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Investigation on Fatty Acids in Leaves, Stems and Fruits of Some Species of *Medicago*

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ABSTRACT

The fatty acids composition of leaf, stem and fruit of *Medicago noeana*, *M. orbicularis, M. polymorpha* var. *vulgaris, M. rigidula* var. *submitis* and *M. rigidula* var. *rigidula* were analyzed by gas chromatography-mass spectrometry. The analysis of fatty acid composition of all the organs of *these* species revealed that palmitic (C16: 0), stearic (C18: 0), oleic (C18: 1), linoleic (C18: 2) and linolenic acids (C18: 3) were major fatty acids. In addition, lauric (C12: 0), miristic (C14: 0), pentadecanoic (C15: 0), heptadecanoic (C17: 0), palmitoleic (C16: 1), arachidic (C20: 0) behenic (C22: 0) and lignoseric acids (C24: 0) were also found in some species. There was fluctuation in fatty acid levels of different organs. Linolenic acid was found in all species, especially at the highest level in leaves. Oleic acid was noted at higher level in fruits of all the species.

Key Words: *Medicago* spp.; Fatty acid; Gas chromatography-mass spectrometry

INTRODUCTION

The homeland of clover (*Medicago* L.) is Transkafkas and Eastern Anatolia, which is known as the strongest for its quality and fruit amongst the feeder plants (Hughes *et al*., 1952). Animal studies have shown that this plant reduces cholesterol and prevent formation of arterial plate. Besides, it includes eight enzymes, which facilitates digestion of protein, fat and carbohydrates. Differences have been observed in fatty acids of animals, fed with various plants (O'Brien & Benson, 1964; Karnezos & Matches, 1993; Hillbrick & Tucker, 1996).

An investigation on seeds of some leguminous plants revealed fat in *Medicago* species (3.3 – 15.9%), *Trifolium* species (3.5 – 19.4%) and *Trigonela coelesyriaca* (5.2%) (Tonnet & Snudden, 1974). Palmitic acid, linolenic acid and linoleic acid were discovered at major levels in sulpholipid and phospholipid fractions extracted in *M. sativa*. However, linolenic acid was found in high ratio in sulpholipid fractions (Klopfenstein & Shigley, 1967). Huang and Grunwald (1990) found linolenic acid as a major seed fatty acid of galactolipits and sulfolipids and it increased greatly during germination of alfalfa (*Medicago sativa*) seeds.

Hawke (1963) reported that young and mature plants contain linolenic acid in higher level then palmitic acid and linoleic acid. Linolenic acid, occurring in large amount in chloroplast, has functional affect on photosynthesis (Poulsan *et al.,* 2002). In investigation on *Vitis vinifera* (Mazruma) and *Olea europaea*, not belonging to Fabaceae family revealed large amount of un-saturated fatty acids in these plants (Demir & Otludil, 1997; Demir & Başhan, 1998).

Physiological and biochemical studies are quite limited on (*Medicago* L.) except some taxonomic studies (Akbayın & Demir, 1994; Akbayın *et al*., 1994). This study aims to determine fatty acids in some *Medicago* species, which grown up naturally and to contribute physiology of these plants partly.

MATERIALS AND METHODS

Rearing of plants. *Medicago noeana*, *M. orbicularis*, *M. polymorpha* var. *vulgaris*, *M. rigidula* var. *submitis* and *M. rigidula* var. *rigidula* are the most common plants which grow up naturally in Diyarbakır region. Samples were collected in between May and July 2004 and kept in deep-freezes until they were analyzed.

Lipid analysis. The plants were processed for lipid extraction and analyzed with the following methods described by Howard and Stanley (1990). Each sample was analyzed three times. For this purpose leave, stem and fruit (3 g each) were used for each analysis. Samples were separated (Bligh & Dyer, 1959) from homogenate using chloroform-methanol (2:1 v/v). Autoxidation of un-saturated components was minimized by adding 50 µL of 2% butylated hydroxytoluene in chloroform to each sample during the extraction process. Samples were taken the reaction tubes by straining with filter paper. The total lipid extracts were dried under a stream of N_2 and scraped into reaction vials, and the associated fatty acids were transmethylated by refluxing the fractions in acidified methanol for 90 min at 85°C. The fatty acid methyl esters (FAMEs) were extracted from the reaction vials three times with hexane and concentrated.

GC-MS analyses were made using a GC-MS equipment (HP 5890-E series GC-System, Hewlett-Packard, Palo Alto, CA, USA) with mass-selective detection. An Innowax column (30 m x 0,25 mm i.e., 0,25 µm film thick-ness) was used and the temperature was programmed from 150 – 230°C at a 2°C min^{-1} increase with an initial hold of 4

Table I. Proportions of fatty acids as percent of total fatty acids in some species of *Medicago* L.*

	Organs	12: 0	14: 0	15: 0	16: 0	17: 0	16: 1	18: 0	18: 1	18: 2	18: 3	20: 0	22: 0	24: 0
M. noeana	Leaf	-	0.80± 0.11	0.81± 0.23	24.57± 1.02	-	4.38± 0.07	5.16± 0.79	2.95± 0.04	10.62± 0.36	50.1± 2.10	-	0.54± 0.16	-
	Steam	-	1.29± 0.27	0.73± 0.13	28.10± 0.92	0.76± 0.02	2.36± 0.14	5.62± 0.80	8.83± 0.87	18.75± 0.94	32.83± 1.19	0.7± 0.07	-	-
	Fruit	-	1.62± 0.16	1.21± 0.11	3.68± 0.40	-	1.14± 0.19	6.07± 0.80	21.37± 1.20	34.11± 1.33	29.89± 1.57	0.87± 0.04	-	-
M. orbicularis	Leaf	0.49± 0.02	1.20± 0.18	-	25.83± 1.09	-	4.21± 0.08	5.01± 0.15	5.17± 0.29	13.16± 0.52	44.84± 2.02	-	-	-
	Steam	0.59± 0.05	1.57± 0.21	0.55± 0.08	29.22± 1.47	0.59± 0.31	2.88± 0.82	6.75± 0.96	5.37± 0.83	16.90± 1.11	32.76± 1.91	1.27± 0.13	0.89± 0.06	0.61± 0.05
	Fruit	0.31± 0.08	0.80± 0.03	0.83± 0.07	38.57± 1.96	-	0.96± 0.11	5.17± 0.87	9.14± 1.03	30.91± 1.18	12.38± 0.60	0.65± 0.07	0.27± 0.10	-
M.polymorpha var.vulgaris	Leaf	0.36± 0.13	0.99± 0.19	-	24.01± 1.01	-	0.94± 0.07	9.17± 0.83	4.07± 0.78	8.32± 0.97	50.72± 2.31	-	0.86± 0.17	0.52± 0.20
	Steam	-	1.00± 0.32	0.46± 0.09	29.76± 1.59	0.55± 0.07	1.01± 0.24	4.46± 0.62	9.27± 0.86	16.73± 1.30	35.58± 1.97	-	0.63± 0.08	0.65± 0.11
	Fruit	-	1.26± 0.43	-	0.55± 0.12	0.65± 0.09	25.69± 0.98	4.24± 0.16	10.27± 0.25	17.28± 0.66	38.15± 2.09	1.10± 0.15	0.75± 0.11	-
M. rigidula var.submitis	Leaf	-	1.33± 0.51	0.38± 0.05	25.67± 1.34	-	3.91± 0.26	4.69± 0.09	5.23± 0.42	12.70± 0.51	42.63± 2.27	1.79± 0.14	1.07± 0.23	0.56± 0.02
	Steam	-	1.34± 0.18	0.71± 0.08	29.80± 1.52	0.73± 0.14	2.94± 0.31	5.58± 0.47	7.18± 0.64	13.99± 1.06	34.04± 1.76	1.88± 0.19	1.05± 0.11	0.60± 0.09
	Fruit	0.37± 0.13	1.21± 0.23	0.79± 0.08	32.06± 1.85	0.43± 0.47	2.43± 0.45	4.04± 0.36	11.32± 0.82	22.83± 0.73	22.04± 1.05	1.40± 0.24	0.70± 0.16	0.33± 0.12
M. rigidula var.rigidula	Leaf	0.33± 0.12	0.97± 0.63	0.33± 0.21	22.56± 1.74	0.30± 0.07	3.21± 0.61	3.91± 0.76	3.11± 0.14	11.65± 0.58	51.33± 2.33	1.43± 0.14	0.52± 0.06	0.28± 0.06
	Steam	0.41± 0.09	1.20± 0.11	0.66± 0.07	28.27± 1.02	0.50± 0.04	2.76± 0.28	5.15± 0.41	5.50± 0.44	20.11± 1.03	33.44± 1.50	0.99± 0.13	0.56± 0.10	0.39± 0.04
	Fruit	0.24± 0.07	0.73± 0.12	0.75± 0.16	32.98± 1.92	0.42± 0.08	1.07± 0.08	4.39± 0.22	10.59± 0.92	24.28± 1.25	22.77± 1.13	1.00± 0.14	0.47± 0.09	0.24± 0.03

*Each value represents mean ±SD of three replicates.

min and a final hold of 36 min. The carrier gas was helium (1 mL min^{-1}) and the split ratio was 1: 50. The injection port and the detector temperatures were 250°C and 300°C, respectively. The mass spectrometer was operated in the electron impact ionization mode (70 eV). FAMEs were identified by comparison with the Wiley 275 and Nist 98 databank. Statistical analysis included ANOVA. Significance tests were conducted at $\alpha = 0.05$ level.

RESULTS AND DISCUSSION

In this study the fatty acids composition of *Medicago noeana*, *M. orbicularis, M. polymorpha* var. *vulgaris, M. rigidula* var. *submitis* and *M. rigidula* var. *rigidula* were analysed. The analysis revealed the presence of lauric, miristic, pentadecanoic, palmitic, heptadecanoic, palmitoleic, stearic, oleic, linoleic, arachidic, behenic and lignoseric acids in this plants. Lauric acid and lignoseric acid could not be discovered in *M. noeana*. But, all the other fatty acids were found in *M. rigidula* var. *rigidula* (Table I).

In all species, linolenic acid was determined in the highest level in leaves compared with stem and fruit. Palmitic acid was at major levels in fruits rather in leaves, following linolenic acid. The exceptions were found in fruit of *M. noeana* and *M. polymorpha* var. *vulgaris*. Oleic acid found in *M. noeana* and, palmitoleic in *M. polymorpha* var. *vulgaris* were determined in higher levels in the fruit parts of the plants. Then, linoleic acid was found in highest levels, followed by oleic, stearic and palmit acids. Lauric and lignoseric acids were determined at lowest levels. In all species oleic acid was found in higher levels in fruits rather in other parts of plant.

Fatty acids have important functions such as being used as a source of energy invertebrate and invertebrate animals and forming structure of biological membranes (Stanley *et al*., 1988). It is possible to generalize fatty acids found in high organized plants and animals. The most common fatty acids have carbon atoms and extend from C14: 0 to C24: 0. Fatty acids (C 16: 0 & C18: 0) are the major ones. The amount of un-saturated fatty acids is more than the amount of saturated fatty acids especially in high organized plants and in animals living under low temperatures. Similar results were found in our study on *Medicago* species. The high proportion of un-saturated fatty acids helps to maintain the static order and dynamic properties of membranes, which is vital for the functioning of cells and whole organisms e.g., bacteria, protozoa, yeast, plants, invertebrates and fish (Hazel, 1995; Tasaka *et al*., 1996; Suutari *et al*., 1997).

Mammals can not synthesize linoleic acid and linolenic acid, but these two un-saturated fatty acids are taken in large amount with plant foods. Arachidonic acid, which is not present in plants, has been synthesized in mammals by priority molecules, which are in the form of linoleic acid. In all species of *Medicago* mainly linolenic acid, palmitic and linoleic acid were found at high level. The

results are in agreement with earlier studies on fatty acids of alfalfa (*M. sativa*), ryegrass, wheetgrass and sainfoin (Karnezos & Matches, 1993).

In all studied species, linolenic acid was determined in larger rate in leaf rather than in stem and fruit. Then, palmitic acid and linoleic acid were found in order. C12: 0 and C24: 0 were found in the lowest rate. The high proportions of C18: 3 in *Medicago* leaf are of interest for certain reasons. Firstly, it is suggested that the high content of linolenic acid in the galactolipits, which occur almost exclusively in the chloroplast, may be related to a functional role of these lipids in photosynthesis (Poulsan *et al*., 2002). Secondly, oxygen is required for the synthesis of linolenic acid from oleic acid (as in yeast), and increased synthesis of this fatty acid after synthesis may be merely a reflection of the requirement for oxygen (O'Brien & Benson, 1964).

Oleic acid was found in larger amounts in fruits of *Medicago* indicates that the oil obtained from these varieties are of good quality and beneficial for health. Because this un-saturated fatty acid constitutes structural component of membranes, high proportion of oleic acids shows that *Medicago* spp. fruits store this oil optionally. The cause of it may be seeds in the fruits.

While Single carbon fatty acids are very rare in territorial animals, there are a lot of fatty acids with single carbon in sea animals (Gözükara, 1997). In this study fatty acids with single carbon C15: 0 and C17: 0 was determined. Variations related to fatty acids exist in different species. The similar situation, were determined in different organs (leaf, stem & fruit) of the same species.

More delicious meat and milk produces can be obtained from animals by changing their diet by *M. noeana*, *M. orbicularis, M. polymorpha* var *vulgaris, M. rigidula* var. *submitis* and *M. rigidula* var. *rigidula,* which are rich especially in the fatty acids such as C18: 1, C18: 2 and C18: 3. The fatty acid profile of yolk are changed by feeding chicken with seeds and sea produces, which are contains more ω-fatty acids (especially ω 3) (Lewis *et al*., 1997). Fatty acid profiles and foods values of obtained from this type of animals can be changed by taking the animals as a biological model.

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REFERENCES

Akbayın, H. and R. Demir, 1994. A Clustering analysis on *Medicago* spescies in the province of Diyarbakır in south-east Anatolia. *Trkish J. Bot.*, 18: 419–23

Akbayın, H., R. Demir and S. Ertekin, 1994. Solution of some taxonomic problems in (*Medicago rigudula* L.) all varieties by discriminant analysis technique. *Trkish J. Bot*., 18: 481–8

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol*., 37: 911–7

Demir, R. and M. Başhan, 1998. A study on the investigation of fatty acids in pulps and seeds of *Olea europae* L. Hırhalı and *Oleae europaea* L. Zoncuk. *Biochem. Arch*., 14: 187–91

Demir, R. and B. Otludil, 1997. Investigation of the saturated and un-saturated fatty acids in the internodes and leaves of *Vitis vinifera* L. Mazruma. *Biochem. Arch*., 13: 223–8

Gözükara, E., 1997. *Biochemistry I,* 3rd edition. İstanbul: Nobel Publishing

Hawke, J.C., 1963. Studies on the properties of New Zealand butterfat: the fatty acid composition of the milk fat of cows grazing on rye-grass at two stages of maturity and the composition of rye-grass lipids. *J. Dairy Res*., 30: 67–75

Hazel, J.R., 1995. Thermal adaptation in biological membranes: is homeoviscous adaptation the explanation? *Annu. Rev. PhysioI*., 57: 19–42

Hillbrick, G.C. and D.J. Tucker, 1996. Effect of nutrition on lipit production and composition of cashmere buck fleece. *J. Small Ruminant Res*., 29: 1441–5

Howard, R.W. and D.W. Stanley–Samuelson, 1990. Phospholipid fatty acid composition and arachidonic acid metabolism in selected tissues of adult *Tenebrio molitor. An. Entomol. Soc. America*, 83: 975–81

Huang, S. and C. Grunwald, 1990. Lipid and fatty acids changes during germination of alfalfa seeds. *J. Phytochem*., 29: 1441–5

Hughes, H.D., M.E. Heath and D.S. Metcalfe, 1952. *Forages: The Secience of Grassland Agriculture*. The Iowa State College Press, Ames, IA

Karnezos, T.P. and AG. Matches, 1993. Fatty acid composition of dock-fat from lambs grazing alfalfa, sainfoin, end wheat-grass. *J. Food Qual*., 16: 409–15

Klopfenstein, W.E. and J.W. Shigley, 1967. Changes in fatty acid composition of sulfolipid and phospholipids during maturation of alfalfa. *J. Lipid Res*., 8: 350–1

Lewis, N.M., K. Schalch and S.E. Scheideler, 1997. *Incorporation of w3 Fatty Acid-enriched Eggs in Low Fat Diets of Hypercholesterolemic Humans.* International Conference on The Return of w-3 Fatty Acids into the Food Supply: I. Land-Based Animal Food Products and Their Health Effects. Bethesda, MD

O'Brien, J.C. and A.A. Benson, 1964. Isolation and fatty acid composition of the plant sulfolipid and galactolipids. *J. Lipid. Res*., 5: 432–4

Poulsan, M.E., G.E. Edwards and J Browse, 2002. Photosynthesis is limited at high leaf to air vapor pressure deficit in a mutant of *Arabidopsis thaliana* that lacks trienoic fatty acids. *Photosynth. Res.,* 72: 55–63

Stanley–Samuelson, D.W., R.A. Jurenka, C. Cripps, G.J. Blomquist and M. De Renobales, 1988. Fatty acids in insects: composition, metabolizm and biological significance. *Arch. Insect Biochem. Physiol*., 9: 1–31

Suutari, M., A. Rintamaki and S. Laakso, 1997. Membrane phospholipids in temperature adaptation of *Candida utilis:* alterations in fatty acid chain length and un-saturation. *J. Lipid. Res.,* 38: 790–4

Tasaka, Y., Z.Gombos, Y. Nishiyama, P. Mohanty, T. Ohba, K. Ohki and N. Murata, 1996. Targeted mutagenesis of acyl-lipid desaturases in *Synechocystis:* evidence for the important roles of poly-unsaturated membrane lipids in growth, respiration and photosynthesis. *EMBO J*., 15: 6416–25

Tonnet, M.L. and P.M. Snudden, 1974. Oil and protein content of the seeds of some pasture legumens. *Australian J. Agric. Res*., 25: 767–74

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