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FATTY ACID COMPOSITION OF FIVE MUSHROOM SPECIES BY GC AND GC-MS WITH A CHEMOMETRIC APPROACH

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Abstract

Fatty acids are recognized as energy sources and membrane components. The biological effects of the fatty acids are composed of influencing cell and tissue metabolism, and responding to hormonal and other signals. This study was planned to characterize the fatty acid compositions of five different mushrooms namely, Cerrena unicolor, Hymenochaete rubiginosa, Inocutis rheades, Leptoporus mollis, and Polyporus squamosus naturally distributed in Turkey by using gas chromatography (GC) and GC-mass spectrometry (GC-MS). A total of sixteen fatty acids were screened in the mushroom species. The most abundant fatty acids were recorded as linoleic (10.35-65.69%), oleic (12.03-53.27%), palmitic (12.68-21.16%), stearic (2.39-4.36%) and palmitoleic (1.40-4.26%) acids in all studied mushrooms. The amounts of unsaturated fatty acids (UFAs) (60.02-80.70%) were calculated higher than saturated fatty acids (SFAs) (19.24-39.58%). The correlations or differences of the mushroom species with regard to fatty acid compositions were chemometrically investigated by using principal component analysis (PCA) and hierarchical clustering analysis (HCA). H. rubiginosa was separated with the highest amount of linoleic acid (65.69%) from other four mushroom species in both analyses.

Keywords: Mushrooms, Hierarchical clustering analysis, Chemometric analysis, Fatty acids, Principal component analysis, Gas chromatography-mass spectrometry (GC-MS).

BEŞ MANTAR TÜRÜNÜN KEMOMETRİK YAKLAŞIM İLE GC VE GC-MS KULLANILARAK YAĞ ASİDİ BİLEŞİMİ

Özet

Yağ asitleri, enerji kaynakları ve zar bileşenleri olarak bilinmektedir. Yağ asitlerinin biyolojik etkileri, hücre ve doku metabolizmasını etkilemek ve hormonal ve diğer sinyallere yanıt vermekten oluşmaktadır. Bu çalışma, Türkiye'de doğal olarak yayılış gösteren Cerrena unicolor, Hymenochaete rubiginosa, Inocutis rheades, Leptoporus mollis ve Polyporus squamosus isimli beş farklı mantarın yağ asidi bileşimlerini gaz kromatografisi (GC) ve GC-kütle spektrometresi (GC-MS) kullanarak karakterize etmek üzere tasarlanmıştır. Mantar türlerinde toplam on altı yağ asidi taranmıştır. Çalışılan bütün mantar türlerinde en fazla bulunan yağ asitleri linoleik (%10,35-65,69), oleik (%12,03-53,27), palmitik (%12,68-21,16), stearik (%2,39-4,36) ve palmitoleik (%1,40-4,26) asitler olarak kaydedilmiştir. Doymamış yağ asitlerinin (UFA) miktarları (%60,02-80,70) doymuş yağ asitlerinden (SFA) (%19,24-39,58) daha yüksek hesaplanmıştır. Mantar türlerinin yağ asidi bileşimleri açısından korelasyonları veya farklılıkları, temel bileşenler analizi (PCA) ve hiyerarşik kümeleme analizi (HCA) kullanılarak kemometrik olarak incelenmiştir. Her iki analizde de diğer dört mantar türünden en yüksek oranda linoleik asit (%65.69) ile H. rubiginosa ayrılmıştır.

Anahtar Kelimeler: Mantarlar, Hiyerarşik kümeleme analizi, Kemometrik analiz, Yağ asitleri, Temel bileşenler analizi, Gaz kromatografisi-kütle spektrometresi (GC-MS).

Cite

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1. Introduction

Mushrooms are described as macro-fungus with a spore and fleshy fruit and have served humanity since the earliest civilizations of history, both with their culinary and therapeutic properties. There are more than 14000 species of mushrooms in the world, of which 3000 species have been identified as edible, about 700 species as medicinally important and about 1400 species as toxic [1]. Mushrooms have been consumed as favourite foods in many countries as edible mushrooms owing to their enticing aroma, delicious taste and nutritive value (high vitamins, fiber, minerals, and protein, low or no cholesterol calories) [2]. The nutrients in mushrooms could be listed as sugars (sucrose, rhamnose, xylose,

fructose, and mannose), amino acids (aspartic, glutamic, cysteine, methionine, and glutamate), fatty acids (linoleic, palmitic, oleic, stearic acids), vitamins (riboflavin, folate, niacin, ascorbic acid, thiamine, cyanocobalamin, and ergocalciferol), proteins, and mineral contents (Ca, Mo, K, Na, Mg, Cu, P, Zn, Cd, and Fe). With their low fat contents and calorie and high dietary fiber properties, mushrooms have been proven to be in the group of healthy foods. It was also revealed that the value of mushrooms was originated with their contents of trace minerals such as selenium and zinc and significant amounts of vitamins which are necessary for quality nutrition [3,4] Additionally, mushrooms are important sources of biologically active molecules (polysaccharides, lectins, proteins, peptides, phenolic compounds, proteoglycans, steroids, terpenes, etc.) that may have therapeutic values [5]. The consumption of mushrooms by people of all ages worldwide is continuously increasing. Mushrooms are considered functional foods because of their versatile bioactive properties (antiviral, immunomodulatory, antibacterial, antioxidant, anti-inflammatory, anti-arthritic, antifungal, antitumor, anticancer, antidiabetic, anti-HIV, anti-cholesterol activities) and high nutritional values (especially protein, dietary fiber, vitamins and minerals) [3,6].

Lipids, which are used as energy sources, are important components that exist in different physiological processes such as synaptic transmission, protein stabilization, cell signalling, transmission and transport. In addition, lipids act as signaling molecules that modulate inflammation, cell survival and ageing. [7]. Fatty acids are the molecules that make up the majority of lipids and are mainly categorized as saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) (monounsaturated (MUFAs) and polyunsaturated (PUFAs)). Fatty acids have an important place both biologically and nutritionally, and clinically, their importance is increasing due to their effects on the initiation or development of many diseases, containing cancer [8]. It is extremely important to supplement PUFAs, which cannot be synthesized by eukaryotic cells since they do not have the necessary enzyme to convert monounsaturated omega-9 (ω-9) fatty acids to polyunsaturated omega-9 (ω-9) fatty acids that can be synthesized endogenously [9]. The daily requirement for PUFAs determined within the scope of the realization of cell formation is essential in daily nutrition and this situation is also of vital importance during cancer treatment [10].

Increasing awareness of the fact that in human fatty acids are necessary parts of basal metabolism and that long-chain PUFAs possess health-promoting abilities has prompted a focus on the fatty acids of different foods. In direct proportion to this, studies on the description of detailed fatty acid compositions of mushrooms have increased. Considering the insufficient data on the importance and fatty acid compositions of mushrooms, this study was planned to characterize the fatty acid compositions of five different mushrooms namely, *Cerrena unicolor, Hymenochaete rubiginosa, Inocutis rheades, Leptoporus mollis,* and *Polyporus squamosus* naturally distributed in Turkey by using gas chromatography (GC) and GC-mass spectrometry (GC-MS). The correlations and differences between the mushroom species with regard to fatty acid compositions were examined chemometrically by with principal component analysis (PCA) and hierarchical clustering analysis (HCA).

2. Materials and Method

2.1. Mushroom Materials

Cerrena unicolor, Hymenochaete rubiginosa, Inocutis rheades, Leptoporus mollis, Polyporus squamosus mushroom species were collected from the area of Fethiye, Muğla, Turkey in April-November, 2018. The specimens have been deposited at Natural Products Laboratory of Muğla Sıtkı Koçman University.

2.2. Extraction

All mushroom samples were macerated with *n*-hexane three times at room temperature and *n*-hexane was removed by using a rotary evaporator to get the *n*-hexane extracts. All extracts were kept at +4°C for identification of fatty acid compositions.

2.3. Analysis of Fatty Acid Compositions

2.3.1. Derivatization of Fatty Acids

The *n*-hexane extracts were dissolved in NaOH (0.5 M), then the mixture was heated in a water bath at 50°C. BF_3: methanol (2 mL) was added in the heated mixture, then the mixture was boiled during 2 min and cooled down to room temperature. The last volume was completed with saturated NaCl solution to 25 mL. *n*-Hexane was used for the extraction of the esters and the organic layer was separated. 4 mL of potassium bicarbonate solution (2%) was used for washing of the *n*-hexane layer. Then anhydrous Na_2SO_4 was used to dry *n*-hexane layer and filtered. The *n*-hexane was evaporated under reduced pressure by a rotary evaporator for obtaining of fatty acid methyl esters [11].

2.3.2. Gas Chromatography (GC)

Methyl esters of fatty acids were analyzed by GC with a flame ionization detector (FID). The analysis conditions were as follows: Column: DB-1 fused silica capillary non-polar column (30m x 0.25 id., film thickness 0.25 mm). Injector temperature: 250°C. Detector temperature: 270°C. Carrier gas: He (1.4 mL/min flow rate). Sample size: 1.0 mL. Split ratio: 50:1. The initial oven temperature was kept for 5 min at 100°C, then increased with 3°C/min increments up to 238°C and kept for 9 min at 238°C. GC solution computer program was used to calculate the relative percentages of fatty acid methyl esters [11].

2.3.3. Gas Chromatography-Mass Spectrometry (GC-MS)

Methyl esters of fatty acids were analyzed by GC with an ion trap mass spectrometer (MS). The analysis conditions were as follows: Column: DB-1 fused silica non-polar capillary column (30m x 0.25mm ID, film thickness 0.25 mm). Ionization energy: 70 eV. Carrier gas: He (15 psi) (1.3 mL/min flow rate). Injector temperature: 220ºC. MS transfer line temperature: 290ºC. Sample size: 0.2 mL. Split ratio: 50:1. The oven temperature was kept for 5 min at 100ºC, then increased with 3ºC/min increments up to 238ºC and kept for 9 min at 238ºC. Sample size: 0.2 mL. Split ratio: 50:1. 70 eV ionization energy was used for EI-MS and mass range was from m/z 28 to 650 amu. Scan time 0.5 sec with 0.1 inters scan delays. NIST and Wiley 2005 (Gas Chromatography-Mass Spectrometry) GC-MS libraries were used for the library search. FAME (fatty acid methyl ester) mixture (SupelcoTM 37, Catalog no: 47885-U) were characterized by matching their retention times with the pure FAMEs standards [11].

2.4. Chemometric Analysis

Chemometric analysis (PCA and HCA) of the fatty acid compositions of the five mushroom species were researched by using MINITAB 16.0 software. Euclidean distance and Ward Linkage method were preferred in the application of cluster analysis to reveal the hierarchical relationship.

3. Results and Discussion

Fatty acid compositions of the mushroom species were detected utilizing the most basic and common methods GC and GC-MS. The list of all identified fatty acids were presented in Table 1. GC-MS chromatograms of the mushroom species was given in Figure 1.

A total of 14 fatty acids were detected in *C. unicolor*, 6 fatty acids in *H. rubiginosa,* 13 fatty acids in *I. rheades,* 12 fatty acids in *L. mollis,* 10 fatty acids in *P. squamosus.* Linoleic (10.35-65.69%), oleic (12.03-53.27%), palmitic (12.68-21.16%), stearic (2.39-4.36%) and palmitoleic (1.40-4.26%) acids were detected as the most abundant fatty acids in all mushroom species. Also, all mushroom species were found as rich in unsaturated fatty acids (UFAs) with the amounts of 60.02-80.70%. Saturated fatty acids (SFAs) were recorded between the range of 19.24 and 39.58%. The highest UFAs/SFAs ratio was measured as 4.19 for *P. squamosus* while the lowest UFAs/SFAs ratio was measured as 1.52 for *C. unicolor.*

Linoleic acid (C18:2) is a polyunsaturated fatty acid and a member of ω-6 fatty acids. Linoleic acid is valued as an essential fatty acid that cannot be produced due to enzyme deficiency and therefore must be taken with dietary supplements. Linolenic acid has been found to prevent systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, neurodegenerative diseases, age-related maculopathy and type 1 diabetes [12]. In addition, anti-inflammatory effect of linoleic acid and its important effects in the therapy of colon and breast cancers have been described in *in vitro* and *in vivo* investigations [8]. Linoleic acid was determined as the most abundant fatty acid in *C. unicolor* (31.84%), *H. rubiginosa* (65.69%) and *I. rheades* (35.59%); the second most abundant in *P. squamosus* (29.28%); the third most abundant in *L. mollis* (10.35%). Oleic acid (C18:1) (monounsaturated fatty acid) is the member of ω-9 fatty acids. The valuable effects of oleic acid on human health are listed as follows: to inhibit platelet aggregation, to lower serum LDL cholesterol and systolic blood pressure in the cardiovascular system.

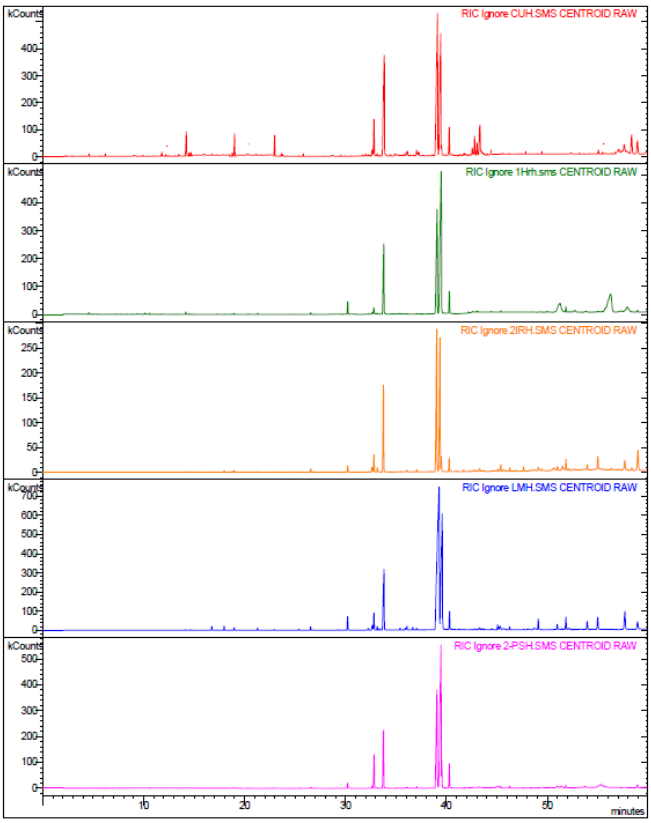

Figure 1. The GC-MS chromatograms of mushroom species. CUH: *C. unicolor* hexane extract, HRH: *H. rubiginosa* hexane extract, IRH: *I. rheades* hexane extract, LMH: *L. mollis* hexane extract, PSH: *P. squamosus* hexane extract.

Also, in the different studies, it has been proven that oleic acid inhibited cancer-causing oncogenes in some types of cancer and had an anti-inflammatory effect on T cells [13]. Oleic acid was recorded as the main fatty acid in *L. mollis* (53.27%) and *P. squamosus* (50.02%); the second most abundant in *C. unicolor* (23.92%) and *I. rheades* (31.15%); the third most abundant in *H. rubiginosa* (12.03%). Stearic acid (C18:0) is a saturated fatty acid. It has been noted that enhanced level of stearic acid in the circulatory system is associated with decreased cancer risk, reduced blood pressure, and enhanced heart function [14]. The inverse relationship between stearic acid consumption and the risk of breast, cervical,

Table 1. The fatty acid compositions of mushroom species (%)

Fatty Acids	*C. unicolor*	*H. rubiginosa*	*I. rheades*	*L. mollis*	*P. squamosus*
Azelaic acid ($C_{9:0}$)	2.92	-[a]	0.28	0.49	-[a]
Sebacic acid ($C_{10:0}$)	2.18	-[a]	-[a]	0.17	-[a]
Lauric acid ($C_{12:0}$)	0.36	-[a]	-[a]	-[a]	-[a]
Tridecanoic acid ($C_{13:0}$)	-[a]	-[a]	-[a]	0.03	-[a]
Myristic acid ($C_{14:0}$)	1.03	-[a]	0.58	0.70	0.14
Pentadecanoic acid ($C_{15:0}$)	1.40	1.40	1.10	2.90	0.85
Palmitic acid ($C_{16:0}$)	21.16	15.62	17.19	21.06	12.68
Palmitoleic acid ($C_{16:1}$)	4.26	1.69	3.16	3.86	1.40
Margaric acid ($_{C17:0}$)	0.68	-[a]	0.41	-[a]	0.28
Stearic acid ($C_{18:0}$)	3.03	3.38	2.39	4.04	4.36
Oleic acid ($C_{18:1}$)	23.92	12.03	31.15	53.27	50.02
Linoleic acid ($C_{18:2}$)	31.84	65.69	35.59	10.35	29.28
Arachidic acid ($C_{20:0}$)	2.31	-[a]	0.63	0.64	0.37
Behenic acid ($C_{22:0}$)	1.81	-[a]	1.90	2.42	0.56
Tricosanoic acid ($C_{23:0}$)	-[a]	-[a]	0.12	-[a]	-[a]
Tetracosanoic acid ($C_{24:0}$)	2.70	-[a]	5.42	-[a]	-[a]
∑ Saturated fatty acids (SFAs)	39.58	20.40	30.02	32.45	19.24
∑ Unsaturated fatty acids (UFAs)	60.02	79.41	69.90	67.48	80.70
UFAs/SFAs	1.52	3.89	2.33	2.08	4.19
ω6/ω9	1.33	5.46	1.14	0.19	0.58

UFAs/SFAs: Unsaturated fatty acids/saturated fatty acids ratio
ω6/ω9: linoleic/oleic acid ratio
[a]: not detected.

prostate and colon cancers and inhibition of these cancer cells has been reported [15,16].

P. squamosus was found to be richest in stearic acid with the amount of 4.36%, followed by *L. mollis* with the amount of 4.04%. Palmitic acid (C16:0) is a saturated fatty acid. An important task of palmitic acid is to be effective in type 2 diabetes pathogenesis in the tissues of the pancreas and liver [17]. It has been proven that supplementation with palmitic acid-rich diets increases the survival rate in newborns due to excess palmitic acid contents in breast milk [18]. *C. unicolor* was found to be richest in palmitic acid with the amount of 21.16%, followed by *L. mollis* with the amount of 21.06% and *I. rheades* (17.19%). Stearic acid (C18:0) is an important saturated fatty acid that reverses the general belief that saturated fatty acids are harmful to human health. In detailed studies, it has been reported that stearic acid lowers cholesterol in coronary heart diseases, diminishes the risk of cancer and cardiovascular disease in diabetic patients, protects cortical neurons from oxidative stress by increasing antioxidant enzymes, and reduces the risk of prostate, breast, and colorectal cancers [8] The amount of stearic acid in decreasing order is as follows: *P. squamosus* (4.36%), *L. mollis* (4.04%), *H. rubiginosa* (3.38%), *C. unicolor* (%3.03), *I. rheades* (%2.39). Palmitoleic acid (C16:1) is an unsaturated fatty acid. The positive effects of the palmitoleic acid in obesity, diabetes, cardiovascular disease, atherosclerosis, breast, pancreatic and prostate cancers have been revealed [19]. All studied mushrooms had considerable amounts of palmitoleic acid with the levels of 1.40-4.26%.

The ω6/ω9 ratio, which is accepted as chemotaxonomically valuable, is used as a useful parameter in the taxonomic evaluation of different species belonging to the same genus [20]. As it seen in Table 1, the lowest ω6/ω9 ratio was calculated for *L. mollis* as 0.19 and the highest ω6/ω9 ratio was 5.46 for *H. rubiginosa.*

In this study, fatty acid compositions of *C. unicolor*, *H. rubiginosa, I. rheades,* and *L. mollis* were characterized for the first time. In an earlier study, thirteen fatty acids were identified in *P. squamosus* by HR-GLC and linoleic, palmitic and oleic acids were found as the highest amounts of 74.60, 11.08, and 8.81%, respectively. SFAs and UFAs amounts were reported as 15.89% and 84.11% [21]. As a result of the identification of *P. squamosus* fatty acids based on GC-FID, linoleic (38.91%), oleic (33.02%), palmitic (17.21%) and stearic (3.21%) acids were detected as the major fatty acids. The level of UFAs (74.91%) was higher than the level of SFAs (25.19%) in the mushroom species [22]. Mocan et al. described the main fatty acids of *P. squamosus* as linoleic (56.55%), oleic (22.27%), palmitic (13.40%) acids by GC. The level of UFAs was 83.97% and SFAs was 17.68% [23]. *Leucopaxillus gentianeus, Armillaria tabescens, Suillus granulatus,* and *Pleurotus eryngii* were studied for fatty acid compositions by GC and GC-MS and linoleic (27.64-68.24%), oleic (10.05-39.78%), palmitic (7.95-19.40%), and stearic (1.29-8.89%) acids were noted for the major fatty acids [24]. The main fatty acids in *Morchella elata, Lactarius delicious, Macrolepiota procera, Helvella lacunosa, Cantharellus cibarius, Boletus edulis, Agaricus bisporus* and *Bovista plumbea* were defined as linoleic (10.78-49.74%), oleic (3.46-45.32%), stearic (8.52-32.67%), palmitic (5.31-29.57%), and pentadecanoic (1.53-17.46%) acids by GC-MS. The level of UFAs was in the range of 51.27-70.87% and SFAs was 29.13-48.74% [25]. In a study on fatty acid compositions of *Hydnum repandum, Pleurotus eryngii,* and *Agaricus silvicola* based on GC-MS, the prominent fatty acids were linoleic (33.57-60.03%), oleic (4.21-39.74%), palmitic (19.65-21.61%) and stearic (3.728.23%) acids. SFAs were calculated as

25.51-32.81% and UFAs were 67.19-74.49% [26]. Olennikov et al. characterized the main fatty acids of fourteen wood-decaying mushroom species namely, *Bjerkandera adusta, Fomitopsis pinicola, Daedaleopsis septentrionalis, Inonotus hispidus, Dichomitus squalens, Fomitopsis cajanderi, Inonotus radiatus, Irpex lacteus, Trametes ochracea, Fomitopsis rosea, Gloeophyllum protractum, Phellinus pini, Lenzites betulina,* and *Trametes gibbosa* as linoleic (19.7-59.1%), oleic (2.0-39.6%), palmitic (1.8-27.1%) and arachinic (0.6-47.9%) acids by GC-MS [27]. In a different study, *Flammulina velutipes, Helvella crispa, Ganoderma lucidum, Mycena vitilis, Lactarius rubidus, Laccaria bicolor, Russula brevipes, Russula nobilis,* and *Xerocomellus chrysenteron* were investigated for fatty acid compositions through GC-MS. Linolenic stearic, oleic, nonadecanoic, linoleic, and palmitic acids were detected as the prominent fatty acids with the amounts of 0.0202-3.978%, 0.413-3.765%, 1.063-4.868%, 0.432-12.547%, 0.258-15.690%, and 1.907-3.768%, respectively [28]. The fatty acid results obtained are fully consistent with the literature results.

Chemometry is defined as a discipline that provides the processing of chemical data with the help of computers using statistics and mathematics for various measurement and evaluation processes. Today, issues related to food and chemistry constitute one of the most important application areas of chemometry.

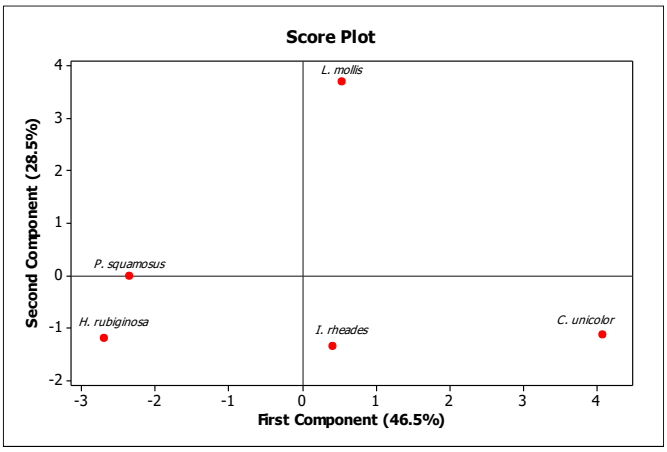

Figure 2. Score plot graphic for PC1 and PC2 in the mushroom species

The advantages of chemometric analysis can be expressed as follows: 1) To provide process monitoring and control as it minimizes the sample preparation process, to allow the measurement of the chemical composition of the unprocessed product, to perform fast analysis and to minimize product loss; 2) To determine the geographical origin, to identify the chemical components and trace elements; 3) To interpret the data obtained in the identification of counterfeit and adulteration products [29].

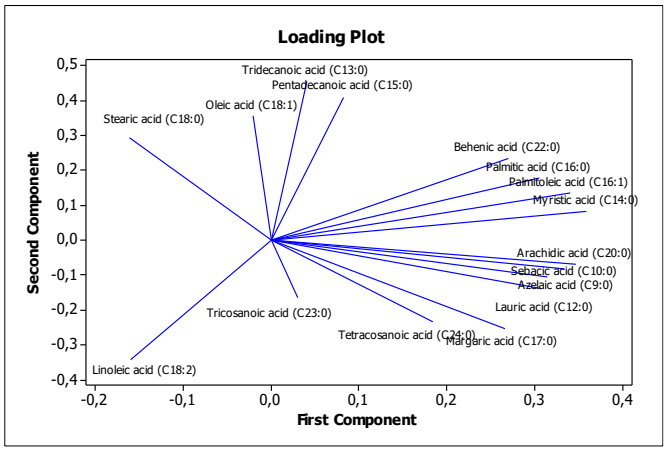

Figure 3. Loading plot graphic for PC1 and PC2 in the mushroom species.

Herein, five mushroom species were chemometrically evaluated for their sixteen fatty acids by using principal component analysis (PCA) and hierarchical clustering analysis (HCA). The use of large data sets is becoming common in many disciplines. PCA is one of the oldest and most widely used statistical methods for interpreting the dimensions of these datasets by preserving the information in the data. The goal here is to preserve as much variability as possible, i.e. statistical information, while reducing the dimensionality of the dataset. [30]. In the PCA method, which enables the visualization of large data sets using two or three-dimensional drawings with minimal information loss, it becomes possible to visualize and remember the similarities and differences in compound collections based on structural and physicochemical parameters, and to design the libraries such as natural products, synthetic drugs, and natural product-like libraries [31]. The similarities and inequalities of the mushroom species according to their fatty acid compositions were analyzed by PCA. For PC1 and PC2 in mushroom species, the score plot graphic was presented in Figure 2 and the loading plot graphic was presented in Figure 3. 1st component (PC1) was estimated for 46.5% and 2nd component (PC2) for 28.5% of total variance. The mushroom species were separated from each other according to differences in their fatty acid compositions. *H. rubiginosa* was separated with the highest amount of linoleic acid; *P. squamosus* was with the highest amount of stearic acid; *L. mollis* was with the highest amounts of oleic, tridecanoic and pentadecanoic acids; *I. rheades* was with the amounts of the tricosanoic and tetracosanoic acids; *C. unicolor* was with the highest amounts of azelaic, sebacic, lauric, margaric and arachidic acids. *P. squoamosus* and *H. rubiginosa* were distributed in very close areas. This may be due to the fact that the amounts of major fatty acids identified are very similar.

Figure 4 presented the dendrogram of HCA documenting the results associated with the resemblances of the mushroom species. Two main clusters and three sub-clusters were obtained according to the amounts of the major fatty acids characterized in the mushroom species. *H. rubiginosa* was in the 1st sub-cluster with the highest

amount of linoleic acid; *L. mollis* and *P. squamosus* were in the 2nd sub-cluster with the highest amounts of oleic acid; *C. unicolor* and *I. rheades* were in the 3rd sub-cluster with similar amounts of linoleic acid as the major fatty acids. Also, when viewed from bottom to top, especially with higher linoleic acid than other mushrooms, *H. rubiginosa* clustered separately by distance discrimination.

PCA and HCA analysis have been successfully applied in a wide variety of studies in the statistical investigations of the relationship between the chemical compositions, collection areas of mushrooms, bioactivities, and/or aroma

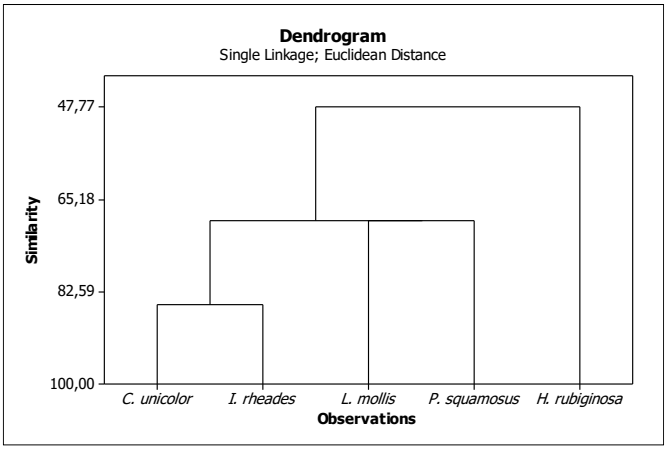

Figure 4. Dendrogram results obtained by Euclidean distance and Ward Linkage method.

compounds. GC and GC-MS analysis led to the characterization of linoleic, oleic, stearic, and palmitic acids as the major fatty acids in 17 mushroom species namely; *Chondrostereum purpureum, Coprinus comatus*, *Gloeophyllum odoratum, Daedalea quercina, Gloeophyllum trabeum, Gloeophyllum sepiarium, Hydnum repandum, Omphalotus olearius, Schizophyllum commune, Phellinus igniarius, Phaeolus schweinitzii, Trametes pubescens, Inonotus radiatus, Trametes versicolor, Macrolepiota procera, Trametes bicolor, and Trametes suaveolens.* The fatty acids of the mushroom species were chemometrically clustered and especially *Daedalea quercina* was reported to distanced from other mushroom species based on the fatty acid profile [11]. The fatty acid compositions of 14 different extracts obtained from 4 different *Ganoderma* mushrooms (*G. lucidum, G.adspersum, G. applanatum, G. resinaceum*) by different extraction methods were analyzed chemometrically by PCA and HCA analysis. It has been reported that *Ganoderma* extracts were divided into 3 different groups in PCA analysis and 6 different groups in HCA analysis based on their fatty acid compositions due to the host tree and extraction technique [32]. In the study of Wang et al., PCA analysis was applied 32 different taste components identified in the stem and cap of *Agaricus bisporus* mushroom and it was assumed that 24 taste components were common components in both stem and cap. Also, 5-GMP, glutamic acid, malic acid, alanine, proline, leucine, and aspartic acid were suggested as the characteristic components of *Agaricus bisporus* fruiting body [33]. In our prior study, we investigated the relationship between the phenolic compounds, anti-cholinesterase, and antioxidant activities of *Chondrostereum purpureum*, *H. rubiginosa*, *Macrolepiota procera, Phaeolus schweinitzii*, and *Phellinus igniariu*s mushroom extracts by the way of PCA and HCA. The mushroom extracts were separated 3 groups by PCA according to the effect of the phenolic compounds and bioactivities and 2 clusters by HCA according to effect of bioactivities [34]. A detailed chemometric study was carried out with PCA and HCA analysis to examine whether 13 multielement in a total of 52 *Thelephora ganbajun* mushroom collected from 4 different regions were an effective tool to distinguish between different origins of the mushroom. The studied multielement were proven to affect 3 main groups in PCA analysis and 4 clusters in HCA analysis [35].

4. Conclusion

In this study, fatty acid compositions of five different mushrooms naturally distributed in Turkey, namely *Cerrena unicolor, Hymenochaete rubiginosa, Inocutis rheades, L. mollis,* and *Polyporus squamosus* by using GC and GC-MS. This is the first study on fatty acid compositions of *H. rubiginosa, C. unicolor*, *I. rheades,* and *L. mollis.* Linoleic, palmitic, palmitoleic, stearic, and oleic acids were found to be the prominent fatty acids in all mushroom species. PCA and HCA were used to investigate the correlations or differences of the mushroom species with regard to fatty acid compositions from a chemometric perspective. Mushrooms were clustered in different groups depending on the amounts of both main fatty acids and other fatty acids in their contents. In this direction, *H. rubiginosa* clustered separately from other mushrooms. In conclusion, it was emphasized in this study that the studied mushroom species are important natural sources of fatty acids. Also, the fatty acid compositions can be used chemometrically for clustering of the mushroom species. In conclusion, the fact that the fatty acids of the studied mushrooms were studied chemometrically here for the first time would be a complement to an important deficiency in the literature. The findings revealed in this direction can be accepted as a reference that will shed light on further studies.

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