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The Characterization of Volatile Compounds Belonging to Some Lupin Genotypes By SPME GC-MS Method

Ela Nur ŞİMŞEK SEZER[1], Mustafa YORGANCILAR[2], Tuna UYSAL[3]
[1,3]Selçuk University Faculty of Science, Department of Biology, Konya/Türkiye, [2]Selçuk University Faculty of Agriculture, Konya/Türkiye
[1]https://orcid.org/0000-0003-2805-7204, [2]https://orcid.org/0000-0003-4938-8547, [3]https://orcid.org/0000-0001-9968-5633
✉: myorg@selcuk.edu.tr

ABSTRACT
In this paper, three *Lupinus albus* L. and two *L. angustifolius* L. genotypes were examined and compared in terms of volatiles or semi volatiles using GC-MS and SPME techniques. For this aim, the seeds of lupins were ground using a hand grinder, and this powder raw material was used to determine the content. Approximately 102 compounds obtained from lupin genotypes were identified for the first time, which mainly included benzene-methyl (1-methyl ethyl), 5-Allyl-4-(1-(p-amino phenyl) ethylidenehyl, 2-propen-1-ol, 3-phenyl-, m-Mentha-4,8,diene-(1S,3S)-(+), gamma terpinene. Thee compounds were determined to exist at different rates in different. As a result, it becomes evident that such studies aiming at revealing the active matters from plants or plant parts and their possible potentials, are of great importance in terms of more specific studies to be carried out in the future.

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Bazı Acıbakla Genotiplerine Ait Ucucu Bileşiklerin SPME GC-MS Yöntemiyle Karakterizasyonu

ÖZET
Bu çalışmada, üç *Lupinus albus* L. ve iki L. *angustifolius* L. genotipi GC-MS ve SPME teknikleri kullanılarak uçucu veya yarı uçucu maddeler açısından araştırılmış ve karşılaştırılmıştır. Bu amaçla acı bakla tohumları el değirmeni yardımıyla öğütülmüş ve bu toz, ham madde içeriğinin belirlenmesinde kullanılmıştır. Bu acı bakla genotiplerinde yaklaşık olarak 102 bileşik ilk defa tanımlanmıştır. Bu bileşiklerden başlıcaları; benzene-methyl (1-methyl ethyl), 5-Allyl-4-(1-(p-aminophenyl) ethylidenehyl, 2-propen-1-ol, 3-phenyl-, m-Mentha-4,8, diene-(1S,3S)-(+), gamma terpinene dir. Bu bileşiklerin farklı genotiplerde ve farklı oranlarda bulunduğu tespit edilmiştir. Sonuç olarak, bitkilerden veya bitki parçalarından gelen aktif maddeleri ve olası potansiyellerini ortaya koymayı amaçlayan bu tür çalışmalar, gelecekte yapılacak daha spesifik çalışmalar açısından büyük önem taşımaktadır.

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INTRODUCTION

Lupin is a legume having being used as human food and animal feed throughout the history dating back to Roman times. Another use medical and pharmaceutical purposes (Abraham et al., 2019). Over 200 lupin species are grown worldwide (Ishaq et al., 2022; Mohamed & Rayas-Duarte, 1995; Święcicki et al., 2015; Yorgancılar et al., 2009). This species have a huge potential as a crop plant due to seed of content mineral, vitamin, oil as well as high protein (Nasir et al., 2022; Pereira et al., 2022; Van de Noort, 2017).

The genus of lupin comprises 275 species, generally including small-seeded New World species (excluding *L. mutabilis* Sweet) and 13-15 Old World species with larger seed size such as *L. albus L., L. angustifolius L.,* and *L. luteus L.*, respectively (Cardoso et al., 2013; Wallace Andrew Cowling et al., 1998; Pascual, 2004; Świ\cecicki et al., 1996; Święcicki et al., 2002). Lupin can show tolerate to frost, water shortage, and degradated soils (Lucas et al., 2015; Shaaban et al., 2022). Besides its ecological requirements, each lupin plant has essential capacity for feeding and agricultural studies (Brummund & Święcicki, 2011).

In the Northern Plains of United States, lupin can be cultivated in North and South Dakota, Wisconsin, and Minnesota. But, utilisation of lupin as its domestication has been restricted, and this may be partly because of its alkaloid ingredient and insufficient yield. Breeding programs have performed on "sweet" varieties possessing as low as 0.002% alkaloid ingredient, which let them safe for consumption of human (Culvenor & Petterson, 1986; Mohamed & Rayas-Duarte, 1995).

Seeds of white lupin are commonly classified as sweet or bitter according to the alkaloid content, which are between 0.01 and 4% (Bhardwaj & Hamama, 2012). The bitter seeds possess the quinolizidine alkaloids sparteine and lupanine. The bitter taste of stem and seeds of the plant is due to their metabolite content which has a toxic effect. The existance of such kind of alkaloids restricts the utilisation of lupin seed as human food or animal feed purposes. Lupins with low alkaloid contents were selected, leading to 'sweet' lupins with alkaloid contents below 0.02% in the protein-rich seeds, which can be used both for human and animal consumption (Gresta, 2017). It has been stated that lupine protein has benefits such as hypertension (Arnoldi et al., 2015), cholesterol lowering (Bähr et al., 2015; Sirtori et al., 2012) and hyperglycemia (Duranti et al., 2008), and these benefits may be related to its natural antioxidant activity (Guo et al., 2018). Elma et al. (2021) were detected metabolite content in local *Lupinus albus* L. seeds, which contained 686.99 mg GAE / 100 mg total phenol, 22.06 mg QE / 100 mg total flavonoid, DPPH 26.04 mg TE / 100 g having antioxidant activity. The Australian standard is 0.02% as the highest treshold for alkaloid content for sweet lupines (W.A Cowling et al., 1998).

In the past decades, headspace solid phase micro-extraction (HS-SPME) has been recognised as an effective isolation methodology for a lot of samples (Arthur & Pawliszyn, 1990; Bicchi et al., 2000; Ercan & Dogru, 2022; Kataoka et al., 2000; Krutz et al., 2003; Ulrich, 2000). This technique dramatically reduces resolution duration and enhancement the minimum detection limits while maintaining resolution, and can be performed in two main modes, overhead configurations and direct isolation. Due to its advantages, HS-SPME is efficiently used for sampling the volatile compounds from medicinal and aromatic plants (Bicchi et al., 2004; Muselli et al., 2009). In order to better reveal and research the medicinal and pharmaceutical aspects of the plant, the contents of the seeds should be well known. The aim of this study is to compare the differences in volatile components of seeds of five different *L. albus* and *L. angustifolius* genotypes using the SPME-GC-MS technique and to reveal especially valuable phytochemicals from a broad point of view.

MATERIAL and METHOD

Five lupin genotypes were studied in terms of volatile oils during our analyses. These are Lutop, Belera, Tanjil, Lublanc and EGY-105, respectively. These are stored in the Department of Field Crops of Agriculture Faculty at the Selçuk University. They were obtained from different sources, as given below in Table 1.

Table 1. The plant materials used in analysis by SPME-GC-MS
Çizelge 1. SPME-GC-MS analizinde kullanılan bitki materyalleri

Genotypes	Form	Place of origin
L. albus cv. Lutop	Sweet	France*,#
L. albus cv. Lublanc	Sweet	France*
L. angustifolius cv. Belera	Bitter	Australia**
L. angustifolius cv. Tanjil	Bitter	Australia**
L. albus line EGY-105	Bitter	Egypt*

*: Kindly provided by Dr. Christian Huyghe and Dr. *Jacques Papineau* from *Institut National de la Recherche Agronomique,* France
**: Kindly provided by Dr. Kedar Adhikari and Dr. Bevan Buirchell from The Department of Agriculture and Food, Western Australia
#Small grain size.

Preparation of Seeds

Seeds were dried at ambient temperature without sunlight exposure. Dried seeds were ground by using a hand grinder. The ground samples (3gr) of five genotypes were diluted in a 10 ml vial. An SPME fibre 50/30 µm DVB/CAR/PDMS was preferred for analysis. The SPME apparatus was directly injected into the upper space of the vial to adsorb volatile compounds and then directly injected into the Shimadzu QP2010ULTRA FID detector GC-MS apparatus using a Restek Rxi-5 MS capillary column.

Analysis of GC-MS

The volatile compounds of lupin samples were analysed by applying the method of (the injector temperature was 250 °C) using SPME-GC-MS. Compounds were isolated by a 15 min. SPME fibre exposure into a GC injector at 250 °C. The extracts from the SPME procedure were analysed on a Shimadzu QP2010 ULTRA FID GC–MS system. A 30 m length Restek Rxi-5 MS column (0.25 mm id, film thickness 0.25 µm) was used. Carrier gas was helium with a flow rate as 1.8 mL/min. The GC oven temperature was programmed to hold at 40 °C for 3 min and then increased to 240 °C at 5 °C/min, finally holding at 240 °C for 3 min. The detector ion source temperature was 200 °C, and the interface temperature was set at 250 °C. Mass spectra were acquired in the electron impact mode at 70 eV, using m/z range of 50–350 and 2 s scan time.

Evaluation of Data

Chromatograms of all samples were subjected to noise reduction prior to peak area integration, and later, the peak areas of components in the chromatogram were integrated. Compounds were identified by comparing with three libraries, W9N11, SWGDR4G4 and SWGDR4G5. Compounds mostly matched in W9N11 library. Identification of components in the sample was based on the retention time (RT). The identification of the components present in the samples was calculated using Kovats retention index. The relative rate of the volatile oil compounds was obtained from peak areas. All analyses were performed in three replications, and all the numeric data are means of three independent analyses.

RESULTS and DISCUSSION

According to our analyses, totally 102 compounds were separated and identified from studied lupin samples (Table2). The total ion chromatograms (TIC) of studied lupin specimens were given in Figure 1. Nine compounds of these were common to each sample. The analysis revealed that there were different components in the samples. In the sample, Belera 61, EGY-105 46, Lutop 41, Lublanc 37 and Tanjil 36 compounds were determined respectively. The major components of Belera were cyclohexanol, 2-methyl-5 (1-methylether) (19.37%), 2-propen-1-ol, 3-phenyl (16.08%) and m-mentha-4,8, diene-(1S,3S) (11.12%). In EGY-105, benzene, methyl (1-methylethyl) (43.32%), pulegone (9.68%) and alpha-pinene (5.27%). In Lutop cultivar, benzene methyl (1-methylethyl), gamma terpinene and pulegone contents were found as 35.83%, 16.21% and 5.85 %, respectively. However, in Lublanc, benzene methyl, cis-Ocimene and 1,3,6-octatriene 3,7-dimethyl-(E) contents were determined as 59.3%, (5.54%) and 4.69%, respectively. In Tanjil, 5 –allyl-4-1, m-mentha-4, 8 diene, pulegone ingredients were detected as 27.69%, 15.74% and 7.64%, respectively (Table 2 and Figure 2). It is crucial that cumene (benzene, methyl (1-methylethyl)), which has a high potential for use as biofuel, is detected high quantity in three *L. albus* samples. Cumene is used as a thinner for paints, lacquers, and enamels and as a component of high octane fuels. Cumene is also used in the manufacture of phenol, acetone, acetophenone, and methylstyrene (National Center for Biotechnology Information, 2022). The production of meta, ortho and para isomers of cumene is usually synthesized by alkylation of toluene. Toluene is the pioneering feedstock in benzene production and a prime octane boosting compound for gasoline blending. Toluene is also utilized as a raw substance in the production of other chemicals (e.g., toluene diisocyanate and benzoic acid) and as a solvent in paints and coatings, inks, adhesives, and pharmaceuticals (Toluene Synopsis, 1990).

Table 2. The % peak area values of identified compounds in studied samples
Çizelge 2. Çalışılan örneklerde tanımlanmış bileşiklerinin % pik alan değerleri

	Sample Name				
Compound name	**Lutop**	**Lublanc**	**Belera**	**Tanjil**	**EGY-105**
Butanoic Acid,2-Methyl-Ester/ Butyric acid	0.22	0.28	0.03	0.08	0.49
P-Xylene	-	-	0.03	-	-
Tricylene	0.06	-	0.02	0.03	0.11
Alpha-Thujene	3.00	2.62	1.13	2.34	3.91
Alpha- Pinene	2.94	2.89	1.16	1.54	5.27
Bicyclo(3.1.0)Hex-2-Ene, 4-Methylene-1-/ Beta-thujene	0.10	-	0.07	0.11	0.11
Bicyclo(2.2.1)Heptane,2,2-Dimethyl-3- Camphene	-	-	0.85	-	2.52
Sabinene	0.18	0.19	0.11	0.16	-
Beta Pinene	0.57	-	0.36	0.47	-
1-Octen-3-One	-	-	0.09		-
1-Octen-3-Ol	0.08	-	0.25	0.11	-
3-Octanone	-	0.73	0.67	0.53	1.45
Geranyl Formate	-	-	3.28	-	-
Ethyl Amyl Carbinol	-	-	0.50	-	-
1-Phellandrene	0.61	-	0.49	0.62	0.25
1,3,6-Octatriene, 3,7-Dimethyl-, (E)-	4.98	4.69	0.04	6.80	3.57
Delta 3-Carene	0.34	0.32	0.19	0.20	0.50
Alpha-Terpinene	3.57	0.13	2.99	-	0.68
2-Propen-1-Ol, 3-Phenyl-	-	-	16.08	-	-
D-Limonene	3.23	3.62	2.76	3.42	2.35
Eucalyptol (1,8-Cıneole)	0.64	0.58	0.87	0.73	0.84
3-Octen-5-Yne, 2,7-Dimethyl-, (E)	-	-	6.17	-	-
Santolina Triene	-	-	6.16	-	-
M-Mentha-4,8,Diene-(1S, 3S)	-	-	11.12	15.74	-

Trans Sabinene Hydrate	0.09	-	0.43	0.17	-
Alpha-Methyl-Alpha-(4-Methyl-3-Pente)	-	-	0.22		-
Alpha-Terpinolene	0.28	-	0.59	0.35	0.18
Cyclohexanol, 2-Methyl-5-(1-Methylethe	-	-	19.37	-	-
2-Cyclohexen-1ol, 1-Methyl-4-(1-Methyl	-	-	0,07	-	-
Alloocimene	-	0.36	0.47	0.44	-
Cis-Epoxy-Ocımene	-	-	0.09	-	-
2-Cyclohexen-1-Ol, 1-Methyl-4-(1-Methyl	-	-	0.05	-	-
2,4,6-Octatriene,2,6-Dimethyl-, (E, Z)	0.11	-	0.19	0.20	0.20
Cis-3-Hexenyl İso-Butyrate	-	-	0,10	-	-
Cyclohexanone, 5-Methyl-2-(1-Methylethe- (Cis)	2.07	0.66	2.12	1.97	2.74
Cyclohexanone, 5-Methyl-2(1-Methylethe (Trans)	0.50	-	0.64	-	-
Bicyclo(2.2.1)Heptan-2-Ol, 1,7,7-Trimethyl	-	-	0.83	-	-
İsopulegone	0.12	-	0.17	0.14	0.15
3-Cyclohexen-1-Ol, 4-Methyl-1-(1-Methyl	0.04	-	0.64	-	-
Benzenemethanol, 4-(1-Methylethyl)-	-	-	0.03	-	-
Cis 3 Hexenyl Butyrate	-	-	0.04	-	-
3-Cyclohexen-1-Methanol, Alpha, Alpha	-	-	0.11	-	-
Dihydrocarvone	-	-	0.25	-	-
Cyclohexanone, 2-Methyl-5-(1-Methylethe-	-	-	0.17	-	-
Z-3-Hexenyl 2-Methylbutanoate	-	-	0.17	-	-
Thyml Methyl Ether	-	-	2.82	-	-
Thymoquinone	-	0.49	1.94	-	-
Phenol, 5-Methyl-2-(1-Methylethyl)-	-	-	0.27	0.07	-
Alpha-Terpinyl Propionate	-	-	0.03	-	-
Phenol, 2-Methyl-5-(1-Methylethyl)-	-	-	3.00	-	-
Carbofurane	-	-	0.02	-	-
Copaene	-	-	0.05	-	-
(-)-Beta, Bourbonene	-	-	0.04	-	-
Caryophyllene	-	-	0.40	-	-
(+)-Aromadendrene	-	-	0.11	-	-
Gamma- Muurolene	-	-	0.03	-	-
Germacrene-D	-	-	0.07	-	-
Bicyclogermacrene	-	-	0.15	-	-
Beta-Bisabolene	-	-	0.37	-	-
Delta-Cadinene	-	-	0.04	-	-
Dodecanoic Acid, İsooctylester	-	0.56	0.06	-	-
1-Pentanol	0.13	0.11	-	-	0.19
1-Hexanol	0.20	0.13	-	-	0.20
Camphene	1.59	1.32	-	1.16	-
Beta-Myrcene	4.51	2.99	-	5.10	3.17
Cis -Ocimene	-	5.54	-	7.46	4.39
Gamma Terpinene	16.21	1.84	-	-	4.24
Azulene	-	-	-	-	0.14
P-Menthone	-	0.28	-	-	0.82
Octane	-	0.08	-	-	0.06
Benzene 1.2-Dimethyl-	0.13	0.24	-	0.06	-
Pentanoic Acid,Ethyl Ester	-	0.25	-	-	-
2,4(10)-Thujadien	-	0.07	-	-	-
Dodecane	0.04	0.27	-	-	0.12
Z-Limonene-1,2epoxide	-	0.11	-	-	-
E-Citral	-	0.06	-	-	-
5-Allyl-4-(1-(P-Aminophenyl)Ethylidenehy	-	-	-	27.69	-
Linalool-L	-	-	-	7.77	-
Carvacrol Methyl Ether	0.39	0.23	-	0.68	0.27
Laurınsaeure,4-Octylester	0.25	-	-	0.14	0.63
Pulegone	5.85	2.09	-	7.94	9.68
Endo-Borneol	-	-	-	0.25	-

Lınalool Oxıde Cıs	-	0.30	-	0.07	-
Cyclopentasiloxane,Decamethyl-	0.06	0.34	-	0.02	0.10
Borneol L	0.09	-	-	-	-
Tetradecane	0.07	-	-	-	0.07
Tridecane	0.02	-	-	-	0.06
Tricyclene	0.06	-	-	-	0.11
Beta Phelllandrane	-	-	-	-	0.23
3,Oxatricyclo [4.1.1.0(2,4)] Octane	-	-	-	-	0.29
Cis-Myrtanol	-	-	-	-	0.09
Limonene Oxide Cis	-	0.30	-	-	0.13
Longifolene	-	-	-	-	0.09
Benzaldehyde	-	0.11	-	-	-
Cyclotetrasiloxane,Octamethyl-	-	-	-	-	0.09
Benzene-Methyl (1-Methylethyl)	35.83	59.3	-	-	43.32
Cyclotrisiloxane, Hexamethyl	-	-	-	-	0.10
3-5-Octadıen -2-One	0.15	-	-	-	0.21
2,5-cyclohexadiene-1,4-Dione,2-Methyl	0.20	-	-	0.13	0.28
Dodecane,2.2.11.11-Tetramethyl	0.05	0.12	-	0.03	0.16
Tetradecane,2,2-Diemthyl	0.06	0.12	-	-	0.17
Propanoic Acid 2-Methyl 2,2 Dimethyl	-	-	-	-	0.17

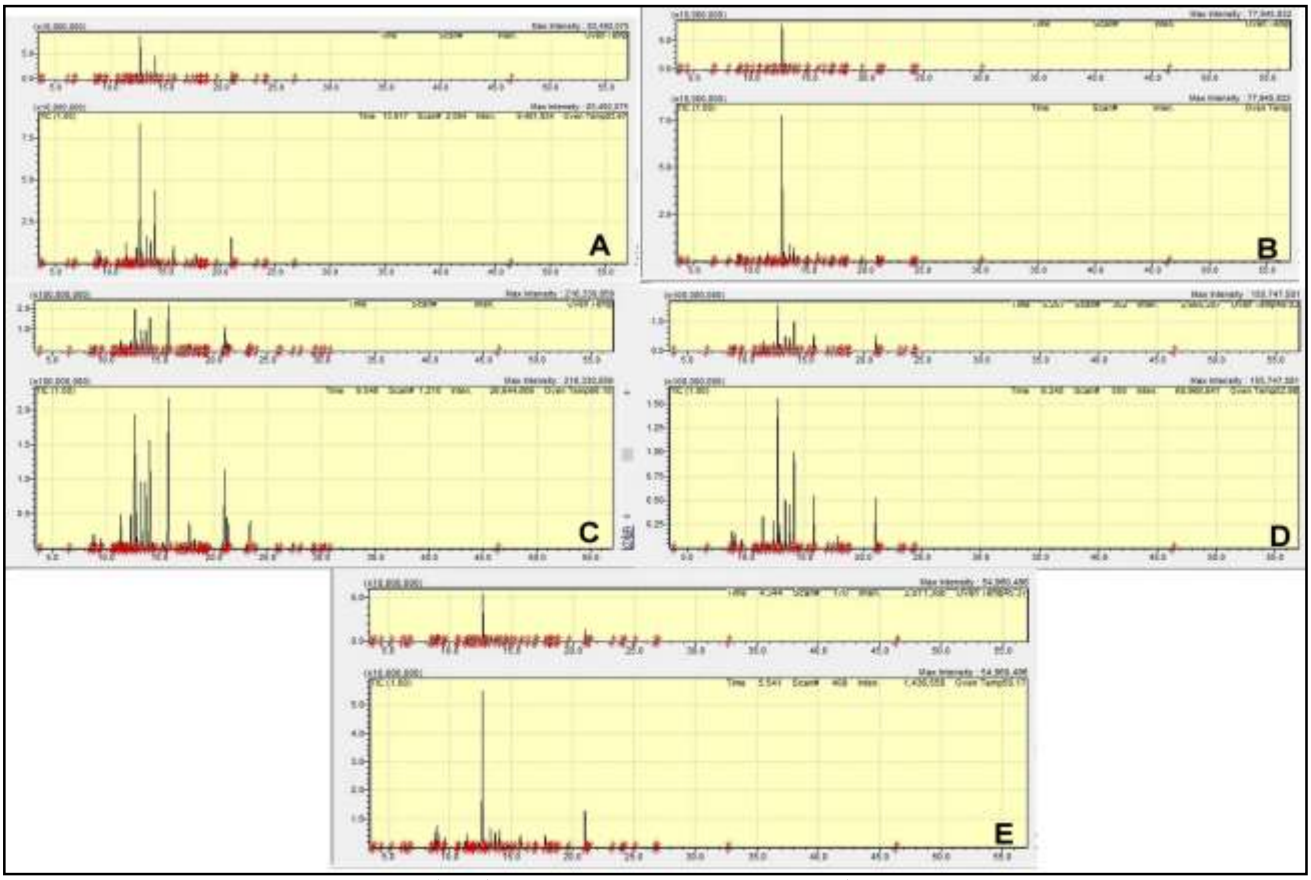

Figure 1. The total ion chromatograms (TIC) of studied Lupin specimens (A: Lutop, B: Lublanc, C: Belera, D: Tanjil, E: EGY-105).

Şekil 1. Çalışılan acı bakla örneklerinin toplam iyon kromatogramları

In common components found in all samples were Butanoic acid, 2-methyl-methyl ester, alpha-thujene, alpha-pinene, 1, 3, 6-octatriene, 3, 7-dimethyl-, (E), delta 3-carene, d-limonene, eucalyptol (1, 8-cineole) cyclohexanone, 5-methyl-2-(1-methylethe- (cis). It was determined that the samples had original components, for example, geraniol formate, 2-propen-1-ol, 3-phenyl-, 3-octen-5-yne, 2,7-dimethyl-, santolina triene, cyclohexanol, 2-methyl-5-(1-methylethe), 2-cyclohexen-1ol, 1-methyl-4-(1-methyl), thyml methyl ether, phenol, 2-methyl-5-(1-methylethyl) components are essential for Belera. On the other hand, azulene and longifolene are determined only EGY-105, 5-allyl-4-(1-(P-aminophenyl) ethylidenehyl and linalool-L are essential for Tanjil.

According to our analyses, α-terpinene is found high rates in Lutop (3.57 %) and Belera (2.99 %) samples (Table 2). It has perfume and flavour properties but is mainly used to give a pleasant odour to industrial liquids and it is present in different essential oils. Interestingly, the other lupin which is the same origin as Belera does not contain this component. One another compound, α-pinene is present in all samples, but the ratio is high in the sample from Egypt (EGY-105). The anti-inflammatory, antimicrobial and acetylcholinesterase inhibitory activities of α- pinene have been reported before (Grundschober, 1979; Sullivan et al., 1979). In particular, this sample can be evaluated pharmacologically and can be an alternative source to *Cannabis* species that contains large amounts of α-Pinene and whose pharmacologically active ingredients have significant drug effects (Kumar et al., 2021).

Another important component, pulegone is available in lupin samples in different proportions except Belera. Pulegone is a naturally synthesized organic plant substance emerged from the essential oils of many kinds of plants such as *Mentha piperita*, *Nepeta cataria* (catnip), and pennyroyal (Nissen et al., 2010; Russo, 2011) and it is used in the perfumery, in flavouring and aromatherapy. Besides, santolina triene, a monoterpene and previously reported to be found in different plant species (*Santolina insularis*, *Santolina corsica* and *Cinnamomum osmophloeum* etc.), was found only in the Belera sample from Austria.

As far as we know, there is a limited number of study about lupins chemical composition, and the volatile compounds of *Lupinus albus* and *L. angustifolius* genotypes has not been studied yet. The chemical compounds of *L. albus* species was studied by Erbaş et al. (2005), and in this study, the nutritional and chemical properties of the white lupin (*L. albus* L.) was characterised via HPLC system. In the other study Yorgancılar and Bilgiçli (2014), investigated the chemical changes of lupin seed during different bulgur process methods by using GC and HPLC.

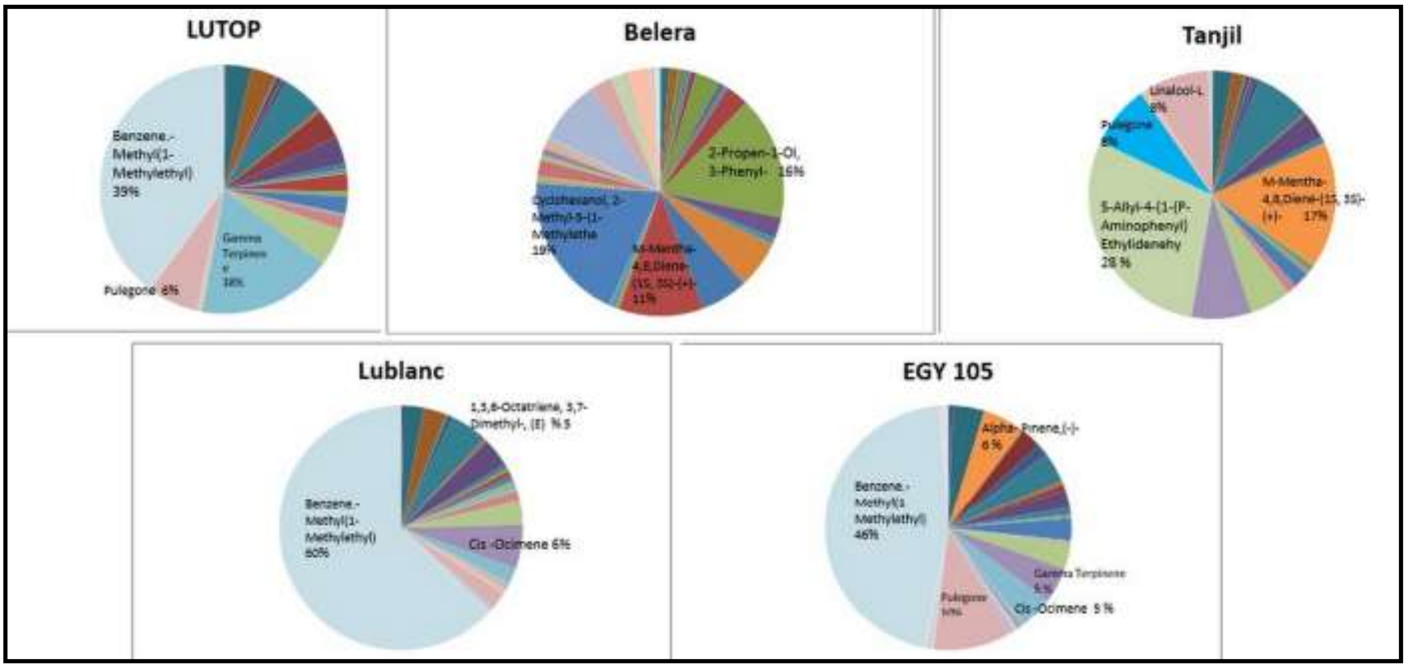

Figure 2. The pie charts of most found compounds in studied Lupin samples
Şekil 2. Çalışılan acı bakla örneklerinde en çok bulunan bileşiklerin dairesel grafikleri

CONCLUSION and RECOMMENDATIONS

In this study, five *Lupinus albus* and *L. angustifolius* genotypes from different regions were analysed using DVB/CAR/PDMS-SPME fibre via GC/MS. To our best of knowledge, interestingly, this study is the first attempt regarding various lupin genotypes volatile compounds with SPME. It is very important to detect the essential natural components which have enormous influences on the lupin samples originating from different geographical locations. Such kind of studies, aiming at revealing the active biochemical compounds from plants or any tissue of plants and their possible potentials, is one of prime issues regarding detailed future investigations.

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Contribution Rate Statement Summary of Researchers

Authors declares the contribution of the authors is equal.

Conflict of Interest Statement

The authors of the article declare that there is no conflict of interest between them.

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