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Fatty Acid Composition of Some *Medicago* L. (Fabaceae) Species From Turkey

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The seed oils of five *Medicago* species (*Medicago lupulina, Medicago sativa, M. minima* var *minima*, *M. rotata* var. *eliezeri, M. rigidula* var. *rigidula*) (Fabaceae) were investigated for their fatty acid composition. The fatty acid composition of these five different species were determined by gas chromatography of the methyl esters of their fatty acids. The seed oils of *Medicago* species contained palmitic acid (10.79-19.18 %), among the saturated acids with a small amount of stearic acid and arachidic acid. The major unsaturated fatty acids found in the seed oils were linolenic acid (43.69-25.51 %) and linoleic acid (41.95-23.99 %). Unsaturated fatty acid concentrations of *Medicago* species were found higher than the saturated fatty acid concentration. The results showed that *Medicago* patterns has linoleic-linolenic and oleic-palmitic type fatty acid. The result were discussed in means of renewable resources and chemotaxonomy.

Key Words: *Medicago*, Seed, Fatty Acid, Renewable resources, Chemotaxonomy.

INTRODUCTION

Legumes have been cultivated for thousand years and played an important role in the traditional diets of many regions throughout the world[1,2]. Legume seeds are rich in many nutrient components including protein, starch, certain fatty acids and micronutrients such as vitamins, trace minerals[3-5]. Therefore important to search for good sources of oil that would have benefical health effects[6]. Some species of family Fabaceae are a source of cheap protein for both humans and animals[7] and Legumes generally are rich sources of proteins[8,9]. Therefore, they are increasingly being looked upon as potential alleviators of the problem of high population to protein ratio in the world[10].

An accurate assessment of the profile and percentage total fatty acids from fresh forage is crucial when studying the biohydrogenation of fatty acid from fresh plants[11]. After harvesting, loss of lipids in forages occurs due to the oxidation of unsaturated fatty acids through the activity of plant lipases[12] and lipoxygenases[13], decreasing the amount of unsaturated fatty acids[14].

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Information on the chemical composition of *Medicago* seed oil is very scanty[15,16]; while previous workers investigated phytochemical properties of some Legumes widely[17-20]. The objective of the present study is to determine the fatty acid composition of some *Medicago* L. species from Turkey (*Medicago lupulina, Medicago sativa* L., *M. minima* L. var *minima*, *M. rotata* Boiss. var. *eliezeri*, *M. rigidula* L. var. *rigidula*). In addition during the course of this study, it is aimed to characterized seed fatty acids used by the animals in field, to establish the nutritional value and to do contributions as the renewable resources of fatty acid.

EXPERIMENTAL

Seed samples: In this research, following plant seeds (*Medicago lupulina*, *Medicago sativa*, *M. minima* var *minima*, *M. rotata*. var. *eliezeri*, *M. rigidula* var. *rigidula*) (Fabaceae) were collected from natural habitats in Eastern Anatolian region of Turkey in years 2007-2008.

Oil extraction and preparation of fatty acid methyl esters (FAME): Impurities were removed from the seeds and the cleaned seeds were ground using a ball mill into powder. Lipids were extracted with hexane/isopropanol (v/v)[17]. The lipid extracts were centrifuged at 10.0 g for 5 min and filtered. The solvent was removed on a rotary evaporator at 40 ºC.

Capillary GLC: Fatty acids in the lipid extracts were converted into methyl esters by means of 2 % sulphuric acid (v/v) in methanol[21]. The fatty acid methyl esters were extracted with *n*-hexane. Then the methyl esters were separated and quantified by gas chromatography and flame ionization detection (Schmiadzu GC, 17 Ver.3) coupled to a glass GC 10 software computing recorder. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25, Machery-Nagel, Germany) using nitrogen as carrier gas (flow rate 0.8 mL/min) the temperatures of the column, detector and injector valve were 130-220, 240-280 ºC, resptectively. Identification of the individual method was performed by frequent comparison with authentic standards mixtures that were analyzed under the same conditions.

RESULTS AND DISCUSSION

In this study, the fatty acid composition of some Medicago plants from Turkey were determined. The results of the fatty acid analysis are shown in Table-1.

The fatty acid composition of *Medicago* plants used as feed crops from Fabaceae family showed different saturated and unsaturated fatty acid concentrations. The main components in the seed oils of these species are linoleic acid and linolenic acid. *Medicago lupulina* and *M. rigidula* were rich by linolenic acid concentrations (41.36, 43.69 %). But *M. sativa* seed oil was rich in view of linoleic acid (41.95 %). The other *Medicago* species comprised the middle linoleic acid and linolenic acid in the seed oils. *M. minima* var. *minima* has 25.33 % linoleic acid and 28.07 % linolenic acid; *M. rotata* var. eliezeri also has 26.85 % linoleic acid 25.51 % linolenic

acid, respectively (Table-1). Linoleic acid is needed for a normal immune response and in essential fatty acids deficiency impairs B and T cell mediated responses[22]. Oleic acid contents of the *Medicago* species has shown more differences among the species studied. While *Medicago lupulina* and *M. rigidula* var. *rigidula* taxa has low oleic acid contents (7.02 and 7.0 %), *M. sativa, M. minima* var. *minima* and *rotata* var. *eliezeri* showed high concentrations of oleic acids (12.10, 15.84, 21.15 %), respectively. *Onobrychis fallax* (Fabaceae) plant was reported as rich by oleic (52.56 %), linoleic (16.93 %), linolenic (8.63 %) and palmitic acids (8.95 %)[23]. Golden (*Trifolium aureum* and *T. repens*) and white clover plants, which are the feed crops, have similar fatty acid composition and also have more linoleic acid (42.53, 51.19 %) concentrations. It is reported that, *Trifolium aureum* has also large amount of linolenic (19.56 %), oleic (13.40 %) and palmitic acids (12.89 %), respectively. *Trifolium repens* plant seeds fatty acid was reported as rich by oleic acid (22.67 %), palmitic acid (9.58 %) and also stearic acid (7.72 %), respectively[23]. Linoleic acid, oleic acid and linolenic acid components were found as main unsaturated fatty acid components in *Lathyrus genus* patterns studied[17].

TABLE-1
FATTY ACID COMPOSITION OF SOME *Medicago* SPECIES FROM TURKEY, DATA SHOWS PEAK AREA % FROM GLC

Plants	Fatty acid composition							
	14:0	16:0	16:1 Δ9	17:0	18:0	18:1 Δ9	18: 2 Δ9,12	18: 3 Δ9,12, 15
Medicago lupulina	–	12.67	0.90	–	2.89	7.02	28.69	41.36
Medicago sativa	0.15	10.79	0.37	0.11	1.88	12.10	41.95	28.53
M. minima var *minima*	0.34	19.18	0.91	0.08	3.72	15.84	25.33	28.07
M. rotata var. *eliezeri*	0.29	18.56	0.87	0.07	1.12	21.15	26.85	25.51
M. rigidula var. *rigidula*	0.17	15.00	0.80	–	1.90	7.00	23.99	43.69
	20:0	20:1	22:0	22:1	22:2	24:0	TSFA	TUSFA
Medicago lupulina	1.79	0.20	0.71	–	0.38	–	18.06	78.55
Medicago sativa	0.65	0.18	0.64	0.19	0.14	–	14.22	83.46
M. minima var *minima*	1.30	0.32	1.12	0.13	0.11	0.31	26.05	70.71
M. rotata var. *eliezeri*	1.32	0.38	1.13	0.13	0.12	0.34	22.83	75.01
M. rigidula var. *rigidula*	1.44	0.27	1.73	–	0.15	0.22	20.46	75.90

TSFA = Total saturated fatty acid; TUSFA = Total unsaturated fatty acid.

The main components in the seed oils of some feed crops were reported as linoleic acid, oleic acid, palmitic acid and linolenic acid. *Vicia ervilia* and *Lotus corniculatus* were rich linoleic acid concentrations (40.16-45.73 %).

Palmitic acid is also has middle concentration in the *Medicago* species (10.79-19.18 %). *M. minima* var. *minima* and *M. rotata* var. eliezeri were shown the high content of palmitic acid (19.18, 18.56 %). *Medicago* species has comprised low amount of stearic acid in their seed oils. It was between 1.12 and 3.72 % in the seed oils of *M. rotata* var. eliezeri and *M. minima* var. *minima* (Table-1). *Medicago* seeds

has also low level of arachidic acid (20:0) and behenic acid (22:0). The amount of the arachidic acid was ranged from 0.65 to 1.79 % in *M. sativa* and *M. lupulina*. But *M. rigidula* var. *rigidula* has highest behenic acid (1.73 %) content and *M. lupulina* has also low level of this fatty acid (0.71 %). The low amounts of behenic acid in legume seed oils is important because of the some researchers have indicated that oils with high levels of behenic acid may be difficult for digestive enzymes in humans and animals[24,25].

Total saturated fatty acid (TSFA) of *Medicago* species were between 14.22 and 26.05 %. *Medicago sativa* has lowest level of saturated acid and *M. minima* var. *minima* has highest amount of saturated fatty acid concentrations (Table-1). On the other hand, the unsaturated fatty acid composition of *Medicago* species were determined as high levels reported as other family members of Fabaceae[18], Lamiaceae[27], Euphorbiaceae[28], Boraginaceae[29], Apiaceae[30] family patterns. *Medicago sativa* has highest level of unsaturated fatty acid (83.46 %) and also *M. lupiluna* (78.55 %), *M. rigidula* var. rigidula (75.9 %), *M. rotata* var. eliezeri (75.01 %) and *M. minima* var. *minima* (70.71 %), respectively. *Vicia ervilia* and *Onobrychis fallax* (Fabaceae) has 80.43 and 79.58 % unsaturated fatty acid concentrations in their seed oils[23].

Molecular and phylogenetic studies revealed that *M. truncatula* is very close to the legumes belonging to genera that comprise economically important crops for human nutrition or animal feed *i.e.*, alfalfa, pea, faba bean and lentil[31,32]. All these traits brought *M. truncatula* to the status of model plant that seems suitable for identifying genes that are important, besides symbiosis, for the improvement of Agronomic traits such as seed quality, pest resistance, *etc.*[32]. The results obtained from these study will be supply some information on the usage of these genus patterns in view of feed crops and different agronomic traits. Most of these plants were pasture plants and α-linolenic acid, linoleic acid and palmitic acid were reported as predominant in pasture plants and also their contents varied during pasture season. The most abundant and most varied fatty acid compound in pasture plants were reported α-linolenic acid[33]. The results obtained from this study supported that this findings as observed in studies of Ryan *et al.*[34].

The results showed that the genera of the Fabaceae genus patterns has qualitative and quantitative different fatty acid, particularly unsaturated fatty acid contents. *Colutea, Hedysarum, Gonocytisus, Lupinus, Vicia, Onobrychis*[18] and *Lathyrus*[17,18], genera patterns has showed high content of linoleic acid. On the other hand, *Trigonella cretica* has oleic acid higher than the other fatty acid mentioned above[18]. It is determined that the linoleic and oleic acid are abundant components in most of the leguminous genera and this may be a characteristic of the family or some genera. But some results showed that the linoleic-palmitic type fatty acid is typical for some genera pattern like *Cassia nodosa, Berlinia auriculata, Bauhinia monandra, Parkia clappertonina*[25], some *Astragalus*[20] and *Ebenus species*[35] or linoleic-oleic-palmitic type, like in *Lathyrus* species[17] and some *Crolataria* species (Fabaceae)[36]. But some genera patterns showed same quantity of linoleic acid and linolenic acid

components in their seed oils like *Medicago* species studied in this study, some *Astragalus* species[20]. The results obtained from this study showed that, in general *Medicago* species has linoleic-linolenic and oleic-palmitic type fatty acid.

The oil contents of the studied legumes belonging to the *Medicago* genus, showed quantitative differences but the seed oils showed uniform fatty acid composition. The results revealed that the seed oils of *Medicago* species studied with a substantial amount of very long chain fatty acids might have attracted attention because of their value of nutritional, industrial and renewable resources.

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