

ARAŞTIRMA MAKALESİ

RESEARCH ARTICLE

Comparative Evaluation of Fatty Acid Profiles, Phenolic Content, and Antioxidant Activity in Seeds of Commonly Grown Forage Legumes

Baklagil Yem Bitkisi Tohumlarında Yağ Asidi Profilleri, Fenolik Madde İçeriği ve Antioksidan Aktivitesinin Karşılaştırmalı Değerlendirmesi

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Abstract

Legume forage crops are among the most important plant species due to their high protein content and bioactive compounds such as fatty acids and phenolic compounds. These compounds play a role in plant physiological processes and exhibit important functional properties in animal and human nutrition. The aim of this study is to determine the oil content, fatty acid composition, total phenolic content, and antioxidant activity of seeds from different legume forage plant species and to elucidate the relationships among these species using multivariate analysis methods. The study included alfalfa (*Medicago sativa*), sainfoin (*Onobrychis sativa*), common vetch (*Vicia sativa*), Hungarian vetch (*Vicia pannonica*), hairy vetch (*Vicia villosa*), narbon vetch (*Vicia narbonensis*), grass pea (*Lathyrus sativus*), and forage pea (*Pisum sativum*). According to the study results, seed oil content, total phenolic content, and antioxidant activity values showed statistically significant differences among species. The highest oil content was found in alfalfa, while the lowest was in common vetch. In all species, the major fatty acids were identified as palmitic, stearic, oleic, linoleic, and linolenic acids. Significant differences were observed in the distribution of fatty acids between species; linoleic acid was found in particularly high proportions in vetch species and sainfoin, whilst linolenic and palmitic acids were found at higher levels in alfalfa and sainfoin. The highest values for total phenolic content and antioxidant activity were observed in alfalfa and sainfoin, and a parallel relationship was observed between the two. Based on principal component and cluster analyses, the species were classified into two main groups based on their fatty acid composition, and it was determined that alfalfa and sainfoin were distinctly different from the other species. In general, it was concluded that the seeds of legume forage crops have significant potential in terms of essential fatty acids, phenolic compounds, and antioxidant activity.

Keywords: Legume forage crops, Fatty acid composition, Phenolic compounds, PCA, Heat map

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Öz

Baklagil yem bitkileri yüksek protein içeriğinin yanında yağ asitleri ve fenolik bileşikler gibi biyoaktif bileşikler bakımından önemli bitki türleri arasında yer almaktadır. Bu bileşikler, hem bitkilerin fizyolojik süreçlerinde rol oynamakta hem de hayvan ve insan beslenmesi açısından önemli fonksiyonel özellikler göstermektedir. Bu çalışmanın amacı farklı baklagil yem bitkisi türlerinin tohumlarında yağ içeriği, yağ asidi kompozisyonu, toplam fenolik madde ve antioksidan aktivite özelliklerini belirlemek ve türler arasındaki ilişkileri çok değişkenli analiz yöntemleri ile ortaya koymaktır. Araştırmada yonca (*Medicago sativa*), korunga (*Onobrychis sativa*), yaygın fiğ (*Vicia sativa*), Macar fiği (*Vicia pannonica*), tüylü fiğ (*Vicia villosa*), koca fiğ (*Vicia narbonensis*), mürdümük (*Lathyrus sativus*) ve yem bezelyesi (*Pisum sativum*) tohumları kullanılmıştır. Araştırma sonuçlarına göre tohum yağ içeriği, toplam fenolik madde ve antioksidan aktivite değerleri türlere göre istatistiki olarak önemli farklılık göstermiştir. En yüksek yağ içeriği yoncada, en düşük yağ içeriği ise yaygın fiğde belirlenmiştir. Tüm türlerde başlıca yağ asitlerinin palmitik, stearik, oleik, linoleik ve linolenik asitler olduğu belirlenmiştir. Türler arasında yağ asidi dağılımı açısından belirgin farklılıklar gözlenmiş; linoleik asidin özellikle fiğ türlerinde ve mürdümükte yüksek oranlarda bulunduğu, linolenik ve palmitik asitlerin ise özellikle yonca ve korungada daha yüksek seviyelerde olduğu tespit edilmiştir. Toplam fenolik madde içeriği ve antioksidan aktivite bakımından en yüksek değerler yonca ve korungada belirlenmiş ve fenolik madde içeriği ile antioksidan aktivite arasında paralel bir ilişki olduğu görülmüştür. Temel bileşenler analizi ve kümeleme analizi sonuçlarına göre türlerin yağ asidi kompozisyonuna göre iki ana gruba ayrıldığı ve özellikle yonca ile korunganın diğer türlerden belirgin şekilde ayrıldığı belirlenmiştir. Genel olarak baklagil yem bitkilerinin tohumlarının esansiyel yağ asitleri, fenolik bileşikler ve antioksidan aktivite bakımından önemli bir potansiyele sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Baklagil yem bitkileri, Yağ asidi kompozisyonu, Fenolik madde, PCA, Isı haritası

1. Introduction

Legume forage crops constitute one of the most important plant groups used in both animal feeding and human nutrition due to their rich chemical composition. In addition to their high protein content, these plants contain significant amounts of primary and secondary metabolites, including lipids, fatty acids, and phenolic compounds (Nizam et al., 2022; Al-Rajhi et al., 2024). Primary metabolites are mainly associated with plant growth and development processes, whereas secondary metabolites are involved in plant defense mechanisms, stress tolerance, and antioxidant activity.

Seed oils and fatty acids are important nutritional components in plant seeds. Unsaturated fatty acids such as oleic, linoleic, and linolenic acids are particularly important because they cannot be synthesized in sufficient amounts in human and animal metabolism and therefore must be supplied through the diet (Stanley-Samuelson et al., 1988). Forage crops are considered important sources of polyunsaturated fatty acids, especially linolenic acid, and they may influence the fatty acid composition of animal products such as milk and meat in ruminant production systems (Khan et al., 2012). Many studies have reported that the dominant fatty acids in legume seeds are generally linoleic, oleic, and linolenic acids, and that fatty acid composition varies depending on species, genotype, and environmental conditions (Akpınar et al., 2001; Bağcı et al., 2004; Solis et al., 2013; Kaplan et al., 2014; Caprioli et al., 2016; Çaçan et al., 2016; Kökten et al., 2022; Arslan et al., 2023).

Differences in fatty acid composition among plants are mainly determined by species, genotype, environmental conditions, and phenological stage, while environmental factors, including temperature, light intensity, and plant growth stage, have been shown to significantly affect fatty acid composition (Clapham et al., 2005; Elgersma et al., 2005; Harwood, 1980; Boufaïed et al., 2003; Falcone et al., 2004; Dewhurst et al., 2006).

Legume species are also known to be rich in phenolic compounds, flavonoids, and other polyphenolic substances, which are major contributors to antioxidant activity. These compounds play a protective role against oxidative stress in plants and have been reported to exhibit antioxidant, anti-inflammatory, and anticarcinogenic activities (Chaudhary et al., 2015; Stanisavljević et al., 2016). Several studies have reported that total phenolic content and antioxidant activity in legume seeds vary depending on genotype and species (Xu and Chang, 2007; Amarowicz et al., 2008; Chaieb et al., 2011; Zia-Ul-Haq et al., 2013; Hegedúsová et al., 2015; Devi et al., 2019; Zhao et al., 2020; Rybiński et al., 2020; Orak, 2019; Orak et al., 2022).

In recent studies, fatty acid composition and bioactive compounds have often been evaluated simultaneously using multivariate statistical approaches such as principal component and cluster analyses. These methods provide important information about similarities and differences among species. Therefore, the aim of this study was to determine the oil content, fatty acid composition, total phenolic content, and antioxidant activity of seeds from different forage legume species, and to evaluate relationships among species using multivariate statistical analyses.

2. Materials and Methods

2.1. Materials

The study was conducted in 2023 at the Department of Field Crops laboratory, Faculty of Agriculture, Isparta University of Applied Sciences. The study included alfalfa (*Medicago sativa*), sainfoin (*Onobrychis sativa*), common vetch (*Vicia sativa*), Hungarian vetch (*Vicia pannonica*), hairy vetch (*Vicia villosa*), narbon vetch (*Vicia narbonensis*), grass pea (*Lathyrus sativus*), and forage pea (*Pisum sativum*) seeds. The seeds were obtained from the Agricultural Education, Research, and Application Farm of the Faculty of Agriculture. The varieties used in the study were Altiva for alfalfa, Özerbey for sainfoin, Alper for common vetch, Budak for Hungarian vetch, Tarman for narbon vetch, Efes 79 for hairy vetch, Gürbüz for grass pea, and Taşkent for forage pea.

A randomized plot design with three replications was used in the experiment. Seeds were ground and passed through a 1-mm sieve prior to analysis, and all laboratory analyses were performed on the ground samples in triplicate for each species.

2.2. Total Phenolic Content and Antioxidant Capacity

10 g of ground sample was mixed with 100 mL of methanol at 100 rpm in a shaker for 30 minutes. The mixtures were left to stand in the dark at room temperature for 24 hours, then filtered. The resulting supernatant was used for analyses of phenolic compounds and antioxidant activity (Pizzale et al., 2002).

The total phenolic content of the samples was determined using the Folin–Ciocalteu method with slight modifications (Folin and Ciocalteu, 1927). Briefly, 0.1 mL of methanol extract was combined with 2.5 mL of deionized water and 0.1 mL of Folin–Ciocalteu reagent (2N). After 6 min of incubation in the dark, 0.5 mL of 20% sodium carbonate solution was added. The mixture was incubated in the dark for 30 min for color development, and absorbance was recorded at 760 nm using a spectrophotometer (Shimadzu UV-1280, Kyoto, Japan). The total phenolic content was expressed as mg gallic acid equivalents per g dry weight (mg g⁻¹ DW).

Two different methods, DPPH and CUPRAC, were used to determine antioxidant capacity. The DPPH assay measures the ability of antioxidants to scavenge the DPPH radical (Bener et al., 2022). For this assay, 1 mL of methanol extract was mixed with 1 mL of 99% ethanol and 2 mL of 0.2 mM DPPH solution. The mixture was kept in the dark at room temperature for 30 min, and absorbance was measured at 515 nm using a spectrophotometer (Shimadzu UV-1280, Kyoto, Japan). Results were expressed as mmol Trolox equivalent per g dry weight (mmol Trolox g⁻¹ DW).

Antioxidant capacity was also determined using the CUPRAC method, which is based on the reduction of the copper(I)–neocuproine complex (Apak et al., 2004). For this method, 0.5 mL of the methanol extract was mixed with Cu(II) chloride (10 mM), neocuproine (7.5 mM), ammonium acetate buffer (1 M), and deionized water. After incubation at room temperature for 30 min, absorbance was measured at 450 nm against a reagent blank using a spectrophotometer (Shimadzu UV-1280, Kyoto, Japan). Results were expressed as mmol Trolox equivalent per g dry weight (mmol Trolox g⁻¹ DW).

2.3. Determination of Crude Oil Content and Fatty Acid Composition

Seed oil content (%) presented in Table 1 was determined directly using a Nuclear Magnetic Resonance (NMR) device (Brüker mq-one, Germany). No solvent extraction was used for the determination of total oil content.

For fatty acid analysis, oil extraction was carried out according to the method of Howard and Stanley (1990). Ground seed samples (3 g) were homogenized in a chloroform–methanol solution (2:1, v/v), and 50 µL of 2% BHT was added to prevent oxidation. After centrifugation at 4000 rpm for 5 min at 10 °C, the supernatant was collected and the solvent was removed at 40 °C using a rotary evaporator (IKA RV 10 digital).

The extracted lipids were then converted to fatty acid methyl esters (FAME) by heating with acidified methanol at 85 °C for 90 min, followed by extraction with hexane. Fatty acid methyl esters were analyzed using a Shimadzu GC-2010 Plus gas chromatograph equipped with a flame ionization detector (FID) and a CP-WAX 52 CB column (60 m × 0.32 mm × 1.20 µm). Helium was used as the carrier gas at a flow rate of 3 mL min⁻¹. Samples (1 µL) were injected with a split ratio of 10:1. Injector and detector temperatures were set to 250 °C and 265 °C, respectively.

The oven temperature program was set from 80 °C (4 min) to 250 °C at a rate of 3 °C min⁻¹ and held at 250 °C for 20 min, with a total analysis time of 85 min. Fatty acids were identified by comparing retention times with those of a commercial FAME standard mixture (Sigma, Supelco® 37-Component FAME Mixture), and results were expressed as relative percentage of total fatty acids (Önder et al., 2025).

2.4. Statistical analyses

Experimental data were analyzed using analysis of variance (ANOVA) in SAS. The experiment was arranged in a randomized plot design, and species were considered as treatments. Treatment means were compared using the Least Significant Difference (LSD) test at $P \leq 0.05$. Principal component analysis (PCA) and heatmap analysis were performed in RStudio (Version 4.3.1) to evaluate relationships among species based on fatty acid composition, total phenolic content, and antioxidant activity (R Core Team, 2023). Prior to PCA, data were standardized to eliminate scale differences among variables.

3. Results

3.1. Oil Content, Total Phenolic Content, and Antioxidant Activity

The seed oil content, total phenolic content, and antioxidant activity values of the eight legume forage crops examined showed statistically significant differences among species ($p < 0.01$). Seed oil content ranged from 0.233% to 6.910%, with the highest oil content observed in alfalfa, followed by sainfoin. Oil content was found to be quite low in other species, and in vetch and pea species it was generally below 1%. The lowest oil content was observed in common vetch (Table 1).

Table 1. Fat content, total phenolic content, and antioxidant activity of some forage legume species

Legume Forage Crops	Fat Content (%)	Total Phenolic Content (mg GAE g ⁻¹ DW)	DPPH (mMol TE)	CUPRAC (mMol TE)
Narbon Vetch	0.473 ± 0.027 d	0.146 ± 0.015 c	0.0168 ± 0.0003 b	0.115 ± 0.002 cd
Hungarian Vetch	0.498 ± 0.017 d	0.169 ± 0.028 c	0.0160 ± 0.0011 bc	0.130 ± 0.005 c
Hairy Vetch	0.299 ± 0.008 f	0.192 ± 0.015 c	0.0160 ± 0.0005 bc	0.112 ± 0.004 d
Common Vetch	0.233 ± 0.008 g	0.195 ± 0.044 c	0.0144 ± 0.0004 c	0.112 ± 0.008 d
Sainfoin	4.634 ± 0.031 b	0.821 ± 0.060 b	0.0334 ± 0.0001 a	0.193 ± 0.007 b
Grass Pea	0.614 ± 0.012 c	0.151 ± 0.029 c	0.0150 ± 0.0003 c	0.108 ± 0.007 d
Forage Pea	0.347 ± 0.007 e	0.153 ± 0.019 c	0.0174 ± 0.0003 b	0.123 ± 0.007 cd
Alfalfa	6.910 ± 0.049 a	1.699 ± 0.055 a	0.0331 ± 0.0024 a	0.228 ± 0.019 a
Significance	**	**	**	**
LSD	0.043	0.068	0.0018	0.016
CV	1.41	8.86	5.02	6.54

Means followed by the same letter are not significantly different according to the LSD test at $P \leq 0.05$. Oil content values were determined using NMR, while solvent extraction was applied only for fatty acid analysis

Species showed significant variation in total phenolic content. The highest value was obtained from alfalfa, with sainfoin ranking second. However, vetch and pea species showed no significant differences and were classified within the same statistical group (Table 1).

Antioxidant activity results showed similar trends according to both the DPPH and CUPRAC methods. The highest DPPH antioxidant activity values were observed in alfalfa and sainfoin, while the lowest value was observed in common vetch (Table 1). According to the CUPRAC method as well, the highest antioxidant activity was observed in alfalfa, followed by sainfoin. In general, it was observed that antioxidant activity was higher in species with higher total phenolic content.

3.2. Fatty Acid Composition

An analysis of the fatty acid composition of legume forage crop seeds revealed that the primary fatty acids in all species are palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). It was observed that fatty acid ratios varied significantly among species (Table 2, Figure 1).

The highest oleic acid content was found in narbon vetch, followed by sainfoin and Hungarian vetch. Linoleic acid was one of the most abundant fatty acids in all species and was particularly high in common vetch, narbon vetch, grass pea, and Hungarian vetch. Linolenic acid, on the other hand, was found in high proportions particularly in sainfoin and alfalfa. The palmitic acid content was notably higher in alfalfa compared to the other species (Table 2, Figure 1).

The total saturated fatty acid (SFA) content was highest in alfalfa and lowest in narbon vetch. Monounsaturated fatty acids (MUFA) were highest in narbon vetch, while polyunsaturated fatty acids (PUFA) were highest in common vetch. Overall, it was determined that polyunsaturated fatty acids constitute the highest proportion of total fatty acids across all species (Table 2).

Table 2. Fatty acid composition (%) of seeds of some forage legume species

Retention Time (min)	Fatty Acid Composition	<i>V. narbonensis</i>	<i>V. pannonica</i>	<i>V. villosa</i>	<i>V. sativa</i>	<i>O. viciifolia</i>	<i>L. sativus</i>	<i>P. sativum</i>	<i>M. sativa</i>
10.958	Capric acid methyl ester (C10:0)	0.033	0.283	0.095	0.593	0.096	0.429	0.478	-
12.232	Undecanoic acid methyl ester (C11:0)	0.038	-	-	-	-	0.092	-	-
13.765	Lauric acid methyl ester (C12:0)	0.013	-	0.145	-	0.018	0.064	-	1.160
18.586	Myristic acid methyl ester (C14:0)	0.229	0.306	0.475	0.471	0.140	0.400	0.421	1.640
20.075	Myristoleic acid methyl ester (C14:1)	-	-	0.216	-	-	-	-	-
22.479	Pentadecanoic acid methyl ester (C15:0)	0.101	0.251	0.246	0.164	0.058	0.157	0.224	1.540
28.035	Palmitic acid methyl ester (C16:0)	5.934	13.473	16.323	12.876	7.527	10.343	14.271	32.240
29.960	Palmitoleic acid methyl ester (C16:1)	0.065	0.126	0.217	-	0.073	0.184	0.163	2.140
35.580	Heptadecanoic acid methyl ester (C17:0)	0.084	0.089	0.179	0.189	-	0.109	0.096	0.960
41.379	Stearic acid methyl ester (C18:0)	1.253	2.558	3.790	3.513	2.869	6.449	5.093	5.220
42.396	Oleic acid methyl ester (C18:1n9c)	34.591	25.838	25.076	15.420	28.760	23.519	20.586	2.480
44.515	Linoleic acid methyl ester (C18:2n6c)	49.916	47.187	39.924	53.548	15.136	48.412	45.863	18.340
47.485	Gamma-linolenic acid methyl ester (C18:3n6)	4.652	8.298	8.008	11.978	44.146	7.109	11.271	32.740
50.247	cis-11-Eicosenoic acid methyl ester (C20:1)	0.913	0.616	1.219	1.248	0.340	0.858	0.462	-
51.196	Alpha-linolenic acid methyl ester (C18:3n3)	0.946	0.358	0.421	-	0.305	0.363	0.202	-
53.301	Heneicosanoic acid methyl ester (C21:0)	0.207	-	0.131	-	-	-	-	-
54.478	cis-11.14-Eicosadienoic acid methyl ester (C20:2)	0.237	-	0.242	-	-	0.285	0.215	-
54.497	Behenic acid methyl ester (C22:0)	-	0.183	-	-	-	-	-	0.320
58.547	Tricosanoic acid methyl ester (C23:0)	0.579	0.434	1.927	-	0.532	0.493	0.298	-
59.507	Arachidonic acid methyl ester (C20:4n6)	0.083	-	0.497	-	-	0.503	-	0.980
61.531	cis-13.16-Docosadienoic acid methyl ester (C22:2)	0.126	-	0.164	-	-	0.231	0.196	-
62.486	Lignoceric acid methyl ester (C24:0)	-	-	0.181	-	-	-	-	0.240
66.552	cis-5.8.11.14.17-Eicosapentaenoic acid methyl ester (C20:5n3)	-	-	0.525	-	-	-	0.161	-
Saturated Fatty Acids (SFA)		8.470	17.577	23.491	17.806	11.240	18.536	20.881	43.320
Monounsaturated Fatty Acids (MUFA)		35.568	26.580	26.728	16.668	29.173	24.561	21.211	4.620
Polyunsaturated Fatty Acids (PUFA)		55.962	55.843	49.781	65.526	59.587	56.903	57.908	52.060

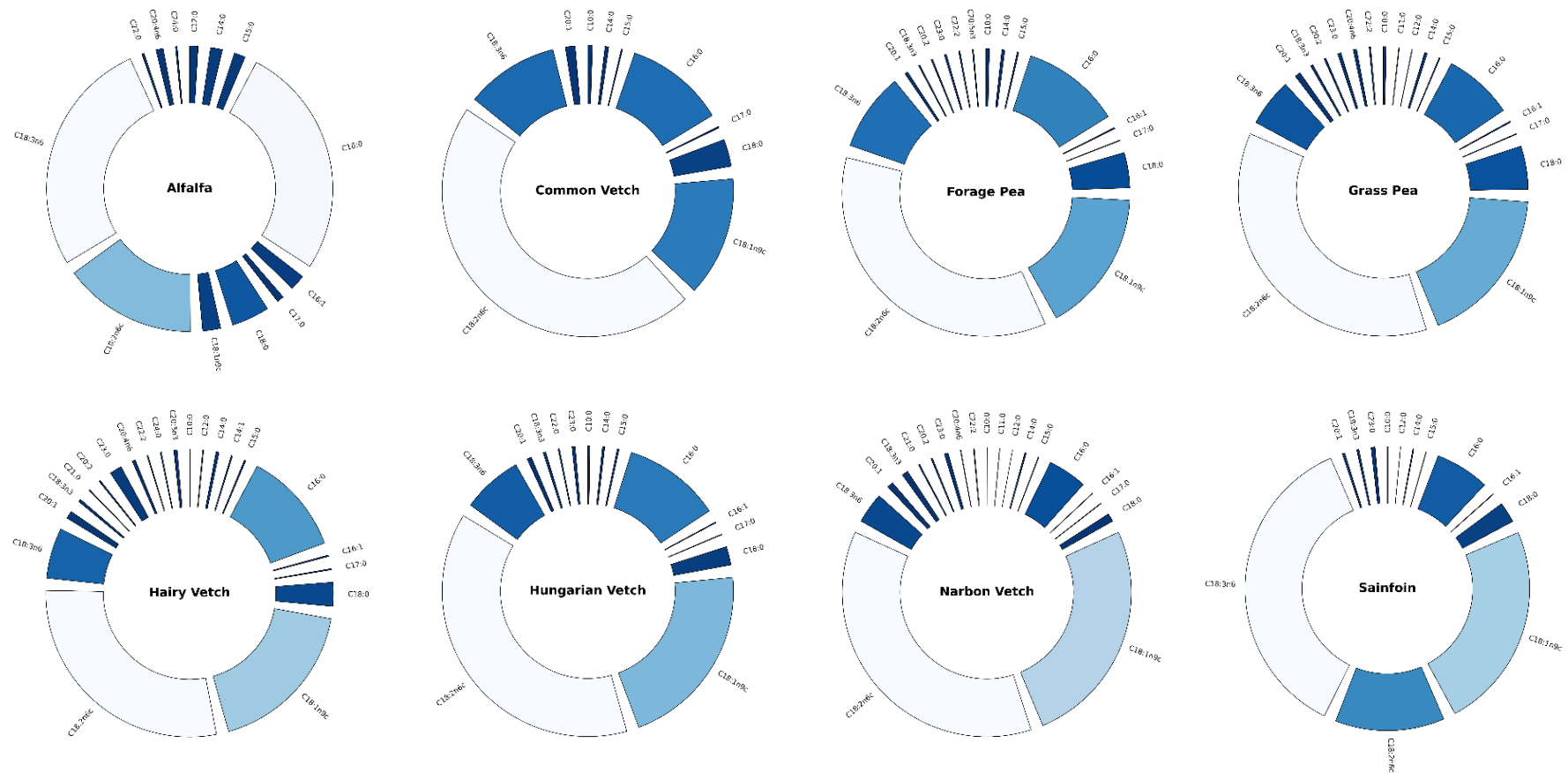


Figure 1. Fatty acid composition of forage legume species presented as doughnut charts

3.3. Heat Map and Principal Component Analysis (PCA)

The heat map and hierarchical clustering analysis based on fatty acid composition revealed distinct groups among the species (Figure 2). According to the clustering analysis, the species were divided into two main groups. The first group included common vetch, narbon vetch, hairy vetch, forage pea, Hungarian vetch, and grass pea, while the second group included alfalfa and sainfoin.

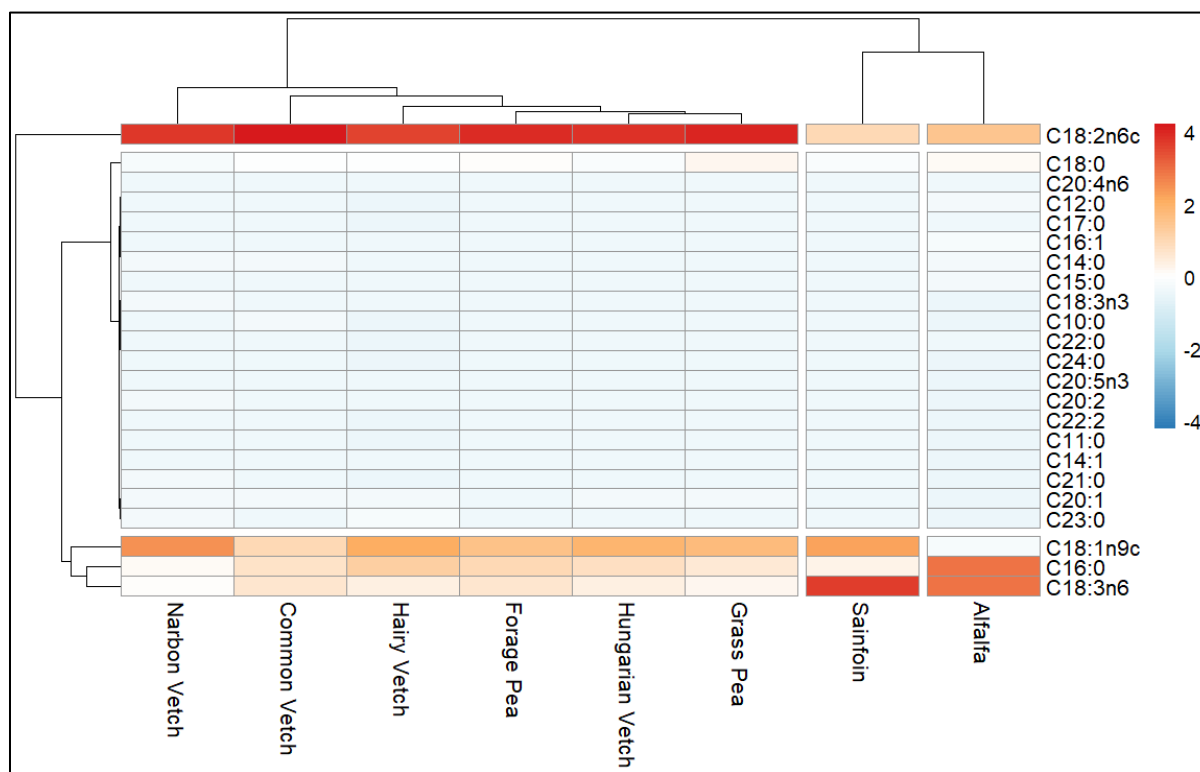


Figure 2. Heatmap and hierarchical clustering analysis of fatty acid composition in seeds of forage legume species

In the heat map, linoleic acid (C18:2), oleic acid (C18:1), palmitic acid (C16:0), and linolenic acid (C18:3) were the primary fatty acids responsible for distinguishing the species. While alfalfa and sainfoin exhibited high values particularly for palmitic and linolenic acids, it was determined that the linoleic acid content was higher in vetch species and pea.

The principal component analysis showed that the first principal component (PC1) explained 82.1% of the total variation, while the second principal component (PC2) explained 12.9%; together, the first two components accounted for 95.0% of the total variation. This result demonstrates that principal component analysis is highly effective in explaining differences in fatty acid composition among species.

According to the PCA biplot graph, alfalfa and sainfoin were positioned along the positive PC2 axis and were found to be particularly associated with linolenic acid (C18:3) and palmitic acid (C16:0). Common vetch, narbon vetch, and some other vetch species, however, were positioned along the PC1 axis and were associated with linoleic acid (C18:2) and oleic acid (C18:1). Forage pea, hairy vetch, common vetch, Hungarian vetch, and grass pea were located in the central region of the PCA plot, placing them in an intermediate group in terms of fatty acid composition (Figure 3).

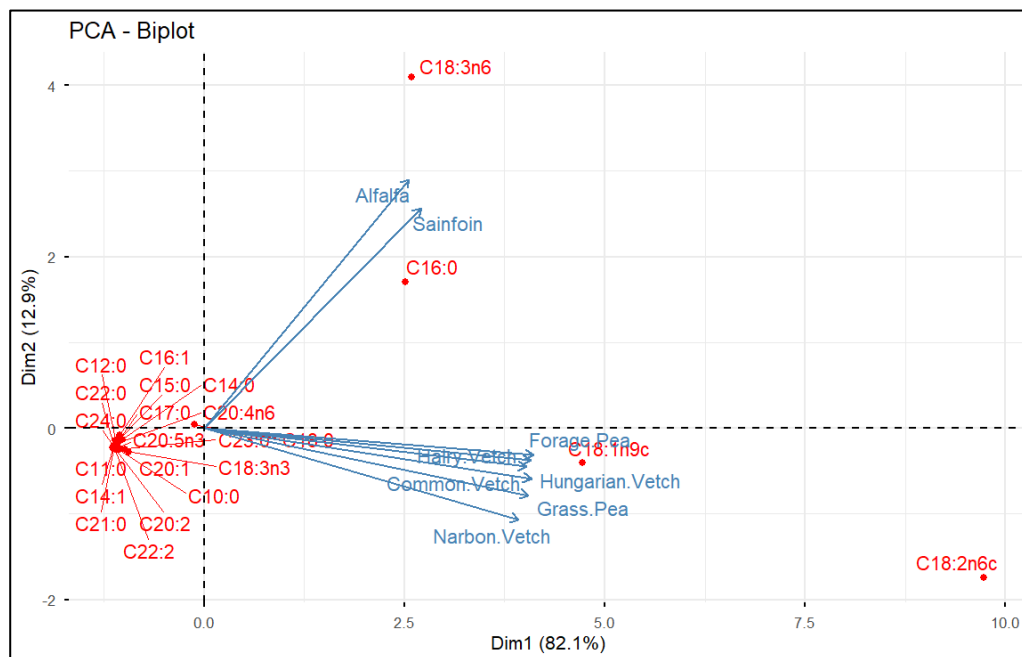


Figure 3. PCA biplot showing relationships among forage legume species and fatty acid components based on the first two principal components

Overall, when PCA and cluster analysis were evaluated together, it was determined that alfalfa and sainfoin were distinctly different from other legume forage plants in terms of fatty acid composition, while the vetch species and the pea had more similar fatty acid compositions.

4. Discussion

4.1. Oil Content and Fatty Acid Composition

Although the oil content in the seeds of legume forage crops is generally low, it is an important nutritional criterion due to the fatty acid composition of these crops. In this study, seed oil content ranged between 0.23% and 6.91%. The oil content of pea and vetch species was lower than values reported by Welch and Griffiths (1984) and Yoshida et al. (2007), who reported oil contents ranging from 1.3% to 3.6% in pea seeds. These differences may be due to species differences, environmental conditions, and extraction methods. Similar variation among legume species was also reported by Caprioli et al. (2016). Additionally, Arslan et al. (2023) noted that the oil content in grass pea genotypes ranged from 0.59% to 0.80%. In the present study, the oil content values for some species were lower than those reported in previous studies, particularly for pea and vetch. These differences may be attributed to species and genotype differences, environmental conditions, seed maturity stage, and the oil determination method used in the study. It is known that oil content values determined by Nuclear Magnetic Resonance (NMR) may differ from those obtained by solvent extraction methods. In addition, ecological conditions during seed development may significantly affect oil accumulation in legume seeds.

Variations in oil content among species may be attributed not only to genetic factors but also to environmental conditions, cultivation practices, and plant developmental stage. Oil and fatty acid synthesis in plants are closely related to carbon metabolism and mainly occur during seed development through the conversion of carbohydrates into fatty acids. Therefore, differences in oil content among species are expected.

The results of this study showed that the major fatty acids in all legume species examined were palmitic, stearic, oleic, linoleic, and linolenic acids. These findings are consistent with many studies reporting that linoleic, oleic, and linolenic acids are the dominant fatty acids in legume seeds. Akpınar et al. (2001), Bağcı et al. (2004), Çağan et al. (2016), and Kökten et al. (2022) reported that the dominant fatty acid in *Vicia* species is linoleic acid. Similarly, Solis et al. (2013) and Caprioli et al. (2016) reported that linoleic acid is the primary fatty acid in pea seeds, while Arslan et al. (2023) reported that linoleic acid is the dominant fatty acid in grass pea, followed by oleic acid.

Unsaturated fatty acids, particularly linoleic and linolenic acids, are essential fatty acids that cannot be synthesized in human and animal metabolism and must therefore be obtained from external sources. These fatty acids are components of cell membranes, play a role in energy metabolism, and serve as precursors in eicosanoid synthesis (Stanley-Samuelson et al., 1988).

Forage crops are considered important sources of polyunsaturated fatty acids, especially linolenic acid, and they may influence the fatty acid composition of milk and meat in ruminant nutrition (Khan et al., 2012). Linoleic and linolenic acids also contribute to the formation of conjugated linoleic acid (CLA) through biohydrogenation processes in the rumen, and these compounds are known to have anticarcinogenic and antiatherogenic effects (Dhiman et al., 1999; Griinari and Bauman, 1999; Rochfort et al., 2008). From a practical perspective, the fatty acid composition of forage legumes is particularly important in ruminant nutrition, as dietary fatty acids can influence the fatty acid profile of milk and meat. For instance, higher levels of linolenic acid in forages may contribute to increased synthesis of beneficial fatty acids such as conjugated linoleic acid (CLA) in milk through rumen biohydrogenation. Therefore, species such as alfalfa and sainfoin, which are characterized by higher levels of certain unsaturated fatty acids, may have greater potential to improve the nutritional quality of animal products.

Fatty acid composition is influenced by species, genotype, phenological stage, and environmental conditions. Clapham et al. (2005) and Elgersma et al. (2005) reported that fatty acid composition in forage crops varies depending on environmental conditions, while Harwood (1980) and Boufaïed et al. (2003) reported that fatty acid levels change during plant maturation. In addition, it has been reported that plants increase the synthesis of unsaturated fatty acids under low-temperature conditions in order to maintain membrane fluidity (Falcone et al., 2004; Dewhurst et al., 2006). Therefore, the differences observed in fatty acid composition among species in this study are consistent with the literature.

4.2. Total Phenolic Content and Antioxidant Activity

Legumes are known to contain significant amounts of phenolic compounds, flavonoids, tannins, and other secondary metabolites. Phenolic compounds play an important role in plant defense mechanisms, UV protection, pathogen resistance, and antioxidant defense systems. Polyphenols found in legume seed coats help protect seeds against oxidative damage and prevent deterioration during storage (Osawa et al., 1985; Chaudhary et al., 2015).

Numerous studies have reported that total phenolic content in legume seeds varies across genotypes. Xu and Chang (2007) reported that total phenolic content in pea seeds ranged from 1.04 to 1.67 mg g⁻¹, while Zia-Ul-Haq et al. (2013) reported values between 0.84 and 0.99 mg g⁻¹. Hegedúsová et al. (2015) reported that total phenolic content in pea varieties ranged from 0.67 to 1.18 mg g⁻¹. Nazir et al. (2020) and Zhao et al. (2020) also reported significant variation in phenolic compounds among pea genotypes. In addition, Orak (2019) and Orak et al. (2022) reported that total phenolic content in *Vicia* species varies depending on genotype, while Rybiński et al. (2020) reported a strong relationship between total phenolic content and antioxidant capacity in grass pea seeds. These findings indicate that phenolic compound content can vary significantly among legume species.

Many studies have reported a positive relationship between phenolic compounds and antioxidant activity (Kähkönen et al., 1999; Cardador-Martínez et al., 2002; Shetty et al., 2002; Orak et al., 2022). Similarly, in this study, higher antioxidant activity values were observed in species with higher total phenolic content, supporting previous findings.

Xu and Chang (2007), Amarowicz et al. (2008), Chaieb et al. (2011), and Devi et al. (2019) reported that antioxidant activity in legume seeds varies among genotypes. Llorent-Martínez et al. (2017) reported a strong correlation between total phenolic content and DPPH and CUPRAC antioxidant activity in *Lathyrus* species. The antioxidant capacity of legumes is mainly associated with flavonoids, phenolic acids, tannins, and other polyphenolic compounds, which are known to have protective effects against chronic diseases (Stanisavljević et al., 2016).

4.3. PCA and Heat Map Analysis

Multivariate statistical analyses are widely used, especially when evaluating datasets containing multiple variables such as fatty acid composition. In this study, PCA and cluster analysis results showed that species formed distinct groups based on their fatty acid composition. It is known that variation in fatty acid composition mainly originates from major fatty acids such as linoleic, linolenic, oleic, and palmitic acids. Previous studies have reported

that differences among species are related to genetic structure, environmental conditions, and metabolic differences (Clapham et al., 2005; Elgersma et al., 2005; Arvidsson, 2009). Therefore, the species grouping observed in PCA and cluster analysis is expected, and the findings of this study are consistent with the literature.

The distinct separation of alfalfa and sainfoin from the other species in both PCA and cluster analyses may be attributed to their unique biochemical composition, particularly their higher levels of palmitic and linolenic acids. In addition, these species are known to differ in terms of lipid metabolism and secondary metabolite profiles, which may contribute to their separation from other legume species. Furthermore, differences in seed structure, physiological maturity, and genetic background may also influence fatty acid accumulation patterns, leading to the observed grouping in multivariate analyses.

5. Conclusions

In this study, significant differences were observed among legume forage crop species in seed oil content, fatty acid composition, total phenolic content, and antioxidant activity. Seed oil content ranged from 0.23% to 6.91%, with the highest observed in alfalfa and sainfoin, and the lowest in common vetch. The dominant fatty acids in all species were palmitic, oleic, linoleic, and linolenic acids, and polyunsaturated fatty acids constituted the largest proportion of total fatty acids in all species. Linoleic acid was particularly high in vetch species and grass pea, whereas linolenic acid and palmitic acid were higher in alfalfa and sainfoin.

Total phenolic content and antioxidant activity showed similar trends across species, with the highest values observed in alfalfa and sainfoin. A positive relationship was observed between total phenolic content and antioxidant activity. Principal component analysis and heatmap analysis revealed that the species were grouped into two main clusters based on their fatty acid composition, with alfalfa and sainfoin clearly separated from the other species due to their higher palmitic and linolenic acid contents.

Overall, the results indicate that legume forage crop seeds are important sources of polyunsaturated fatty acids and phenolic compounds, and alfalfa and sainfoin seeds stand out for their oil content, phenolic compounds, and antioxidant activity. These species may be considered as potential sources of functional compounds and quality feed resources. In this context, the selection of appropriate forage legume species may play an important role in improving the fatty acid composition and nutritional quality of animal-derived products.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Alagöz, M., Türk, M.; Design: Alagöz, M., Türk, M.; Data Collection or Processing: Alagöz, M., Türk, M.; Statistical Analyses: Alagöz, M., Türk, M.; Literature Search: Alagöz, M., Türk, M.; Writing, Review and Editing: Alagöz, M., Türk, M.

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