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Seed Fatty Acid Composition of Some *Medicago* L. and *Melilotus* L. (Fabaceae) Taxa From Turkey

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Abstract: Fatty acids composition of plant oil of *Medicago disciformis* DC., *Medicago orbicularis* (L.) Bart., *Medicago intertexta* (L.) Mill var. *ciliaris* (L.) Heyn., *Medicago scutellata* (L.) Mill., *Melilotus alba* Desr. and *Melilotus officinalis* (L.) Desr. were analyzed. The fatty acid composition of these six different taxa were determined by gas chromatography. The fatty acid composition of plants used to this study showed different saturated and unsaturated fatty acid concentrations. The main fatty acids found were linoleic acid (19.43-56.25 %), linolenic acid (22.10-36.35 %), oleic acid (18.56-31.21 %), stearic acid (3.16-5.09 %) and palmitic acid (11.93-23.37 %); while other fatty acids were found in minor proportions. As a result, present study determined that all taxa had the highest total unsaturated fatty acid amounts (69.81-82.53 %) and the lowest total saturated fatty acid amounts (17.47-30.19 %). The higgest unsaturated fatty acid determinated to *Melilotus officinalis* (82.53 %), the lowest in *Medicago intertexta* (69.81 %). In the study of species, palmitic and stearic acid were found, the major saturated fatty acids. The other hand oleic, linoleic and linolenic acids in major unsaturated fatty acids. Fatty acid composition of studied plants oils could be used as a chemotaxonomical marker.

Key words: *Medicago*; *Melilotus*; seed; fatty acid.

Introduction

Legumes are important crops valued for their place in crop rotations and as food, feed and protein sources. Legumes are an important food source and play a significant role in traditional diets in many regions of the world. Among the legume seeds, some are used as vegetables and others as supplementary sources of protein in animal diets; therefore, Fabaceae taxa increasingly being looked upon as potential alleviators of the problem of high population to protein ratio in the world [1]. The widespread use of legumes makes this food group an important source of lipid and fatty acids in animal and human nutrition. Some publications dealing with the total lipid and fatty acid composition are reviewed by a few researchers [2-4]. The possible role of forage legumes in modern livestock production is being reconsidered [5]. Greater use of these species in pastures provides economic and agronomic benefits to agropastoral systems [6]. Biological nitrogen fixation by legumes results in high protein herbage and improved nutrient balance of the sward [7].

Legume-based pastures may increase the self provision of the protein sources, the feeding value of forages and sustainability of grazing systems [8]. Information on the chemical composition of *Medicago* seed oil is very scanty [9]. Polyunsatu-

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rated fatty acids (PUFA) function as major nutrients, constituents of cell membranes and precursors of various signal molecules [10]. They are important in both the medical and, as they are involved in the human inflammatory response, blood-pressure regulation, cholesterol metabolism, and infant retinal and brain development [11]. Information on the chemical composition of *Medicago* and *Melilotus* seed oil is lack, while previous workers investigated phytochemical properties of some Fabaceae taxa [2-4]. The objective of the present study was to determine fatty acid contents of the seeds of some *Medicago* L. taxa (*M. disciformis, M. orbicularis, M. intertexta* var. *ciliaris, M. scutellata*) and *Melilotus* L. species (*M. alba* and *M. officinalis*).

Material and methods

Plant samples

In this research, matured dried seeds of *Medicago disciformis, Medicago orbicularis, Medicago intertexta* var. *ciliaris, Medicago scutellata, Melilotus alba* and *Melilotus officinalis* were collected from natural habitats in Eastern Anatolian region of Turkey in years 2012-2013. The voucher specimens were deposited in ISTE and Department of Field Crops, Faculty of Agriculture, University of Bingol.

Oil extraction and preparation of fatty acid methyl esters (FAME)

Impurities were removed from the seeds and the cleaned seeds were ground. Lipids were extracted with hexane/isopropanol (2 v/v) [12]. The lipid extracts were centrifuged at 10.0 g for 5 min and filtered. The solvent was a rotary evaporator at 40°C.

Capillary GLC

Fatty acids in the lipid extracts were converted into methyl esters by means of 2 % sulphuric acid (v/v) in methanol [13]. 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 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°C, resptectively.

Identification of the individual method was performed by frequent comparison with authentic standards mixtures that were analyzed under the same conditions.

Statiscial analysis

The statistical software Cropstat (IRRI 2005) was used to perform the ANOVA and pattern analysis. Standard analyses of variance (anova) were used to analyze the data obtained. Cluster analysis of studied samples seen in Figure 1; fatty acid composition of the studied samples are reported in Table 1. ANOVA is used to detect is the difference between more than two groups is important statistically [14]. Hierarchical cluster analysis is a technique that aims to unify units at specific levels by considering their similarities [15]. Hierarchical clustering techniques are Unifying Hierarchical Technique and Separative Hierarchical Technique. In Seperative Technique, all units are considered a cluster at the beginning. In Unifying Technique, on the other hand, all units are considered separate clusters at the beginning [15]. In Hierarchical clustering techniques, dendogram is used in order to understand the process easily. At the beginning of clustering process every individual is a cluster; at the end of the process all individuals are gathered in one cluster. When applying Hierarchical clustering methods Single Connection Method or the Nearest Neighbour Method is used [16].

Results and discussion

Total fatty ratio in stadied plants showed different concentrations. The higgest ratio was *M. scutellata* (11.31 %), the lowest to *M. intertexta* var. *ciliaris* (0.50 %). The fatty acid composition of *Medicago* and *Melilotus* 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 seen in Table 1. *M. orbicularis* and *M. disciformis* were rich by linolenic acid

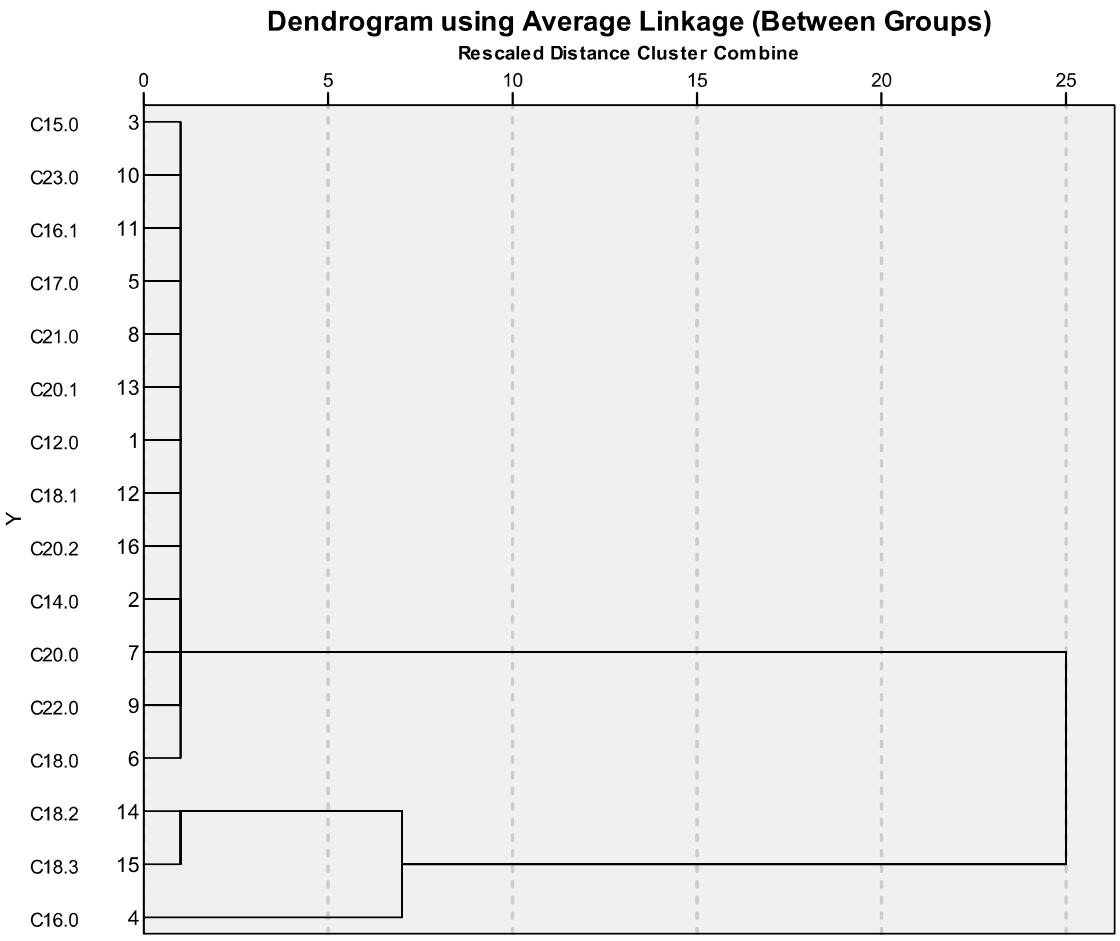

Fig. 1. Hierarchical cluster analysis fatty acid of studied taxa

concentrations (36.35, 35.59 %). But *M. officinalis* and *M. alba* seed oil was rich in view of linoleic acid (56.25, 51.92 %). *M. scutellata* (31.21 %) and *M. intertexta* var. *ciliaris* (18.56 %) have oleic acid, but not other species. The other species comprised the middle linoleic acid and linolenic acid in the seed oils. *M. disciformis* has 37.28 % linoleic acid; *M. orbicularis* has 36.80 %, *M. intertexta* var. *ciliaris* 25.81 %, *M. scutellata* 19.43 %; *M. intertexta* var. *ciliaris* has 25.06 % linolenic acid, *M. scutellata* 22.10 %, *M. alba* 28.15 % and *M. officinalis* 25.85 %, 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 [17]. Oleic acid contents of the *Medicago* species has shown more differences among the species of studied. *Medicago* taxa were reported as rich by oleic (7.00-21.15 %), linoleic (23.99-41.95 %) and linolenic (25.51-43.69 %) [4]. *Onobrychis fallax* Freyn & Sint. (Fabaceae) plant was reported as rich by oleic (52.56 %), linoleic (16.93 %), linolenic (8.63 %) and palmitic acids (8.95 %) [3]. Golden (*Trifolium aureum* Poll. and *Trifolium repens* L. var. *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 %) [3]. *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 %) [3]. Linoleic acid, oleic acid and linolenic acid components were found as main unsaturated fatty acid components in *Lathyrus* L. genus patterns of studied [18] has also low level of arachidic acid (20:0 %) and be-

Table 1. Seed fatty acid composition of *Medicago* and *Melilotus* samples (%)

Fatty acids	***Medicago disciformis***	***Medicago orbicularis***	***Medicago intertexta* var. *ciliaris***	***Medicago scutellata***	***Melilotus alba***	***Melilotus officinalis***
Total fatty ratio	**2.41**	**3.36**	**0.50**	**11.31**	**5.59**	**7.37**
C12.0	-	0.02	-	-	-	-
C14:0	0.19	0.41	0.54	0.27	0.13	0.08
C15:0	0.06	0.09	0.09	0.07	0.10	0.09
C16:0	20.39	20.01	23.37	20.02	14.72	11.93
C17:0	0.09	0.11	0.07	0.05	0.13	0.18
C18:0	3.16	3.54	3.92	5.09	3.32	4.37
C20:0	1.34	1.31	1.26	1.02	0.67	0.56
C21:0	0.09	0.11	-	-	0.06	0.05
C22:0	1.52	0.84	0.85	0.36	0.28	0.16
C23:0	-	0.09	0.07	-	0.07	0.05
SFA	26.84	26.54	30.19	26.88	19.48	17.47
C16:1	0.07	0.10	0.06	0.11	0.14	0.15
C18:1	-	-	18.56	31.21	-	-
C20:1	0.22	0.15	0.33	0.27	0.23	0.19
MUFA	0.29	0.25	18.95	31.60	0.37	0.34
C18:2	37.28	36.80	25.81	19.43	51.92	56.25
C18:3	35.59	36.35	25.06	22.10	28.15	25.85
C20:2	-	0.05	-	-	0.08	0.08
PUFA	72.87	73.20	50.86	41.52	80.15	82.19
UFA	73.16	73.46	69.81	73.12	80.52	82.53

Lauric acid methyl ester (C12:0)
Myristic acid methyl ester (C14:0)
Miristoleic acid methyl ester (C14:1)
Pentadecanoic acid methyl ester (C15:0)
Palmitic acid methyl ester (C16:0)
Palmitoleic acid methyl ester (C16:1)
Heptadecanoic acid methyl ester (C17:0)
cis 10–heptadecenoic acid methyl ester (C17:1)
Stearic acid methyl ester (C18:0)
Oleic acid methyl ester (C18:1)
Linoleic acid methyl ester (C18:2)
Linolenic acid methyl ester (C18:3)
Arachidic acid methyl ester (C20:0)
cis-11,14-eicosadienoic acid methyl ester (C20:2)
Heneicosanoic acid methyl ester (C21:0)
Behenic acid methyl ester (C22:0)
Tricosanoic acid methyl ester (C23:0)
MUFA= Monounsaturated Fatty Acid
PUFA= Polyunsaturated Fatty Acid
SFA= Saturated Fatty Acid
UFA= Unsaturated Fatty Acid

henic acid (22:0 %). In this study, The amount of the stearic acid was ranged from 3.16 % (*M. disciformis*) to 5.09 % (*M. scutellata*); palmitic acid was ranged from 11.93 % (*M. officinalis*) to 23.37 % (*M. intertexta* var. *ciliaris*). *M. orbicularis* has only lauric acid (0.02 %). The amount of arachidic acid was ranged from 0.56 % (*M. officinalis*) to 1.34 % (*M. disciformis*), behenic acid ranged from 0.16 % (*M. officinalis*) to 1.52 % (*M. disciformis*) (Table 1). The amount of the arachidic acid was ranged from 0.56 to 1.79 % in *M. sativa* L. subsp. *sativa* and *M. lupulina* L., but *M. rigidula* (L.) All. var. *rigidula* has the highest behenic acid (1.73 %) content and *M. lupulina* has also low level of this fatty acid (0.71 %) [4]. 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 [19].

Total saturated fatty acid of stadied species were between 17.47 % and 30.19 %. *Melilotus officinalis* has the lowest level of saturated acid and *Medicago intertexta* var. *ciliaris* the highest amount of saturated fatty acid concentrations. On the other hand, the unsaturated fatty acid composition of species were determined as high levels reported as other family members of Fabaceae [20-22], Apiaceae [23-27], Lamiaceae [28-31], Asteraceae [32-34] family patterns. *Melilotus officinalis* has higgest level of saturated fatty acid (82.53 %), and also *M. alba* (80.52 %), *M. orbicularis* (73.46 %), *M. disciformis* (73.16 %), *M. scutellata* (73.12 %) and *M. intertexta* var. *ciliaris* (69.81 %) respectively.

Bakoglu *et al*., determined that *Medicago sativa* has the highest level of unsaturated fatty acid (83.46 %) and also *M. lupiluna* (78.55 %), *M. rigidula* var. *rigidula* (75.9 %), *M. rotata* Boiss. var. *eliezeri* Eig. (75.01 %) and *M. minima* (L.) Bart. var. *minima* (70.71 %) [4]. Studied taxa manufactured many similar constituents in their fatt acid composition that could be verified by the same ecological conditions of their habitat; but also differences were detected that could ecological needs and conditions to evaluate if the pedoclimatic circumstances could effect the fatty acid composition cause chemical convergence and approve their taxonomic separation.

In conclusion, the oil contents of studied legumes belonging to the *Medicago* and *Melilotus* genus, showed quantitative differences but the seed oils showed uniform fatty acid composition. The results revealed that the seed oils of *Medicago* and *Melilotus* taxa 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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