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Tannin, protein contents and fatty acid compositions of the seeds of several *Vicia* L. species from Turkey

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RESUMEN

Taninos, contenido proteico y composición de ácidos grasos de semillas de algunas especies de *Vicia* L. de Turquía.

Las semillas oleaginosas de seis especies de *Vicia* (Leguninoas) fueron investigadas por su proteína, contenido en taninos y composición en ácidos grasos. El contenido de proteína en las semillas vario entre un 21.87%-31.33%. El contenido de taninos en las semillas vario entre un 0.13%-1.07%. La composición de ácidos grasos de estas seis diferentes especies fue determinada por GC mediante los esteres metílicos de sus ácidos grasos. Las semillas oleaginosas de las especies de *Vicia* contienen ácidos palmítico y esteárico como componentes mayoritarios entre los ácidos grasos saturados, con pequeñas cantidades de ácido mirístico, ácido palmitoleico y ácido margárico. Los ácidos grasos insaturados mayoritarios encontrados en las semillas oleaginosas fueron el ácido oleico, ácido linoleico y ácido linolénico. En este estudio, el contenido total de ácidos grasos saturado de las especies de *Vicia* vario entre 18.5 y 22.4% mientras que el de insaturados vario entre 71.1 y 80.3%.

PALABRAS CLAVE: Ácidos grasos – Especies de Vicia *– Proteína – Taninos.*

SUMMARY

Tannin, protein contents and fatty acid compositions of the seeds of several *Vicia* L. species from Turkey.

The seedoils of six *Vicia* species (Leguminosae) were investigated for their protein, tannin contents and fatty acid compositions. The protein contents of the seeds were found to be between 21.87%-31.33%. The tannin contents of the seeds were found to be between 0.13%-1.07%. The fatty acid compositions of these six different species were determined by the GC of the methyl esters of their fatty acids. The oilseeds of *Vicia* species contain palmitic and stearic acids as the major component of their fatty acids, among the saturated acids, with small amounts of myristic, palmitoleic and margaric acids. The major unsaturated fatty acids found in the oilseeds were oleic, linoleic and linolenic acids. In this study, the total saturated fatty acids of *Vicia* species were between 18.5 and 22.4% while the total unsaturated fatty acids were between 71.1 and 80.3%.

KEY-WORDS: Fatty acids – Protein – Tannin – Vicia *species.*

1. INTRODUCTION

The major use of vetches has been mixtures with cereals in hay, forage and green manure crops (Walton, 1992). The assumption being that this would allow for more immediate financial returns to the farming community without the necessity for an extra value-adding process involving livestock production, and would thus constitute an additional end-use option.

Polyunsaturated fatty acids (PUFAs) function as major nutrients, constituents of cell membranes and precursors of various signal molecules (Needleman *et al.,* 1986, Sakuradani *et al.,* 1999). They are important in both the medical and pharmaceutical fields, as they are involved in the human inflammatory response, blood-pressure regulation, cholesterol metabolism, and infant retinal and brain development (Horrobin, 1992; van Gool *et al.,* 2003).

The need for protein in human and animal diets is increasing all over the world. Protein, especially for use in animal feed, is becoming more scarce and more expensive. This is particularly relevant as far as traditional protein sources are concerned, such as fish meal and meat and bone meal. Throughout the world today there is also a growing resistance against the use of animal protein sources in animal feeding (Brand, 2002). It is desirable, therefore, that the nutritional potential of alternative plant protein feeds, such as grain legumes is fully exploited (Brand *et al.,* 2004).

The use of the *Vicia* species as grain legumes requires an understanding of their nutritive value and the potential toxicity of the grain of the different species to the various types of livestock (fish, poultry, pigeons, pigs, horses, cattle, sheep, goats) and humans (Enneking, 1995). The presence of tannins, saponins, phytic acid and other antinutritional substances hinder their use for human consumption (Morrow, 1991). Tannins affect the availability of amino acids and the utilization of protein while they inhibit the activities of digestive enzymes (Griffiths and Mosely, 1980; Sharma and Sehgal, 1992; Chi-Fai *et al.*, 1997).

The widespread use of legumes makes this food group an important source of lipid and fatty acids in animal and human nutrition. Some reports dealing with the total lipid and fatty acid compositions were published by a few researchers (Welch and Griffiths, 1984; Grela and Gunter, 1995; Akpinar *et al.,* 2001; Bakoglu *et al.,* 2009; Pastor-Cavada *et al*., 2009b; Yoshida *et al.,* 2009).

The objective of the present study was to determine the tannin and protein contents of the seeds of several *Vicia* L. species (*V. angustifolia* Reichard., *V. peregrina* L., *V. narbonensis* L., *V. hybrida* L., *V. ervilia* (L.) Willd. and *V. cracca* L. subsp. *cracca*), to characterize seed fatty acids used by animals in field, to establish the nutritional value and to make contributions as renewable resources of FA and other chemical patterns in these crops.

2. MATERIAL AND METHODS

2.1. Seed samples

The *Vicia* species used in this study were *V. angustifolia* Reichard., *V. peregrina* L., *V. narbonensis* L., *V. hybrida* L., *V. ervilia* (L.) Willd. and *V. cracca* L. subsp. *cracca*. Mature seeds of these species were collected from various locations in the Adana province of 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 clean 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 by means of 2% sulphuric acid (v/v) in methanol (Christie, 1990). The fatty acid methyl esters were extracted with 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 analyzed under the same conditions.

2.4. Determination of protein and tannin contents

Seed samples were cleaned and 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 replications. Data were analyzed using the SAS packet program.

3. RESULTS AND DISCUSSION

In this study, the total protein, fatty acid composition and tannin contents of several *Vicia* species from Turkey were determined. The results of the fatty acid analysis are shown in Table 1 and Figure 1, and the total protein and tannin contents are shown in Table 2.

The fatty acid composition of these plants used as feed crops from the Fabaceae family showed different saturated and unsaturated fatty acid concentrations. The main components in the oilseed of feed crops were linoleic, linolenic, oleic and palmitic acid. In all *Vicia* species studied, linoleic acid was found to be the major component (43.65%-63.84%). The oleic acid content was at its highest level in *V. narborensis* (29.87%), but found to be a its lowest in *V. ervilia* (9.20%) and *V. cracca* subsp. *cracca* (7.70%). Linoleic acid was found in the greatest proportion in the seed oil. The linoleic acid content was at its highest level in *V. cracca* subsp. *cracca* (63.84%), but found to be at its lowest level in *V. hybrida* (43.65%). The oilseeds of all the species were richer in linoleic than linolenic acid. The linolenic acid content was the highest in *V. hybrida* (21.98%) and *V. ervilia* (19.69%). Linolenic acid was also detected at a low level in *V. narborensis* (3.98%) (Table 1). Linoleic acid is needed for a normal immune response and in essential fatty acids, a deficiency impairs B and T cell mediated responses (Meydani *et al.,* 1991). The linoleic acid contents of the *Vicia* species has shown the greatest differences among the species studied. It is reported that, the oilseeds *Vicia* species also have large amounts of oleic (14.6-35.0%), linoleic (4.33-9.42%), and linolenic acid (1.95-9.20%) (Akpinar *et al.,* 2001).

We have used the biplot (Figure 1) to compare *Vicia* species on the basis of fatty acid compositions and to identify species or groups of *Vicia* that are particularly good in certain aspects (Rubio *et al.,* 2004). Specifically, the comparison between *V. angustifolia* and *V. narbonensis* indicates that *V. angustifolia* was better in palmitic acid, whereas *V. narbonensis* was better in arachidic, myristic, palmitoleic, oleic acid and lignoceric acid. Similarly, *V. hybrida* and *V. ervilia* had a greater value than *V. angustifolia*, *V. peregrina* and *V. cracca* subsp. *cracca* in all fatty acid compositions except palmitic acid and linoleic acid. The interpretation of the biplot also shows that *V. peregrina* and *V. cracca*

Table 1
Fatty acid composition of some *Vicia* species from Turkey

Vicia species	Fatty Acid Components												
	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	22:0	24:0	TSFA	TUSFA
V. angustifolia Reichard.	--	17.77	--	--	3.84	15.82	53.19	8.57	0.81	--	--	22.4	77,6
V. peregrina L.	--	13.87	--	--	4.20	12.44	62.08	7.40	0.10	--	--	18.0	81.9
V. narbonensis L.	1.04	14.45	1.76	--	1.95	29.87	44.49	3.98	1.02	--	1.43	19.9	80.1
V. hybrida L.	0.26	11.2	0.24	0.27	5.80	12.63	43.65	21.98	1.31	0.53	0.25	19.6	78.5
V. ervilia (L.) Willd.	--	10.33	0.40	0.26	5.0	9.20	53.77	19.69	0.51	0.27	--	16,4	83.1
V. cracca L. subsp. *cracca*	--	14.9	0.23	--	2.36	7.70	63.84	8.50	0.28	0.71	0.40	18.7	80.3

14:0: myristic acid, 16:0: palmitic acid, 16:1Δ9: palmitoleic acid, 17:0: margaric acid, 18:0: stearic acid, 18:1Δ9: oleic acid, 18:2Δ9,12: linoleic acid, 18:3Δ9,12, 15: linolenic acid, 20:0: arachidic acid, 22:0: behenic acid, 24:0: lignoceric acid, TSFA: Total saturated fatty acid, TUSFA: Total unsaturated fatty acid

Table 2
Total protein (%) and tannin contents (%) of some *Vicia* species from Turkey

Vicia Species	Protein	Tannin
V. angustifolia Reichard.	29,07 b	0,90 ab
V. peregrina L.	29,30 b	0,67 cd
V. narbonensis L.	24,10 d	0,83 bc
V. hybrida L.	27,07 c	1,07 a
V. ervilia (L.) Willd.	21,87 e	0,13 e
V. cracca L. subsp. *cracca*	31,33 a	0,47 d
LSD	1,6772	0,22

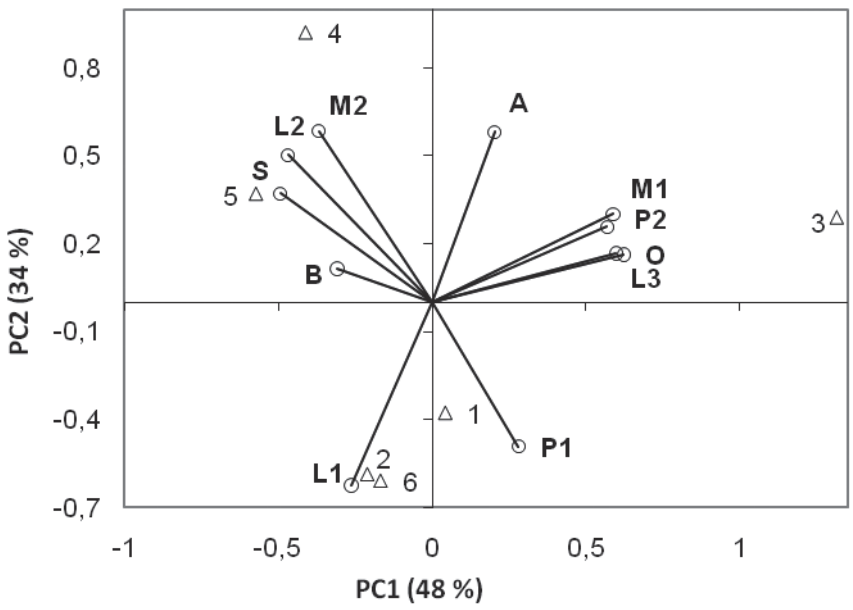

Figure 1.
The biplot of 6 *Vicia* species for fatty acid composition. (*Vicia* species: 1: *V. angustifolia,* 2: *V. peregrina,* 3: *V. narbonensis,* 4: *V. hybrida,* 5: *V. ervilia,* 6: *V. cracca* subsp. *Cracca*. Fatty acids: M1:myristic acid, P1: palmitic acid, P2: palmitoleic acid, M2: margaric acid, S: stearic acid, O: oleic acid, L1: linoleic acid, L2: linolenic acid, A: arachidic acid, B: behenic acid, L3: lignoceric acid.

subsp. *cracca* were the highest in linoleic acid while *V. angustifolia* was the highest in palmitic acid. *V. narbonensis* had the highest levels of myristic acid, palmitoleic acid, oleic acid and lignoceric acid. *V. hybrida* had the highest contents of margaric, linolenic and stearic acids (Figure 1).

The total saturated fatty acids (TSFA) of the *Vicia* species were between 18 and 22.4%. *Vicia peregrina* had the lowest level of saturated acid and *V. angustifolia* had the highest amount of saturated fatty acid (SFA) concentration (Table 1). On the other hand, the unsaturated fatty acid composition of the *Vicia* species was determined at levels as high as those reported for other family members of the *Vicia* species (Pastor-Cavada et al., 2009a), *Fabaceae* (Bagci *et al.,* 2004a), *Lamiaceae* (Bagci, 2007), *Boraginaceae* (Bagci *et al.,* 2004b), *Apiaceae* (Bena *et al.,* 1998) family patterns. *Vicia ervilia* had the highest level of unsaturated fatty acid (83.1%), along with *V. paregrina* (81.9%), *V. cracca* subsp. *cracca* (80.3%), *V. narborensis* (80.1%), *V. hybrida* (78.5%), *V. angustifolia* (77.6%), respectively. *Vicia ervilia* and *Onobrychis fallax* (Fabaceae) had 80.43 and 79.58% unsaturated fatty acid concentrations in their oilseeds (Bakoglu *et al.,* 2009).

The results showed that the genera of the Fabaceae genus patterns have qualitatively and quantitatively different fatty acids, particularly unsaturated fatty acid contents. But some results showed that the linoleic-palmitic type of fatty acid is typical for some genera patterns like *Cassia nodosa*, *Berlinia auriculata*, *Bauhinia monandra*, *Parkia clappertonina* (Balogun and Fetuga, 1985), some *Astragalus* (Bagci, 2006) and *Ebenus* species (Azcan *et al*., 2001) or the linoleic-oleic-palmitic type, like in the *Lathyrus* species (Bagci and Sahin, 2004) and some *Crolataria* species (Fabaceae). But some genera patterns showed the same quantity of linoleic acid and linolenic acid components in their oilseeds, like the *Vicia* species studied in this work, several *Astragalus* species (Bagci, 2006).

The total protein of the different grain legumes is presented in Table 2. *V. cracca* subsp. *cracca* had the highest protein content and differed significantly ($P \leq 0.05$) from the others. The total protein amounts of the *Vicia* species studied were between 21.87- 31.33% in *Vicia ervilia* and *Vicia cracca* subsp. *cracca* and 24.10, 27.07, 29.07 and 29.30% in *V. narbonensis, V. hybrida, V. angustifolia* and *V. peregrina* . The Crude Protein (CP) content of narbon beans (*V. narbonensis*) may vary between 26.0 and 32.0% (Abd El Moneim, 1992). These values were higher than the values found in the present study. Brand *et al.* (2004) found the CP level of narbon beans to be 23.7% and Bakoglu *et al.* (2009) found the CP level bitter vetch to be 20.09%. These values were slightly lower than the values found in the present study.

The tannin contents of the different grain legumes belonging to the *Vicia* genus are presented in Table 2. *V. hybrida* had the highest tannin and differed significantly ($P \leq 0.05$) from the others. The tannin amounts of the feed crops studied were between 0.13- 1.07% in *Vicia ervilia* and *Vicia hybrida* and 0.47, 0.67, 0.83 and 0.90% in *V. cracca* subsp. *cracca, V. peregrina, V. narbonensis* and *V. angustifolia* respectively. It was reported that condensed tannin may act as an antihelmintic against parasitic nematodes or indirectly by improving the nitrogen supply (Niezen *et al.,* 1995; Robertson *et al.,* 1995; Butter *et al.,* 1998).

The consequences of the complexes between tannin and protein (protein bound) or carbohydrate (fiber bound) and decreased digestibility are that the microbial population is denied access to essential amino acids and decreased N availability which may lead to restricted growth and depressed fermentative activity (Longland *et al*., 1995).

4. CONCLUSION

The oil contents of the studied legumes belonging to the *Vicia* genus showed quantitative differences but the oilseeds showed uniform fatty acid composition. The legumes are rich in proteins and complex carbohydrates and are an important source of minerals and vitamins (Mahadewamma and Tharanathan, 2004). The results revealed that the oilseeds of the *Vicia* species studied with a substantial amount of very long chain fatty acids might have attracted attention because of their value for nutritional, industrial and renewable resources.

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