GRASAS Y ACEITES, 62 (4),
OCTUBRE-DICIEMBRE, 383-388, 2011,
ISSN: 0017-3495
DOI: 10.3989/gya.125210

Chemical analyses of the seeds of some forage legumes from Turkey. A chemotaxonomic approach

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RESUMEN

Análisis químico de semillas de algunas leguminosas forrajeras de Turquía. Un enfoque quimiotaxonómico.

Se han estudiado las semillas procedentes de seis leguminosas forrajeras (leguminosas) en relación a sus proteínas, contenido de taninos y composición de ácidos grasos. El contenido de proteínas de las semillas de leguminosas forrajeras osciló entre un 23,1 y un 37,2%. Por otro lado, el contenido de taninos de las mismas semillas se encontraron entre 0,67 y 6,33%. La composición en ácidos grasos de estas seis especies diferentes se determinó por cromatografía gaseosa como ésteres metílicos de los ácidos grasos. Los aceites de las semillas de diferentes taxones de leguminosas contenían los ácidos linoleico y linolénico como principales componentes. Los aceites de semillas de las especies *Helianthus Lathyrus*, *Onobrychis crista-galli*, *Trigonella foenum-graceum*, *Lotus corniculatus*, y *Leuceana Leucocephala* contienen 50.0, 59.4, 69.5, 37.06 y 52.6%, respectivamente de ácido linoleico, como principal componente de los ácidos grasos, mientras que el aceite de semillas de *Cicer Lathyrus* contiene ácido linolénico en un 29,7% como principal componente de los ácidos grasos. Las proporciones de los ácidos grasos en los géneros de leguminosas resultaron ser muy variable. En el estudio de los taxones, el ácido palmítico se encuentra entre los ácidos grasos saturados más importante, por otro lado los ácidos oleico, linoleico y linolénico se encuentra entre los principales ácidos grasos insaturados.

PALABRAS CLAVE: Aceites de semillas – Ácidos grasos – Leguminosas – Proteínas – Taninos

SUMMARY

Chemical analyses of the seeds of some forage legumes from Turkey. A chemotaxonomic approach.

The seeds of six forage legumes (Leguminosae) were investigated for their protein, tannin contents and fatty acid compositions. The protein contents of forage legume seeds were found to be between 23.1 and 37.2%. On the other hand, the tannin contents of the same seeds were found to be between 0.67 and 6.33%. The fatty acid compositions of these six different species were determined by gas chromatography of the methyl esters of their fatty acids. The seed oils of different *Leguminous taxa* contained linoleic and linolenic acids as their major components. The seed oils of *Lathyrus annuus*, *Onobrychis crista-galli*, *Trigonella foenum-graceum*, *Lotus corniculatus*, and *Leuceana leucocephala* species contain 50.0, 59.4, 69.5, 37.06, and 52.6% linoleic acid, respectively, as the major component fatty acids, whereas in the seed oil of *Lathyrus cicer,* linolenic acid accounts for 29.7% as the major component fatty acid. The ratios of these fatty acids in the *Leguminous* genera were found to be highly variable. In the study of taxa, palmitic acid was found in the major saturated fatty acids, on the other hand oleic, linoleic and linolenic acids were found in major unsaturated fatty acids.

KEY-WORDS: Fatty acid – Leguminosae – Protein – Seed oil – Tannin.

1. INTRODUCTION

Legumes as source of protein for both forage and grain have a great chance of increasing their importance in the farming systems of the 21st century. Legume plants include many economically important crop plants that are utilized in human foodstuffs, herbal medicines, oil materials and as animal forages.

In Turkey, the *Lathyrus* species has been cultivated in small field areas and used mainly as forage and rarely as food. The *Lathyrus* species contains some toxic substances. One of the most important of these substances is a neurotoxin-oxalyl diamino propionic acid - (ODAP), which can result in paralysis in humans and the lower limbs of animals by affecting the central nervous system. This disease, named Lathyrism, generally occurs when seeds are consumed in large quantities for 3-4 months (Basaran *et al*. 2007).

Sainfoin (*Onobrychis viciifolia* Scop.), also known as holy clover, is a perennial forage legume with a deep taproot often grown in conjunction with forage grasses to reduce bloat hazard as well as to improve soil fertility due to its nitrogen fixing ability. Another desirable trait is that sainfoin has an early growth habit, sprouting earlier than alfalfa in spring to give good forage yields. While the availability of early fresh forage for stock is appreciated by farmers it is the ability of the feed to reduce

the incidence of bloating and to increase animal performance which provided the main incentive for its incorporation into farm management. Bloating is a major worldwide problem and frequently occurs in dairy cattle affecting up to 90% of the herds in a single district, causing many deaths (Lu *et al*. 2000).

Trigonella foenum-graecum is native to the area from the eastern Mediterranean to Central Asia and Ethiopia, and much cultivated in Pakistan, India, and China. Its dried ripe seeds are referred to as *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 (Petit *et al*. 1995). The seeds are also rich in protein and contain a unique major free amino acid 4-hydroxyisoleucine, which has been characterized as one of the active ingredients in fenugreek seeds (Broca *et al*. 1999).

Forages play an important role in ruminant nutrition in terms of providing energy, protein and minerals. Birdsfoot trefoil (*Lotus corniculatus*) is one of the self-generating plants in native pastures in Turkey. The nutritive value of birdsfoot trefoil was comparable with other legumes (Waghorn and Shelton 1992).

Tree legumes, such as *Leuceana leucocephala*, have been used as protein supplements for low quality forages and resulted in improved ruminant productivity (Norton 1994). It is reported that in over 26 *Leucaena* species studied, *Leucaena colinsii* has the lowest content of condensed tannin (CT) whereas *Leucaena pallida* has the highest CT content (Dalzell *et al*. 1998). Condensed tannin has been reported to strongly bind protein and other components in the diet, rendering them unavailable for digestion and absorption, which in turn affects animal performance (Mangan 1988).

Pulses are also important as potential sources of natural tocopherols, tocotrienols and fatty acids all over the world (Krishna *et al*. 1997; Bagci *et al*. 2003). Some leguminous species are groundnut (*Arachis hypogea*), cowpea (*Vigna anguiculata*), soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), lentil (*Lens culinaris*) and broad bean (*Vicia faba*). Groundnut and soybean have received considerable attention because of their high oil as well as high protein contents. Therefore, their fat characteristics and fatty acid components have been extensively investigated (Grela and Gunter 1995).

The objective of the present study was to determine the tannin and protein contents of the seeds of some forage legumes (*Lathyrus annuus* L., *Lathyrus cicera* L., *Onobrychis crista-galli* (L.) Lam., *Trigonella foenum-graceum* L., *Lotus corniculatus* L., and *Leuceana leucocephala* Lam.). An additional aim was to characterize seed fatty acids used for animals in the field, to establish their nutritional value and determine their potential as renewable resources of FA and other chemical patterns.

2. MATERIALS AND METHODS

2.1. Seed samples

The Forage legumes used in this study were *Lathyrus annuus* L., *Lathyrus cicera* L., *Onobrychis crista-galli* (L.) Lam., *Trigonella foenum-graceum* L., *Lotus corniculatus* L., and *Leuceana leucocephala* Lam. Matured seeds of these species were collected from various locations in Adana (Turkey) between June and August, 2009.

2.2. 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 (Hara and Radin 1978). 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.

2.3. Capillary GLC

Fatty acids in the lipid extracts were converted into methyl esters with 2% sulphuric acid (v/v) in methanol (Christie 1990). 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 the 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 fatty acids was made by frequent comparisons with authentic standard mixtures that were analyzed under the same conditions.

2.4. Determination of protein and tannin contents

Seed samples were cleaned and their protein content was analyzed according to the method of AOAC (1990). The Tannin contents of the seeds were determined by the method of Makkar *et al*. (1995). Protein and tannin analyses were carried out in triplicate.

2.5. Statistical analysis

The experimental design was a completely randomized design with 3 replicates. Standard analyses of variance (anova) were used to analyze the data obtained. The means of tannin and protein contents were compared by an LSD test using an SAS packet program.

3. RESULTS AND DISCUSSION

In this study, the total protein amount, fatty acid composition and tannin contents of some forage legumes from Turkey were determined. The results of the protein, tannin and fatty acid analyses are shown in Tables 1 and 2.

The protein contents of forage legume species varied from 23.1 to 37.2% (Table 1). Generally, the amount of protein detected was lower in *Lathyrus cicera* (23.1%) and *Leuceana leucocephala* (23.9%) than in *Trigonella foenum-graceum* (24.8%) and *Lathyrus annuus* (26.1%). The protein content of *Leuceana* was similar to the value reported by Abdulrazak *et al*. (2006). The highest protein contents were found to be 34.0 and 37.2% in *Lotus corniculatus* and *Onobrychis crista-galli*. These results are similar to those reported by Hanbury *et al*. (2000), Abdulrazak *et al*. (2006) and Bakoglu *et al*. (2009).

On the other hand, the tannin contents of forage legume species were determined to be in high levels in *Lathyrus cicera* (6.33%) and in low levels in *Trigonella foenum-graceum* (0.67%). In the other forage legumes, the tannin contents were determined as 0.70, 0.77, 1.63 and 2.17% in *L. leucocephala, O. crista-galli, L. corniculatus* and *L. annuus*, respectively (Table 1).

Unlike red clover (*Trifolium pratense*) and Lucerne (*Medicago sativa*) (Jackson *et al*. 1996; Wang *et al*. 1996), lotus (*Lotus corniculatus*) and sainfoin (*Onobrychis viciifolia*) contain significant amounts of condensed tannins (Waghorn *et al*. 1987; Wang *et al*. 1996). These are polyphenolic secondary compounds that can react by hydrogen-bonding with plant protein in the near neutral pH range to form condensed tannin±protein complexes which are insoluble at pHs found in the rumen, but dissociate below pH 3,5 (Waghorn *et al*. 1987; Wang *et al*. 1996). It is now thought that the nutritional role of condensed tannins for ruminants depends on their concentration, structure and molecular weight in plants, and, while high levels of condensed tannins may depress intake and the digestion of protein and fiber (Barry and Manley 1984; Terrill *et al*. 1992), low levels may increase absorption in the small intestine by reducing rumen protein degradation (Waghorn *et al*. 1994). Thus, increased animal performance has been documented for sheep grazing lotus compared with sheep grazing non-tanniniferous legumes (Wang *et al*. 1996).

Table 1
Total protein (%) and tannin contents (%) of some forage Legume species from Turkey

Forage Legume Species	Protein	Tannin
Lathyrus annuus (T-1)	26.1 c	2.17 b
Lathyrus cicera (T-2)	23.1 e	6.33 a
Onobrychis crista-galli (T-3)	37.2 a	0.77 d
Trigonella foenum-graceum (T-4)	24.8 cd	0.67 d
Lotus corniculatus (T-5)	34.0 b	1.63 c
Leuceana leucocephala (T-6)	23.9 de	0.70 d
LSD	1.4194	0.22

Table 2
Fatty acid composition (%) of some forage Legume species from Turkey.

Forage Legume species	Fatty Acid Components												
	14:0	16:0	16:1Δ9	18:0	18:1Δ9	18: 2Δ9,12	18:3Δ9, 12, 15	20:0	20:1	22:0	24:0	TSFA	TUSFA
Lathyrus annuus	0.99	17.0	–	6.69	12.04	50.0	13.30	0.11	–	–	–	24.68	75.45
Lathyrus cicera	0.50	22.2	–	2.22	27.9	12.3	29.7	0.67	0.94	1.73	0.78	24.92	74.02
Onobrychis crista-galli	1.01	17.95	0.73	4.79	5.92	59.4	7.38	0.69	–	–	0.89	24.48	74.28
Trigonella foenum-graceum	–	12.4	–	2.66	12.0	69.5	3.46	–	–	0.10	–	15.06	85.06
Lotus corniculatus	0.76	22.03	0.64	2.53	30.52	37.06	0.89	1.28	1.12	0.66	0.75	25.96	72.28
Leuceana leucocephala	0.44	16.83	0.33	4.39	12.4	52.6	11.27	0.47	–	0.74	0.57	21.99	78.05

14:0: myristic acid, 16:0: palmitic acid, 16:1 9: palmitoleic acid, 18:0: stearic acid, 18:1 9: oleic acid, 18:2 9,12: linoleic acid, 18:3 9,12, 15: linolenic acid, 20:0: arachidic acid, 20:1: gadoleic acid, 22:0: behenic acid, 24:0: lignoceric acid, TSFA: Total saturated fatty acid, TUSFA: Total unsaturated fatty acid

The fatty acid composition of some plants used as feed crops from the 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. Analyses showed that low myristic and palmitoleic acid from the fatty acid (FA), and these fatty acids were present at trace levels in the Leguminosae seed oils. Palmitic acid (16:0) was the highest SFA in *Lathyrus cicer* (22.2%), *Lotus corniculatus* (22.03%), *Onobrychis crista-galli* (17.95%), *Lathyrus annuus* (17.0%), *Leuceana leucocephala* (16.83%), and *Trigonella foenum-graceum* (12.4%) respectively (Table 2). Bagci *et al*. (2004) reported that palmitic acid was the major component in *Trigonella cretica* (12.9%), *Lupinus varius* (12.8%) and *Colutea melanocalyx* (10.7%). This is also a very constant lipid constituent in most leguminous genera seed oils. It is possible to say that this fatty acid is not a highly variable component in the leguminous genera pattern. Palmitoleic acid (16:1Δ9Δ was either not detected or detected in very small amounts. Similarly, *Trigonella foenum-graceum* and *Lathyrus annuus* had no palmitoleic, gadoleic or lignoceric acid (Table 2).

Stearic acid (18:0) was generally found in low levels, but it was shown in higher concentrations in *Lathyrus annuus* (6.69%), *Onobrychis crista-galli* (4.79%) and *Leuceana leucocephala* (4.39%). This is also reported for some *Vicia* sp. from Turkey like *V. hyrcanica* Fisch. et Mey. (19.4%), *V. peregrina* L. (7.3%), *V. hybrida* L. (9.1%) (Akpinar *et al*. 2001). On the other hand, this fatty acid was reported as lower in some *Vicia* sp. from different countries; *Vicia faba* (1.4%), *V. sativa* (1.3%). *Colutea melanocalyx* and *Onobrychis altissima* (1.38% and 1.79%) seed oils showed low stearic acid amounts. This FA was reported as 3.1% in *Colutea arborescens* (Ivanov and Aitzetmuller 1998). Some researchers indicated that oils with high levels of long chain SFA such as behenic acid may be difficult for the digesting enzymes in humans and animals (Balogun and Fetuga 1985; Akpinar *et al*. 2001). In addition, *Lathyrus cicera* had the highest amounts of linolenic and behenic acids (Table 2).

Among the unsaturated fatty acids (USFA), oleic, linoleic and linolenic acids were the major constituents of the studied legume seed oil. The greatest percentages of oleic acid were determined in *Lotus corniculatus* (30.52%), *Lathyrus cicera* (27.9%), *Leuceana leucocephala* (12.4%), *Lathyrus annuus* (12.04%) and *Trigonella foenum-graceum* (12.0%) respectively (Table 2). *Trigonella foenum-graceum* (69.5%), *Onobrychis crista-galli* (59.4%), *Leuceana leucocephala* (52.6%), *Lathyrus annuus* (50.0%), *Lotus corniculatus* (37.06%), *Lathyrus cicera* (12.3%) showed the highest linoleic acid composition in the seed oils. On the other hand, linolenic acid was determined as the major constituent of the all seed oils of *Lathyrus cicera* (29.7%). A high content of this component was found to be characteristic of legume seed oil. The seed oils of all the species analyzed were rich in palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. In the Akpinar *et al*. (2001) study, while most of the studied *Vicia* samples showed this result, *Vicia* hybrid concentrated from the other *Vicia* taxa. At the same time, oleic and linoleic acids were determined to be the major unsaturated fatty acids in *Psophocarpus tetragonnolobus* L. (Fabaceae) DC. (Winged bean) oil which is used as a food in the diets of some countries (Higuchi *et al*. 1982).

The total saturated fatty acid (TSFA) concentrations of the oils were between 15.06% and 25.96%. Total unsaturated fatty acid (TUSFA) contents were found between 72.28 and 85.06%. Linoleic, oleic and linolenic components were reported as the main TUSFA components in *Lathyrus* (Grela and Gunter 1995; Bagci *et al*. 2001), *Colutea*, *Gonocytisus*, *Lupinus*, *Vicia*, *Hedyserum*, *Onobrychis*, *Trigonella* (Bagci *et al*. 2004) and *Astragalus* (Bagci and Vural 2001; Bagci 2006) genera patterns and also in some other family patterns like Euphorbiaceae (Bagci, 2007). The high TUSFA contents in these seed crops have nutritional significance.

The results of the present study, as far as unsaturated fatty acid content is concerned, is supported by previous leguminous studies (Sengupta and Basu 1978; Daulatab *et al*. 1987; Tharib and Veitch 1983; Hamberg and Fhalstadius 1992; Liu *et al*. 1995). All these studies showed that the saturated and particularly unsaturated FA contents of Fabaceae seed oils are closely allied to each other and the main components in the oils are linoleic-oleic type fatty acids.

In general, legumes occupy an important place in human and also animal nutrition. Legumes are rich in proteins and complex carbohydrates and are important sources of minerals and vitamins (Mahadewamma and Tharanathan 2004). Grain legumes are potential sources of energy and micronutrients but their application is still limited because of uncertainty about the amount and the effect of antinutritional factors they may contain (Wiryavan and Dingle 1999).

4. CONCLUSIONS

The oil contents of the studied legumes belonging to the *Lathyrus*, *Onobrychis*, *Trigonella*, *Lotus*, and *Leuceana* genus, showed quantitative differences but the seed oils showed uniform fatty acid compositions. The seed oils of the all the investigated species were rich in palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. In the Akpinar *et al*. (2001) study, most of the studied *Vicia* samples showed the same results as ours. The results revealed that the seed oils of *Lathyrus*, *Onobrychis*, *Trigonella*, *Lotus*, and *Leuceana* 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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Recibido: 3/12/10
Aceptado: 3/3/11