were calculated from data obtained in three separate experiments performed in triplicate. All statistical analyses were performed using SPSS statistical software (SPSS 15.0 for Windows, Chicago, IL, USA). Statistical comparisons of the FA percentages were carried out by an analysis of variance (ANOVA) and comparisons between means were performed by using Tukey's test. *P* values less than 0.05 were considered statistically significant.

Results and Discussion

Total lipid, phospholipid and triacylglycerol fatty acid composition of Hyoscyamus reticulatus

The results of the analysis of the FA compositions of the *Hyoscyamus* species are summarised in Table 2.

Table 2: Total fatty acid (FA) composition of *Hyoscyamus* species

Fatty Acids	*H. reticulatus*	*H. leptocalyx*	*H. aureus*	*H. albus*
C 14:0	0.23±0.01^{a**}	2.28±1.01^{b}	1.41±1.13^{c}	1.36±0.62^{c}
C 15:0	0.05±0.02^{a}	0.19±0.22^{b}	0.09±1.03^{c}	0.42±1.20^{d}
C 16:0	8.69±1.81^{a}	13.83±2.24^{b}	15.06±2.98^{b}	20.80±1.85^{c}
C 17:0	0.15±0.38^{a}	0.51±0.29^{b}	1.68±1.11^{c}	5.92±2.03^{d}
C 18:0	3.33±1.00^{a}	4.94±2.08^{a}	4.55±1.92^{a}	5.41±0.95^{a}
ΣSFA	**12.45**	**21.75**	**22.79**	**33.91**
C 16:1 n-7	0.18±0.05^{a}	0.43±0.07^{b}	0.84±1.03^{c}	1.94±1.25^{d}
C 18:1 n-9	16.39±1.43^{a}	15.10±2.48^{a}	12.86±1.93^{a}	5.72±1.01^{b}
ΣMUFA	**16.57**	**15.53**	**13.70**	**7.66**
C 18:2 n-6	68.02±5.41^{a}	52.05±4.23^{b}	49.43±3.54^{b}	24.50±4.28^{c}
C 18:3 n-3	2.95±1.36^{a}	10.64±4.11^{b}	14.07±2.48^{b}	33.92±2.21^{c}
ΣPUFA	**70.97**	**62.69**	**63.50**	**58.42**

$^{**a, b, c}$ means followed by different letters in same line are significantly different ($P < 0.05$) by Tukey's test.

It is clear to see in the table that total PUFA percentages were meaningfully higher than total monounsaturated fatty acids (MUFAs) and total saturated fatty acids (SFAs) for *H. reticulatus*, *H. leptocalyx*, *H. aureus and H. albus*. In the aerial parts of *Hyoscyamus* species, C18:2 n−6 (LA) (72.14% in in TG fraction, 32.45% in PL fraction, 68.02% in FA fraction) was the major fatty acid among all *Hyoscyamus* species. Five different SFA including C14:0 (MA),

C15:0 (APA), C16:0 (PA), C17:0 (AMA), C18:0 (SA) were identified in the samples. In the aerial parts of *H. reticulatus*, the percentages of total SFA ranged from 10.32% to 31.51%. Only two MUFA including PLA and OLA were identified and qualificated in targeted fractions that determined as C18:1 n−9 (OLA) in the range of 13.35–16.68%, C16:1 n−7 (PLA) in the range of 0.09–1.32%. The total MUFA ranged from 14.67% to 16.77%. LA and ALA as members of PUFA were qualificated. LA was the predominant fatty acid for TL, TG and PL in PUFAs. The percentages were determined from 32.45% to 72.14%. In PL fraction, ALA (21.36%) fatty acid was significantly higher than TL (2.95%) and TG (0.76%).

Total lipid, phospholipid and triacylglycerol fatty acid composition of Hyoscyamus leptocalyx

In the aerial parts of *H. leptocalyx*, main FA was LA in TL and TG fractions. However, in PL fraction ALA was found as the dominant fatty acid. *H. leptocalyx* samples contain five SFAs (C14:0, C15:0, C16:0, C17:0 and C18:0) with the total percentages of 18.18–41.67%. C16:0 was the major fatty acid among SFAs with the total proportion of 10.72–27.71%. OLA was the main fatty acid to all of MUFAs, ranges from 6.50% to 25.63%. Total PUFA ranged from 51.04% to 62.69%. It should be noted that MUFAs especially OLA have great importance in terms of the effect on oxidative stability of oils and their nutritional implications and the high intake of a very long-chain (n-3) fatty acids such as ALA from fish was associated with reduced risk of fatal ischemic heart disease in prospective cohort studies [22].

Total lipid, phospholipid and triacylglycerol fatty acid composition of Hyoscyamus aureus

LA was found in as a major constituent in TL (49.43%) and TG (56.56%) fraction, whereas ALA (36.97%) was major fatty acid in PL fraction. Total SFA percentages of TL, TG and PL were 22.79%, 18.54% and 36.59% respectively. OLA was found higher in TG (20.31%) fraction than the others. PUFA contents in view of LA and ALA were %55.53–63.50% of total fatty acids that are clearly found higher than SFA and MUFA in TL, TG and PL fractions. Total SFA ratio of total FA (22.79%), TG (18.54%), and PL (36.59%) fractions did not show a significant difference.

Total lipid, phospholipid and triacylglycerol fatty acid composition of Hyoscyamus albus

In the aerial parts of *H. albus*, ALA was significantly higher than other *Hyoscyamus* species in TL and TG fractions. In addition, the amount of C17:0 fatty acid that known as odd numbered fatty acid was higher than other species in TL, TG and PL fractions. Although most naturally occurring lipids contain FAs with an even number of carbon atoms, FAs with an odd number of carbons are found in significant amounts in the lipids of many plants and some marine organisms. The fatty acid composition of the different lipid subclasses included SFA: C14:0, C16:0 and C18:0 acids and unsaturated fatty acid: PLA, OLA, LA and ALA, in agreement with the literature [18,19]. On the other hand, no data was found concerning fatty acid composition of PL from *Hyoscyamus* species. Many studies have demonstrated that PUFA has beneficial effects on coronary heart disease, hypertension, rheumatoid arthritis, breast and colon cancer, Alzheimer disease inflammation and autoimmune disorders [23-25]. In conclusion, this investigation on TL, TG and PL fatty acid profile of different *Hyoscyamus* species, it could be said that the lipids seem to be a good source of essential fatty acids.

Evaluation of SFA, MUFA and PUFA levels

In the present study, total SFA, MUFA and PUFA percentages of *Hyoscyamus* species were determined as 10.32–41.67%, 6.24–26.78% and 49.55–72.90%, respectively. Consumption percentages of SFA, MUFA and PUFA, in daily diet, are an important parameter in health. It was noted that the replacement of SFAs with PUFAs, rather than with MUFAs, prevents coronary heart disease [26]. Total SFA, MUFA and PUFA percentages of selected cereal-based Turkish foods were found in the ranges of 19.7–51.0%, 14.2–59.4% and 4.4–60.9%, respectively [27]. It was reported that LA and ALA have an important role in metabolism of mammals. From the experimental results it was possible to conclude that risks of cancer and cardiovascular diseases were reduced by consumption of them in daily

diet [28]. A recent study reported that LA contents in edible plants were found as 0.14, 0.12, 0.89, 0.10, and 0.12 mg/g (wet weight) in spinach, mustard, purslane, buttercrunch lettuce, and red leaf lettuce, respectively, and ALA contents in edible plants were found as 0.89, 0.48, 4.05, 0.26, and 0.31 mg/g (wet weight) in spinach, mustard, purslane, buttercrunch lettuce and red leaf lettuce, respectively [29].

The possible critical roles of fatty acids in prostate cancer were highlighted in recent studies in details [30,31]. Intakes values of MUFA as PLA and OLA of prostate cancer were reported as 23.4±0.7 and 23.7±0.7 g/day respectively for cases and controls for PLA, 9.0±0.3 and 9.2±0.3 g/day respectively for cases and controls for OLA [31]. It was also reported that the ratio of stearic acid to OLA was lower in red blood cell (RBC) membranes from cancer patients than RBCs from patients without cancer [32]. PLA has an important role in different cell functions, from growth and proliferation to endoplasmic reticulum stress [33].

Table 3: Phospholipid (PL) composition of *Hyoscyamus* species

Fatty Acids	***H. reticulates***	***H. leptocalyx***	***H. aureus***	***H. albus***
C 14:0	1.11±0.28^{a}	2.77±1.14^{b}	1.42±0.98^{a}	1.11±0.36^{a}
C 15:0	0.32±0.42^{a}	0.77±0.25^{b}	0.25±0.32^{a}	0.68±0.55^{b}
C 16:0	21.64±2.22^{a}	27.71±3.21^{b}	23.03±2.98^{a}	25.00±4.28^{b}
C 17:0	0.78±0.98^{a}	0.86±0.55^{a}	3.46±0.77^{b}	5.68±1.03^{c}
C 18:0	7.66±1.26^{a}	9.56±2.45^{a}	8.44±3.20^{a}	6.19±2.47^{a}
ΣSFA	**31.51**	**41.67**	**36.59**	**38.66**
C 16:1 n-7	1.32±0.66^{a}	0.78±0.02^{b}	2.71±0.05^{c}	3.11±1.02^{c}
C 18:1 n-9	13.35±1.25^{a}	6.50±2.24^{b}	5.16±1.98^{b}	3.13±0.96^{c}
ΣMUFA	**14.67**	**7.28**	**7.87**	**6.24**
C 18:2 n-6	32.45±4.22^{a}	20.11±3.20^{b}	18.56±5.02^{b}	18.98±4.03^{b}
C 18:3 n-3	21.36±3.00^{a}	30.93±4.24^{b}	36.97±3.99^{c}	36.11±3.78^{c}
ΣPUFA	**53.81**	**51.04**	**55.53**	**55.09**

$^{a, b, c}$ means followed by different letters in same line are significantly different ($P < 0.05$) by Tukey's test.

Table 4: Triacylglycerol (TG) composition of *Hyoscyamus* species

Fatty Acids	***H. reticulatus***	***H. leptocalyx***	***H. aureus***	***H. albus***
C 14:0	0.12±0.11^{a**}	1.51±0.66^{b}	0.58±0.23^{c}	2.92±1.04^{d}
C 15:0	0.02±0.12^{a}	0.24±0.17^{b}	0.13±0.31^{c}	1.03±0.14^{d}
C 16:0	7.15±2.99^{a}	10.72±3.25^{b}	12.49±3.78^{b}	22.24±3.25^{c}
C 17:0	0.06±0.01^{a}	0.40±0.03^{b}	0.31±0.26^{b}	4.45±1.07^{c}
C 18:0	2.97±0.65^{a}	5.31±1.66^{b}	5.03±1.03^{b}	8.39±2.04^{c}
ΣSFA	**10.32**	**18.18**	**18.54**	**39.03**
C 16:1 n-7	0.09±0.02^{a}	1.15±0.97^{b}	1.26±0.92^{b}	0.93±0.23^{b}
C 18:1 n-9	16.68±2.02^{a}	25.63±3.33^{b}	20.31±3.06^{c}	10.48±1.98^{d}
ΣMUFA	**16.77**	**26.78**	**21.57**	**11.40**
C 18:2 n-6	72.14±7.03^{a}	52.27±4.09^{b}	56.56±4.21^{b}	23.85±2.15^{c}
C 18:3 n-3	0.76±0.22^{a}	2.76±0.96^{b}	3.32±1.08^{b}	25.70±4.01^{c}
ΣPUFA	**72.90**	**55.03**	**59.88**	**49.55**

$^{a, b, c}$ means followed by different letters in same line are significantly different ($P < 0.05$) by Tukey's test.

Conclusion

Detailed GC analyses of the FA compositions of TL, TG and PL fractions of *H. reticulatus, H. leptocalyx, H. aureus and H. albus* species from Turkey were presented, and they remain as an important source for the discovery of new bioactive secondary metabolites. The results of this study show us the nutritional potential of the high PUFA contents (especially linoleic acid) of *Hyoscyamus* species, which can offer nutritional importance in the application of the food industries and medical purposes. Further information on the FA composition properties of *Hyoscyamus*

species could be nutritionally considered as a new non-conventional supply for pharmaceutical industries, industrial applications for health and edible purposes.

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Conflict of Interest
There are no conflicts of interest among the authors.

References

1. Baytop, A. (1978). *Hyoscyamus*. In (Ed. Davis PH) Flora of Turkey and the East Aegean Islands. University Press, Edinburgh, Scotland, U.K. Vol. 6, pp. 454–455.
2. Guler, G.O. (2012). Studies on antioxidant properties of the different solvent extracts and fatty acid composition of *Hyoscyamus reticulatus* L. *Journal of Food Biochemistry*, 36(5):532–538.
3. Mateus, L., Cherkaoui, S., Christen, P., & Veuthey, J.L. (1998). Capillary electrophoresis for the analysis of tropane alkaloids: Pharmaceutical and phytochemical applications. *Journal of Pharmaceutical Biomedical Analysis*, 18(4-5):815–825.
4. Zehra, M., Banerjee, S., Naqvi, A.A., & Kumar, S. (1998). Variation in the growth and alkaloid production capability of the hairy roots of *Hyoscyamus albus*, *H. muticus* and their somatic hybrid. *Plant Science*, 136(1):93–99.
5. Zayed, R., Abbas, F., & Wink, M. (2003). Study of alkaloid metabolism during seedling development in *Hyoscyamus muticus* L. and *Hyoscyamus niger* L. and elicitation of tropane alkaloid formation in root cultures of *Hyoscyamus niger*. *Egyptian Journal of Pharmaceutical Science*, 44(2):293–306.
6. Kartal, M., Kurucu, S., & Altun, L. (2003). Quantitative analysis of l-hyoscyamine in *Hyoscyamus reticulatus* L. by GC-MS. *Turkish Journal of Chemistry*, 27(5):565–569.
7. Zayed, R., & Wink, M. (2004). Induction of tropane alkaloid formation in transformed root cultures of *Brugmansia suaveolens* (Solanaceae). *Zeitschrift für Naturforschung C*, 59(11-12):863–867.
8. Sakouhi, F., Herchi, W., Sebei, K., Absalon, C., Kallel, H., & Boukhchina, S. (2011). Accumulation of total lipids, fatty acids and triacylglycerols in developing fruits of *Olea europaea* L. *Scientia Horticulturae*, 132: 7–11.
9. Visioli, F., & Galli, C. (2002). Biological properties of olive oil phytochemicals. *Critical Reviews in Food Science and Nutrition*, 42(3): 209–221.
10. Sorci-Thomas, M., Wilson, M.D., Johnson, F.L., Williams, D.L., & Rudel, L.L. (1989). Studies on the expression of genes encoding apolipoproteins B100 and B48 and the low density lipoprotein receptor in nonhuman primates. Comparison of dietary fat and cholesterol. *Journal of Biological Chemistry*, 264(15): 9039–9045.
11. Rebey, I.B., Kefi, S., Limam, F., & Marzouk, B. (2013). Variations in fatty acid composition during maturation of cumin (*Cuminum cyminum* L.) seeds. *African Journal of Biotechnology*, 12(34):5303–5307.
12. Daviglus, M.L., Stamler, J., Orencia, A.J., Dyer, A.R., & Liu, K. (1997). Fish consumption and the 30-year risk of fatal myocardial infarction. *New England Journal of Medicine*, 336(15):1046–1053.
13. Murase, T., Mizuno, T., Omachi, T., Onizawa, K., & Komine, Y. (2001). Dietary diacylglycerol suppresses high fat and high sucrose diet-induced body fat accumulation in C57BL/6J mice. *Journal of Lipid Research*, 42(3): 372–378.

14. Hosamani, K.M., & Katagi, K.S. (2008). Characterization and structure elucidation of 12-hydroxyoctadec-*cis*-9-enoic acid in *Jatropha gossypifolia* and *Hevea brasiliensis* seed oils: A rich source of hydroxy fatty acid. *Chemistry and Physics of Lipids*, 152(1): 9–12.
15. Aparicio, R., & Aparicio-Ruiz, R. (2000). Authentication of vegetable oils by chromatographic techniques. *Journal of Chromatography A*, 881(1-2): 93–104.
16. Wannes, W.A., Mhamdi, B., Sriti, J., & Marzouk, B. (2010). Glycerolipid and fatty acid distribution in pericarp, seed and whole fruit oils of *Myrtus communis* var. *italica*. *Industrial Crops and Products*, 31(1): 77–83.
17. Hamama, A.A., Bhardwaj, H.L. (2004). Phytosterols, triterpene alcohols, and phospholipids in seed oil from white lupin. *Journal of the American Oil Chemists' Society*, 81(11):1039–1044.
18. Ramadan, M.F., Zayed, R., & El-Shamy, H. (2007). Screening of bioactive lipids and radical scavenging potential of some solanaceae plants. *Food Chemistry*, 103(3): 885–890.
19. Nejadhabibvash, F., Rahmani, F., Heidari, R., & Jamei, R. (2012). Heritability and correlation studies of fatty acid composition within *Hyoscyamus* accessions. *International Research Journal of Applied and Basic Sciences*, 3(9): 1837–1844.
20. Kayhan, H., Bashan, M., & Kacar, S. (2015). Seasonal variations in the fatty acid composition of phospholipids and triacylglycerols of brown trout. *European Journal of Lipid Science and Technology*, 117(5): 738–744.
21. Keskin, C., & Kaçar, S. (2013). Fatty acid composition of root and shoot samples of some *Astragalus* L. (Fabaceae) taxa growing in the East and Southeast of Turkey. *Turkish Journal of Biology*, 37(1):122–8.
22. Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E.B., Wolk, A., Colditz, G.A., Hennekens, C.H., & Willett, W.C. (1999). Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. *American Journal of Clinical Nutrition*, 69(5):890–897.
23. Finley, J.W., & Shahidi, F. (2001). The chemistry, processing, and health benefits of highly unsaturated fatty acids: An overview. In (Eds. Shahidi F, Finley JW) Omega-3 fatty acids: Chemistry, nutrition, and health effects. Vol.788, pp. 2–11, ACS Symposium Series.
24. Riemersma, R.A. (2001). The demise of the n-6 to n-3 fatty acid ratio? A dossier. *European Journal of Lipid Science and Technology*, 103(6): 372–373.
25. Kamel, B.S., & Kakuda, Y. (2007). Fatty acids in fruits and fruit products. In (Ed. Chow CK) Fatty acids in foods and their health implications, (3rd Edition); pp. 263–301, CRC Press.
26. Roehm, E. (2009). The evidence-based Mediterranean diet reduces coronary heart disease risk, and plant-derived monounsaturated fats may reduce coronary heart disease risk. *American Journal of Clinical Nutrition*, 90(3): 697–698.
27. Daglioglu, O., Tasan, M., & Tuncel, B. (2002). Determination of fatty acid composition and total trans fatty acids in cereal-based Turkish foods. *Turkish Journal of Chemistry*, 26(5):705–710.
28. Nagao, K., & Yanagita, T. (2008). Bioactive lipids in metabolic syndrome. *Progress in Lipid Research*, 47(2):127–146.
29. Simopoulos, A.P. (2004). Omega-3 fatty acids and antioxidants in edible wild plants. *Biological Research*, 37(2): 263–277.
30. Mamalakis, G., Kafatos, A., Kalogeropoulos, N., Andrikopoulos, N., Daskalopulos, G., & Kranidis, A. (2002). Prostate cancer vs hyperplasia: Relationships with prostatic and adipose tissue fatty acid composition. *Prostaglandins, Leukotriens and Essential Fatty Acids*, 66(5-6): 467–477.
31. Mannisto, S., Pietinen, P., Virtanen, M.J., Salminen, I., Albanes, D., Giovannucci E., & Virtamo, J. (2003). Fatty acids and risk of prostate cancer in a nested case-control study in male smokers. *Cancer Epidemiology Biomarkers and Prevention*, 12(12): 1422–1428.
32. Jackson, J.A., Riordan, H.D., Hunninghake, R., Meng, X., & Sarwar, Y. (1997). Red blood cell membrane fatty acids as a diagnostic test. *Journal of Orthomolecular Medicine*, 12(1):20–22.

33. De Fabiani, E. (2011). The true story of palmitoleic acid: Between myth and reality. *European Journal of Lipid Science and Technology*, 113(7): 809–811.