

# Seed fatty acid composition of some Fabaceae taxa from Turkey, a chemotaxonomic approach

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**Summary.** Seed fatty acids composition of *Lathyrus nissolia* L., *Lathyrus hirsutus* L., *Pisum sativum* L. var. *arvense* (L.) Poiret., *Onobrychis montana* DC. subsp. *cadmea* (Boiss.) P.W.Ball., *Trigonella monantha* C.A.Mey. subsp. *monantha*, *Trigonella foenum-graceum* L. were analyzed by gas chromatography of the methyl esters of their fatty acids. The fatty acid composition of the studied taxa were found as identical qualitatively, but some quantitative differences were determined in interspecific and intergenus level. The fatty acid composition of studied plants showed different saturated and unsaturated fatty acid concentrations. The major fatty acids were found to be linoleic acid (11.94-53.09%), linolenic acid (7.70-47.05%), oleic acid (0.00-28.01%), palmitic acid (12.40-26.14%) and stearic acid (2.82-10.25%); while other fatty acids were found in minor percentages. As a result, in this research we detected that all taxa had the higher total unsaturated fatty acid (UFA) (68.11-80.67%) than saturated fatty acid (SFA) (19.33-31.89%) amounts. The highest UFA detected to *Trigonella monantha* subsp. *monantha* (80.67%), lowest to *Onobrychis montana* DC. subsp. *cadmea* (68.11%). In this study, palmitic and stearic acid were found in the major saturated fatty acids; while oleic, linoleic and linolenic acids were found to be the major unsaturated fatty acids. Chemotaxonomic implications of the components of the studied plant taxa are discussed and the main components could be used as a chemotaxonomical marker.

**Key words:** chemotaxonomy, fatty acid, *Lathyrus*, *Pisum*, *Onobrychis*, *Trigonella*

## Introduction

Fabaceae (Leguminosae) is one of the largest families of angiosperms and represented in Turkey with 71 genus, 1013 species which 400 of are endemic (1). *Lathyrus* L., *Pisum* L., *Onobrychis* Adans. and *Trigonella* L. genera are all in the Fabaceae family that these genera are represented in Turkey about 64, 6, 60 and 61 taxa respectively (2-4). "Legumes are important crops valued for their place in crop rotations as food, feed sources and play an important role in traditional diets in many regions of the world that some the legume seeds are used as vegetables and others as supplementary sources of protein in animal diets" (5). Some taxa of Leguminosae family are a source of cheap

protein for both humans and animals (6). "The pulses are also important as potential sources of natural tocopherols, tocotrienols and fatty acid composition all the world" (7). Some *Lathyrus* taxa are economic and agriculture plants throughout the world and Turkey (8); this genus includes a range of grain, forage, pasture and ornamental crops (9). "*Trigonella* seeds or as fenugreek and are well known for their pungent aromatic properties; the seeds contain the alkaloid trigonelline along with mucilage, tannic acid, yellow coloring matter, fixed and volatile oils, a bitter extractive, diosgenin, gitogenin, a trace of trigogenin and vitamin A" (10). *Onobrychis* comprises about 130 taxa and its distribution ranges from the Mediterranean region to Caucasia, the Zagros Mountains and central Asia; especially

in Iran and Turkey (11). Field pea is a common forage legume in the semiarid regions of the Anatolia and Mediterranean area; used for seed, hay, pasture, silage, and green manure and this plant is rich in high quality protein, phosphorus, calcium; and also a good source of vitamins A and D. "These qualities make field pea one of the best feeds for animals and almost indispensable for efficient, economical livestock feeding" (12).

Pea is one of the most common food plants in Turkey grown for fresh consumption and raw material of canned food industry. The green pea contains 6.7% protein, 0.5% oil and 13.9% carbohydrates; shyperlink, 1988 ome of Fabaceae taxa like *Arachis hypogea* L. and *Glycine max* (L.) Merr. have received considerable attention because of their high oil as well as high protein contents; therefore, their fat characteristics and fatty acid compositions have been extensively investigated (13). Polyunsaturated fatty acids function as main nutrients, constituents of cell membranes, precursors of various signal molecules (14); and involved in the human inflammatory response, blood-pressure regulation and cholesterol metabolism (15). The fatty acid composition of plant seed oils can provide characteristic information in order to confirm taxonomical and phylogenetic relationships in the plant kingdom (16) and fatty acid composition of some seed oils of Fabaceae taxa were first used for chemotaxonomic purposes by Wolff and Kwolek (17). Some publications dealing with the total lipid and fatty acid composition are reviewed by a few researchers (18-20).

In this study, fatty acid content of six plant samples from different genera (*Lathyrus*, *Pisum*, *Onobrychis*, *Trigonella*) were investigated and obtained results might provide new information, some contributions on the chemotaxonomic relationships, renewable resources and natural product. In order to extend our knowledge of the FA composition of the *Lathyrus*, *Pisum*, *Onobrychis*, *Trigonella* seed oils, it was considered desirable to investigate more members of these genus with modern analytical technics.

## Materials and Methods

In this research, maturated plant seeds (*Lathyrus nissolia*, *Lathyrus hirsutus*, *Pisum sativum* var. *arvense*,

*Onobrychis montana* subsp. *cadmea*, *Trigonella monantha* subsp. *monantha*, *Trigonella foenum-graceum*) were collected from natural habitats in Eastern Anatolian region of Turkey (Bingol) in years 2012-2013; to determine the seed fatty acids of studied samples. The voucher specimens were deposited in the Herbarium of ISTE and Department of Field Crops, Faculty of Agriculture, University of Bingol. Impurities were removed from the seeds and the cleaned seeds were ground using a ball mill into powder. Lipids were extracted with hexane/isopropanol (2 v/v) (21). The lipid extracts were centrifuged at 10.0 g for 5 min and filtered. The solvent was removed on a rotary evaporator at 40°C. Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol (22). 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, Perma-bound 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, respectively. Identification of the individual method was performed by frequent comparison with authentic standards mixtures that were analyzed under the same conditions.

## Statistical 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 determine is the difference between more than two groups is important statistically (23). Hierarchical cluster analysis is a technique that aims to unify units at specific levels (cluster distance measurement) by considering their similarities (24). Hierarchical clustering techniques are Unifying Hierarchical Technique and Separative Hierarchical Technique. In Separative 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 (24). In Hierarchical clustering techniques, dendrogram is used in order to understand the process easily. At the beginning of clustering process every individual is a cluster (branches of a tree); at the end of the process all individuals are gathered in one cluster (trunk of a tree). When applying Hierarchical clustering methods Single Connection Method or the Nearest Neighbour Method is used (25).

## Results

In this study, seed fatty acid composition of *L. nissolia*, *L. hirsutus*, *P. sativum* var. *arvense*, *O. montana* subsp. *cadmea*, *T. monantha* subsp. *monantha*, *T. foenum-graceum* were detected and the results are shown in Table 1. Total fatty acid ratio in studied plants showed different concentrations. The highest ratio was *T. foenum-graceum* (4.19%), lowest to *O. montana* (0.10%). The fatty acid composition of studied plants used as feed crops from Legume family showed different saturated and unsaturated fatty acid compositions. The main components in the seed oils of these taxa are linoleic (C18:2), linolenic (C18:3), oleic (C18:1), stearic (C18:0) and palmitic (C16:0) acid. Studied plant samples generally showed similar fatty acid composition, with few exceptions. *L. nissolia*, *L. hirsutus*, *P. sativum* var. *arvense* were rich by linoleic (49.30%-52.40%-53.09%) and palmitic acid (20.24%-15.74%-14.96%) concentrations respectively; *T. foenum-graceum* and *T. montana* were rich by linoleic (51.32%-33.41%) and linolenic acid (24.31%-47.05%) concentrations respectively; *Onobrychis montana* subsp. *cadmea* was rich by linolenic (28.17%) and oleic acid (28.01%) concentrations respectively (Table 1). It is noteworthy that, oleic acid was found to be high percentages, except for no percentages in the *T. foenum-graceum* and *T. monantha* subsp. *monantha* fatty acid compositions (Table 1).

## Discussion

Bakoglu *et al.*, (2010) detected that, *Medicago* L. taxa were rich by oleic (7.00%-21.15%), linoleic (23.99%-41.95%) and linolenic (25.51%-43.69%) ac-

ids (26). In another study with *Onobrychis fallax* Freyn & Sint was reported as rich by oleic (52.56%), linoleic (16.93%), linolenic (8.63%) and palmitic acids (8.95%) (27). In the studied plant samples, linoleic, oleic and linolenic acids were the main USFA components and analysis of this research showed that unsaturated fatty acids comprised most of the oil. Oleic acid (18:1) was not found the *Trigonella foenum-graceum* and *Trigonella monantha* subsp. *monantha* oil; however *Onobrychis montana* subsp. *cadmea* (28.01%) and *Lathyrus hirsutus* (19.4%) have the highest oleic acid (18:1) composition. Linoleic acid was the predominant component of seed oils of all studied samples. This unsaturated fatty acid (USFA) was highest in *Pisum sativum* var. *arvense* (53.09%), *L. hirsutus* (52.40%) and in *Trigonella foenum-graceum* (51.32%); in this plants linoleic acid comprised more of the half of the oils (Table 1). *Trifolium aureum* Poll. has high percentage of linolenic (19.56%), oleic (13.40%) and palmitic acids (12.89%); *Trifolium repens* L. var. *repens* plant seeds fatty acid was reported as rich by oleic (22.67%), palmitic (9.58%) and stearic acid (7.72%) concentrations (27). "Linoleic acid, oleic acid and linolenic acid components were found as main unsaturated fatty acid components in *Lathyrus* genus patterns studied" (16). In this research, the amount of the stearic acid was ranged from 2.82% (*O. montana* subsp. *cadmea*) to 10.25% (*L. hirsutus*); behenic acid were found to be low percentages in all studied taxa (Table 1). "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" (28).

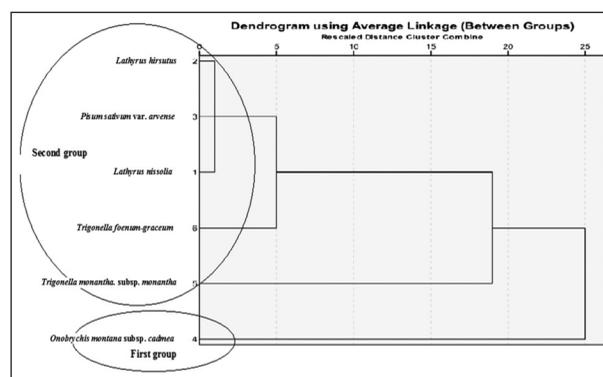


Figure 1. Hierarchical cluster analysis fatty acid of studied samples.

Omega-3 fatty acids have been associated with many health benefits (29). "Omega-3 fatty acids modulate prostaglandin metabolism and decrease triglycerides and, in high doses, lower cholesterol and have antithrombotic and anti-inflammatory properties". These studies were extensively reviewed and reported" (30,31). "A new arena for omega-3 fatty acids has emerged as adjuvants to drug treatment leading to synergism (potentiating the effects of drugs) or to decreasing their toxicity" (32). "Similarly, increasing the intake of ome-

ga-3 fatty acids while decreasing the omega-6 fatty acids in the diet has led to improvements and a decrease of non-steroidal anti-inflammatory agents in patients with rheumatoid arthritis (33) and asthma" (34). "The importance of omega-3 essential fatty acids in the diet is now evident, as well as the need to return to a more physiologic omega-6/omega-3 ratio of about 1-4/1 rather than the ratio of 20-16/1 provided by current Western diets. In order to improve the ratio of omega-6/omega-3 essential fatty acids, it will be necessary to decrease the

**Table 1.** Seed fatty acid composition of studied samples (%).

<i>Fatty acids</i>	<i>Lathyrus nissolia</i>	<i>Lathyrus hirsutus</i>	<i>Pisum sativum</i> subsp. <i>arvense</i>	<i>Onobrychis montana</i> subsp. <i>cadmea</i>	<i>Trigonella monantha</i> subsp. <i>monantha</i>	<i>Trigonella foenum-graceum</i>
<b>Total oil</b>	0.76	1.10	1.08	0.10	0.46	4.19
C 14:0	0.36	0.26	0.15	0.40	0.27	0.11
C 15:0	0.12	0.14	0.16	0.15	0.14	0.12
C 16:0	20.24	15.74	14.96	26.14	14.92	12.40
C 17:0	0.12	0.20	0.16	0.10	0.12	0.60
C 18:0	6.06	10.25	6.28	2.82	3.02	7.82
C 20:0	0.75	0.67	0.67	0.59	0.39	1.89
C 21:0	0.00	0.00	0.00	0.00	0.06	0.13
C 22:0	0.00	0.00	0.00	1.70	0.34	0.64
C 23:0	0.00	0.00	0.00	0.00	0.06	0.11
<b>TSFA</b>	<b>28.41</b>	<b>27.27</b>	<b>22.38</b>	<b>31.89</b>	<b>19.33</b>	<b>23.84</b>
C 16:1	0.00	0.00	0.00	0.00	0.06	0.05
C 17:1	0.00	0.00	0.00	0.00	0.00	0.13
C 18:1	10.52	12.63	15.01	28.01	0.00	0.00
C 20:1	0.00	0.00	0.00	0.00	0.15	0.27
<b>MUFA</b>	<b>10.52</b>	<b>12.63</b>	<b>15.01</b>	<b>28.01</b>	<b>0.21</b>	<b>0.45</b>
C 18:2	49.30	52.40	53.09	11.94	33.41	51.32
C 18:3	12.51	7.70	9.52	28.17	47.05	24.31
C 20:2	0.00	0.00	0.00	0.00	0.00	0.08
<b>PUFA</b>	<b>61.81</b>	<b>60.10</b>	<b>62.61</b>	<b>40.11</b>	<b>80.46</b>	<b>75.71</b>
<b>TUSFA</b>	<b>71.59</b>	<b>72.73</b>	<b>77.62</b>	<b>68.11</b>	<b>80.67</b>	<b>76.16</b>
PUFA/TSFA	2.17	2.32	2.86	1.34	4.19	3.24
n-3	12.51	7.70	9.52	28.17	47.05	24.31
n-6	49.30	52.40	53.09	11.94	33.41	51.40
n-9	10.52	12.63	15.01	28.01	0.21	0.45
TSFA+TUSFA	100.00	100.00	100.00	100.00	100.00	100.00

*Myristic acid (C14:0); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Arachidic acid (C20:0); Heneicosanoic acid (C21:0); Behenic acid (C22:0); Tricosanoic acid (C23:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:1); Oleic acid (C18:1); Paullinic acid (C20:1); Linoleic acid (C18:2); Linolenic acid (C18:3); Eikosadienoic acid (C20:2); Omega-3 fatty acid (n-3); Omega-6 fatty acid (n-6); Omega-9 fatty acid (n-9); MUFA=Monounsaturated Fatty Acid; PUFA= Polyunsaturated Fatty Acid; SFA= Saturated Fatty Acid; UFA= Unsaturated Fatty Acid.*

intake of omega-6 fatty acids from vegetable oils and to increase the intake of omega-3 fatty acids by using oils rich in omega-3 fatty acids and increase the intake of fish to two to three times per week or take supplements. Omega-3 fatty acids have been part of our diet since the beginning of time. It is only for the past 150 years that omega-3 fatty acids have been decreased in Western diets due to agribusiness and food processing. The need to return the omega-3 fatty acids into the food supply has been recognized by industry, which is already producing omega-3 enriched products” (35). In this study omega-3, omega-6, omega-9 fatty acids were found to be high percentages in all studied taxa (Table 1). Total unsaturated fatty acid (USFA) of studied taxa were between 68.11% and 80.67% (Table 1). Bakoglu *et al.*, (2010) determined that *Medicago sativa* has highest level of unsaturated fatty acid (83.46%) and also *Medicago lupulina* (78.55%), *M. rigidula* var. *rigidula* (75.9%). In another study, *Vicia ervilia* (80.43%) and *Onobrychis fallax* (79.58%) have unsaturated fatty acid concentrations in their seed oils (27). Total saturated fatty acid (SFA) of studied taxa were between 19.33% and 31.89%. *O. montana* subsp. *cadmea* has highest level of SFA (31.89%); also in the *L. nissolia* (28.41%) and *L. hirsutus* (27.27%). “The results of the this study, as far as unsaturated fatty acid content is concerned, is supported by previous leguminous studies (36). All these studies showed that the saturated and particularly unsaturated fatty acid contents of Leguminosae seed oils are closely allied to each other and the main components in the oils are linoleic and linolenic type fatty acids. Hierarchical cluster analysis essential of studied samples is seen in Figure 1. Results of cluster analysis based on the distribution of fatty acid compounds show two main groups; linoleic acid (first) and the other fatty acids (second) (Figure 1). Seed oils of some Fabaceae taxa have attracted attention because of their value for industrial purposes and compounds of seed oils can be chemotaxonomic significance of studied taxa (7). *O. montana* subsp. *cadmea* was very far apart from all the other taxa. We can separate second main group in three groups; first *Trigonella monantha* subsp. *monantha*, second *Pisum sativum* subsp. *arvense* and *Trigonella foenum-graceum*, third *L. nissolia* and *L. hirsutus* samples. In fact, in the second main group *T. monantha* subsp. *monantha* was showed different fatty acid composition from all the other taxa in the second

group. *Lathyrus nissolia* and *Lathyrus hirsutus* which were in the same genus (*Lathyrus*), are very close in the dendrogram in terms of major fatty acid components. It is noteworthy that, *P. sativum* subsp. *arvense* and *T. foenum-graceum* which were not in the same genus, but these species are close in the dendrogram in terms of major fatty acid components. *Onobrychis montana* subsp. *cadmea* were collected in a single cluster; according to these results, some of comments can be made about relationships of studied samples (Figure 1).

In conclusion, the oil contents of the studied samples showed quantitative differences but the seed oils generally showed uniform fatty acid compositions. The seed oils of the all the investigated taxa were rich in palmitic, stearic, oleic, linoleic and linolenic acid. The fatty acid chemotypes of studied taxa were determined as; linoleic and palmitic acid in *L. nissolia*, *L. hirsutus*, *P. sativum* subsp. *arvense*; linolenic and palmitic acid in *O. montana*; linoleic and linolenic acid in *T. monantha* subsp. *monantha* and *T. foenum-graceum* (Table 1). It appears from aforementioned studies that there are many Fabaceae taxa whose fatty acids contents have not been studied enough. Thus we believe that the results of our study encourage further screening for the fatty acids of other Fabaceae taxa that have not been studied earlier. With regard to the fatty acid composition of the family Fabaceae requires further investigation, and our research team is currently engaged in an intensive study on this research areas. The fatty acid results from this study might be helpful in potential usefulness and chemotaxonomy of studied taxa. In addition, the results revealed that the seed oils of *Lathyrus*, *Onobrychis*, *Trigonella* and *Pisum* patterns studied with a substantial amount of very long chain fatty acids might have attracted attention because of their value as nutritional, industrial and renewable resources.

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