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Fatty acids, protein contents and metal composition of some feed crops from Turkey

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Abstract

In the course of investigations of new sources of higher plant lipids, fatty acid and metal compositions of some feed crops -*Vicia ervilia* (bitter veitch), *Lotus corniculatus, Onobrychis fallax*, *Trifolium aureum* (golden clover), *Trifolium repens* (white clover) (Fabaceae)- from Turkey were investigated by using GC and ICP-OES system. All the legume crops showed the same pattern of fatty acids. The fatty acid composition of plants used as feed crops showed different saturated and unsaturated fatty acid concentrations. Linolenic, oleic, palmitic and linoleic acids were found as the abundant compounds. The seeds are rich in unusual fatty acids, protein and also some elements. The protein content ranged from 20.09 to 28.6% in bitter vetch and golden clover. Cu, Mn, Cr, Ni, Zn, Fe, Mg, Al and Pb were detected in the crop seeds in different amounts. The proximate analysis indicated that the seeds contained Cu 4.93-12.59, Mn 10.17-20.0, Zn 33.67-54.48, Fe 51.6-372.8, Mg 258.2-793.2 and Al 10.38-15.50 $\mu g\ g^{-1}$, respectively. The results were discussed in means of nutrition, agricultural values and phytochemicals.

Key words: Feed crops, protein contents, fatty acid, trace elements, GC, ICP-OES.

Introduction

Some species of family Fabaceae are a source of cheap protein for both humans and animals [1] and legumes generally are rich sources of proteins [2, 3]. Therefore, they are increasingly being looked upon as potential alleviators of the problem of high population to protein ratio in the world [4]. Some legumes are rich, not only in protein, but also in other chemical entities such as starch, oils, vitamins and mineral elements [5]. They have, therefore, been exploited economically for some of these chemical components. Some of these leguminous species are groundnut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), soybean (*Glycine max*), common bean (P*haseolus vulgaris*), pea (*Pisum sativum*), lentil (*Lens culinaris*) and broad bean (*Vicia faba*). Therefore, their fat characteristics and fatty acid components have been extensively investigated [6-10].

The problems of industrial waste are becoming harder to solve, and much effort will be needed to develop the nutritional and industrial potential of by–products, waste and underutilized agricultural products. Only a small portion of plant material is utilized directly for human consumption [11]. There is an increasing awareness of the need to pay greater attention to the role of trace elements in plant and animal nutrition. The term trace element is useful but imprecise because it can refer to any element in the soil-plant-animal system regardless of its role [12].

The seed lipid content of 704 legume samples studied averaged 5.5% while many leguminous seeds used for food contain only 1-2% lipids, the unsaponifiable fraction of leguminous seed fixed oils ranged from 0.5 to 4.0% and the presence of myristic, palmitic, stearic, oleic, linoleic and linolenic acids in the seed oils of certain *Vicia* species was revealed [13]. Metal ions, metal complexes and vitamins are materials having an important role in vital functions of organisms [14].

The objective of the present study was to determine the protein contents and trace elements of some seed crops (*Vicia ervilia* (L.) Willd. (bitter vetch), *Lotus corniculatus* L., *Onobrychis fallax* Freyn & Sint., *Trifolium aureum* Poll. (golden clover) and *Trifolium repens* L. (white clover)). In addition during the course of this study, it was aimed to characterize seed fatty acids used by animals in field, to establish the nutritional value and to do contributions as the renewable resources of FA and other chemical patterns in these crops.

Materials and Methods

Seed samples: In this research, seeds of *Vicia ervilia, Lotus corniculatus, Onobrychis fallax, Trifolium aureum* and *Trifolium repens* (Fabaceae) were collected from natural habitats in Eastern Anatolian region of Turkey in 2007-2008.

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 2v/v [15]. 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.

Capillary GLC: Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol[16]. 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 (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 analysed under the same conditions.

Determination of protein and metal contents: Seed samples were cleaned and protein content was analysed according to the method of AOAC [17]. Seed samples were also analysed for metals. For this purpose, they have digested by domestic microwave oven (Premier). A 2.0 g portion of each sample was dried at 80°C and accurately 0.50 g directly weighed into PTFE bombs. For the acid digestion of samples 4 ml HNO_3 (65% w, Merck, Darmstadt) and 1 ml $HClO_4$ (60% Merck, Darmstadt) were added. In a tightly closed system, the following six-step microwave digestion program was applied according to literature [18]. PTFE bomb was left for an hour to cool and carefully opened. Colorless solution was transferred into a beaker and evaporated to dryness with hot plate. Afterwards final volume was diluted with 10 ml of 0.1 M HNO_3. The blank digests were carried out in the same way. Stock standard solutions of the metals (1000 mg l^{-1}) were supplied by Merck. Sample solutions were analyzed with method of direct calibration curve by ICP-OES. The samples were analyzed in triplicate. Though the FAAS [19] technique have been used in many trace element studies of plant samples, we have used the ICP-OES technique because it has the advantage of being multi-elemental and also requires only very small samples.

Results and Discussion

In this study, the total protein amount, fatty acid composition and trace elements of some feed crops from Turkey were determined. The results of the fatty acid analysis are shown in Table 1 and total protein and trace elements in Table 2.

The fatty acid composition of some plants used as feed crops from Fabaceae family showed different saturated and unsaturated fatty acid concentrations. The main components in the seed oils of feed crops are linoleic, oleic, palmitic and linolenic acids. *Vicia ervilia* and *Lotus corniculatus* were rich in linoleic acid (40.16-45.73%). Linoleic acid, undoubtedly, is one of the most important polyunsaturated fatty acids in human food because of its preventention of distinct heart and vascular diseases [20]. There are large amounts of oleic (28.2-16.31%), palmitic (12.66-11.73%), linolenic (9.26-11.0%) and stearic acid (5.84% - only in *Lotus corniculatus*) in the seed oils. On the other hand, *Onobrychis fallax* is rich in oleic (52.56%), linoleic (16.93%), linolenic (8.63%) and palmitic acid (8.95%) (Table 1). Golden and white clover have similar fatty acid composition and also more linoleic acid (42.53, 51.19%). *Trifolium aureum* has also large amount of linolenic (19.56%), oleic (13.40%) and palmitic acid (12.89%), respectively. *Trifolium repens* seeds FA was as rich in oleic (22.67%), palmitic (9.58%) and also stearic acid (7.72%), respectively. Linolenic acid concentration in white clower (3.45%) was lower than in golden clover (19.56%). There is a controversy between golden and white clover according to the stearic and linolenic acid contents of the seed oil. The presence and ratio of one of the three essential fatty acids in the seed oils make them nutritrionally valuable.

The contents of other PUFA and PSFA components of the seed oils of feed crops studied were not higher than 1%. Total saturated fatty acid (TSFA) concentrations of the feed crop oils studied were between 15.79 and 19.75%. On the other hand, total unsaturated FA contents of the seed oils were higher than TSFA, between 74.62 and 80.76%. TUSFA contents of the oils were found as similar. Linoleic, oleic, linolenic and also palmitic acid components were reported as main USFA components in *Lathyrus*[6, 21], *Colutea, Gonocytisus, Lupinus, Vicia, Hedysarum, Onobrychis, Trigonella* [22] and *Astragalus* [23, 24] genera patterns and also in some other family patterns like Euphorbiaceae [25]. The high USFA contents in these seed crops has nutritional significance.

As far as unsaturated fatty acid content is concerned, the present study is supported by previous reports [22, 24, 26-28] which suggest that the unsaturated FA contents of *Lathyrus* and other legume seed oils closely resemble each other and the abundant components are the linoleic–palmitic and/or oleic acids type FA.

Behenic acid (22:0) was not found or it was in very low amounts in the legume seed oils studied, except *Trifolium aureum* (1.38%). The low amounts of behenic acid in legume seed oils is important because of some researchers have indicated that oils with have high levels of behenic acid may be difficult for digestive enzymes in humans and animals [29, 30]. Despite its low bioavailability compared with oleic acid, behenic acid is a cholesterol-raising

Table 1. Total protein contents and fatty acid composition of some feed crops from Turkey. Data shown are peak area % from GLC.

Plant	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	20:0	20:1	22:0	22:1	22:2	24:0	TSFA	TUSFA
Vicia ervilia (L.) Willd.	0.75	12.66	0.99	-	1.93	28.20	40.16	9.26	1.30	0.70	-	-	1.12		16.64	80.43
Lotus corniculatus L.	0.18	11.73	0.76	0.77	5.84	16.31	45.73	11.00	0.79	0.28	0.43	0.27	0.27	-	19.74	74.62
Onobrychis fallax Freyn & Sint.	-	8.95	-	-	4.82	52.56	16.93	8.63	0.92	-	1.10	-	1.76	-	15.79	79.58
Trifolium aureum Poll.	-	12.89	0.53	-	3.80	13.40	42.53	19.56	0.82	0.18	1.38	-	0.18	0.16	19.05	76.38
Trifolium repens L.	?	9.58	0.51	-	7.72	22.67	51.19	3.45	0.53	0.18	0.14	0.31	0.25		17.97	78.56

TSFA Total saturated fatty acid, TUSFA Total unsaturated fatty acid.

Table 2. Total protein (%) and trace elements (μg.g^{-1} dry weight) of some feed crops from Turkey.

Plant	Total protein (%)	Trace elements								
		Cu	Mn	Cr	Ni	Zn	Fe	Mg	Al	Pb
Vicia ervilia (L.) Willd.	20.09	11.09	5.99	0.96	1.28	34.58	34.40	258.2	18.53	2.24
Lotus corniculatus L.	21.2	7.28	22.62	1.79	5.81	48.56	91.37	476.7	10.37	5.19
Onobrychis fallax Freyn & Sint.	25.4	2.55	30.04	2.37	4.62	14.48	136.88	593.5	33.20	3.77
Trifolium aureum Poll.	28.6	19.55	9.74	1.65	3.29	38.50	68.67	650.6	20.15	4.09
Trifolium repens L.	26.1	16.79	12.38	2.94	6.92	43.88	518.91	793.2	7.78	5.30

fatty acid in humans and therefore not a suitable substitute for palmitic acid in manufactured triacylglycerols [31].

Total protein amounts of feed crops studied were between 20.09-28.6% in *Vicia ervilia* and *Trifolium aureum* and 21.2, 25.4 and 26.1% in *Lotus corniculatus, Onobrychis fallax* and *Trifolium pratense,* respectively. The crude protein content was for *Brachystegia eurycoma* 11.82±0.25 [11], for cereal seeds such as corn, triticale and wheat [32] 8.40–14.8 and for pork and oyster 10.0–11.0 g/100 g dry matter, respectively. The data for *Tamarindus indica* (24.28±0.5 g/100 g dry matter) and *Mucuna flagellipes* (24.94±0.18 g/100 g dry matter) are higher than the protein contents in seeds of important legumes (18.0–25.0 g/100 g dry matter [33] and also the protein content of high protein animals such as lamb, marine fish and beef (16.0–18.0 g/100 dry matter) [34] but are close to seed contents of underutilized legumes such as *Ganavalia ensiforms* (26 g/100 g dry matter) [35]. The protein concentrations of studied legume seeds suggest that they can contribute to the daily protein need of 23.6 g/100 g for adults as recommended by the National Research Council [36].

The concentrations of the elements in the seeds are presented in Table 2. All data are averages of three measurements on each sample. The levels of metals were calculated on μg.g^{-1}dry weight. Nine elements (Cu, Mn, Cr, Ni, Zn, Fe, Mg, Al and Pb) were detected in the crop seeds in different amounts. The contents of elements in the crop seeds were Cu 4.93-12.59, Mn 10.17-20.0, Cr 0.91-5.59, Ni 2.83-5.02, Zn 33.67-54.48, Fe 51.6-372.8, Mg 258.2-793.2, Al 10.38-15.50 and Pb 1.39-5.46 μg g^{-1} (Table 2). The role of trace elements in human nutrition and disease cannot be overemphasized. Even though the mineral elements form a small proportion of the total composition of most plant materials and total body weight and do not contribute to the energy value of the food, they are of great physiological importance particularly in body metabolism [37].

It is of interest to note that the prevalent mineral element in the seeds is Mg which is as high as 793.2 μg.g^{-1} dry matter in *Trifolium pratense* and low in *Vicia ervilia* (258.2 μg.g^{-1}) (Table 2). The high quantity of potassium, magnesium and calcium together with the quantity of sodium plus the content of the essential elements iron, manganese, zinc and copper allow the seeds to be considered as excellent sources of bioelements [38]. It is recommended that these seeds could be used in the preparation of diets of individuals with low levels of these mineral elements.

Bitter veitch has the lowest proportion of the whole trace elements studied (Table 2). *Lotus corniculatus* has high proportion of Ni (5.81 μg.g^{-1}) and Zn (48.58 μg.g^{-1}) between the elements studied. While *Onobrychis fallax* has low amounts of Cu, Zn and Pb, it has high proportion of Mn (30.04 μg.g^{-1}), Al (33.20 μg.g^{-1}) and Mg (593.5 μg.g^{-1}), respectively. *Trifolium aureum* has high proportion of Cu and Mg. On the other *Trifolium* species, *T. repens* has high proportion of Cu, Cr, Ni, Zn, Fe, Mg and Pb, respectively.

Metals are unique nutrients because of their important role in metabolism. They are essential part of many important enzymes and they also play roles as catalysts and antioxidants. Iron and copper, for example, are essential in blood formation, and copper is also involved in normal carbohydrate and lipid metabolism [39]. Chromium regulates the action of insulin and is also essential in carbohydrate and lipid metabolism [40]. Zinc for its part is a multi-functional nutrient involved in glucose and lipid metabolism, hormone function and wound healing [39] and is also associated with proper hair growth [41]. The heavy metal concentrations in plants are mainly affected by pH and organic matter contents of the ecosystem and soil. The uptake of metal ions in plants is in many respects different, thus, the concentrations of metals depend on species, ecosystem and soil [42].

In general, legumes occupy an important place in human and also animal nutrition. Legumes are rich in proteins and complex carbohydrates and are important source of minerals and vitamins[43]. Grain legumes are potential sources of energy and micronutrients but their use is still limited because of incertainty about the amount and the effect of antinutritional factors they may contain [44].

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