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CHEMICAL COMPOSITION OF THE SEEDS OF SOME *Medicago* SPECIES

Kagan Kokten,[1] Adil Bakoglu,[2] Alpaslan Kocak,[3] Eyup Bagci,[4] Mevlut Akcura,[1] and Mahmut Kaplan[5]*

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Legume seeds are rich in many nutrient components, including protein, starch, certain fatty acids, and micronutrients such as vitamins and trace minerals [1–3]. Some species of the family Fabaceae are a source of cheap protein for both humans and animals [4], and legumes are generally rich sources of proteins [5, 6].

Information on the chemical composition of *Medicago* seed oil is very scant [7, 8], while previous workers investigated phytochemical properties of some legumes widely [9–12]. The objective of the present study was to determine the tannin, protein contents, and fatty acid composition of the seeds of *Medicago* L. species (*M. disciformis* DC., *M. polymorpha* L., *M. intertexta* (L.) Mill., *M. scutellata* (L.) Mill., *M. truncatula* Gaertn. and *M. orbicularis* var. *marginata* (L.) Bart.). The results of the fatty acid analysis are shown in Table 1, and total protein and tannin contents in Table 2.

The total protein amounts of the feed crops studied were found to be between 30.50–47.17% in *Medicago orbicularis* var. *marginata* and *M. truncatula.* These were 34.87, 34.93, 36.20, and 38.30% in *M. intertexta, M. scutellata, M. disciformis,* and *M. polymorpha,* respectively.

The protein levels were determined in fruit, leaf, and steam of *Medicago noeana, M. orbicularis, M. polymorpha* var. *vulgaris, M. rigidula* var. *submitis,* and *M. rigidula* var. *rigidula* [7]. According to them, the protein levels studied were found to be between 20% and 30%. The protein levels of fruits were found to be higher than those in leaf and steam. On the other hand, the protein contents of fruit of *M. noeana, M. orbicularis,* and *M. polymorpha* var.*vulgaris* were found to be higher than in fruits of other species.

The tannin amounts of feed crops studied were found to be between 0.27–1.23% in *M. orbicularis* var. *marginata* and *M. scutellata.* The others comprise 0.30% (*M. intertexta*) and 0.70% (*M. disciformis*, *M. polymorpha* and *M. truncatula*), respectively. It was reported that condensed tannin may act as anthelmintics against parasitic nematodes or indirectly by improving nitrogen supply [13–15].

The main components in the seed oils of *Medicago* species were palmitic, oleic, linoleic, and linolenic acids.

The other fatty acids of the legume seed oils (arachidic and lignoceric acid) in the studied species were shown to be lower than 1% except for *M. truncatula* (Table 1). This is similar to those reported [12]. On the other hand, behenic acid was at the highest level in *M. disciformis* (1.11%). Some researches have indicated that oils with high levels of long-chain SFA such as behenic acid may be difficult for digesting enzymes to digest in humans and animals [16].

The results of the present study, as far as unsaturated fatty acid content is concerned, are supported by previous leguminous studies [12, 17, 18]. All these studies showed that the saturated, and particularly unsaturated, fatty acid contents of Fabaceae seed oils are closely allied to each other and that the main components in the oils are linoleic-oleic type fatty acids.

Seed Samples. Matured seeds of these species were collected from various locations in Adana (Turkey) between June and August 2009.

Oil Extraction and Preparation of Fatty Acid Methyl Esters (FAME). Impurities were removed from the seeds, and the cleaned seeds were ground into powder using a ball mill. Lipids were extracted with hexane/isopropanol, 2 v/v [19].

1) Bingol University, Faculty of Agriculture, Department of Field Crops, Bingol, Turkey; 2) Bingol University, Vocational School of Higher Education, Program of Field Crops, Bingol, Turkey; 3) Bingol University, Faculty of Science and Arts, Department of Biology, Bingol, Turkey; 4) Firat University, Faculty of Science and Arts, Department of Biology, Elazig, Turkey; 5) Erciyes University, Faculty of Agriculture, Department of Field Crops, Kayseri, Turkey, e-mail: mahmutk@erciyes.edu.tr. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 548–549, July–August, 2011. Original article submitted February 3, 2010.

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TABLE 1. Fatty Acid Composition of Some *Medicago* Species from Turkey

Fatty Acid	*M. disciformis*	*M. polymorpha*	*M. intertexta*	*M. scutellata*	*M. truncatula*	*M. orbicularis* var. *marginata*
14:0	0.35	0.21	0.39	0.38	0.32	–
16:0	17.5	19.5	18.8	17.7	20.4	12.2
16:1	–	–	–	–	–	0.51
18:0	1.71	1.92	3.04	2.28	3.5	1.71
18:1	10.63	5.82	31.5	6.67	13.6	22.5
18:2	14.6	26.3	19.5	32.05	42.2	61.0
18:3	51.8	43.8	25.3	37.2	16.3	1.21
20:0	0.93	0.82	0.72	0.80	1.46	0.30
20:1	0.32	0.27	0.38	–	0.25	0.28
22:0	1.11	0.74	0.21	0.46	0.60	0.36
24:0	0.79	0.73	0.20	2.40	1.41	0.32
TSFA	22.4	23.8	23.2	24.0	27.7	14.4
TUSFA	77.3	76.0	76.7	75.9	72.3	85.5

TSFA: total saturated fatty acids; TUSFA: total unsaturated fatty acids.

TABLE 2. Total Protein and Tannin Contents of some *Medicago* Species from Turkey, %

Medicago Species	Protein	Tannin	*Medicago* Species	Protein	Tannin
M. disciformis	36.20	0.70	*M. truncatula*	47.17	0.70
M. polymorpha	38.30	0.70	*M. orbicularis* var. *marginata*	30.50	0.27
M. intertexta	34.87	0.30	LSD	1.6543	0.3764
M. scutellata	34.93	1.23			

The lipid extracts were centrifuged at 10.0 g for 5 min and filtered; then the solvent was removed on a rotary evaporator at 40°C. Fatty acids were converted into methyl esters by means of 2% sulfuric acid (v/v) in methanol [20]. Then the methyl esters were separated and quantified by gas chromatography and flame ionization detection (Schimadzu 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 and 240–280°C, respectively. Identification of the individual method was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Determination of Protein and Tannin Contents. Seed samples were cleaned, and protein content was analyzed according to the method of AOAC [21]. Tannin contents of seeds were determined by the method of Makkar et al. [22]. Protein and tannin analyses were carried out in triplicate.

Statistical Analysis. The experimental design was completely randomized with three replications. Data were analyzed by the SAS packet program. Biplot analysis was done to differentiate between fatty acids and *Trifolium* species by the method of Yan and Kang [23].

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