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FATTY ACID ANALYSIS FOR SEEDS OF SOME ORCHID SPECIES SPREADING IN VAN PROVINCE

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ABSTRACT

Fatty acids have many important biochemical properties such as energy, metabolic and other structural activities. Orchids, which are among the common species worldwide, are used as beverages, food additives and drugs in societies. Orchid seeds are one of the smallest seeds that do not contain nutrient tissue naturally and are rich in fatty acids.

Plants dried at room temperature were extracted using chloroform/methanol (2:1). Their methyl esters were analyzed using a Gas Chromatography device. As a result of the analysis, a total of 16 fatty acids were determined. Out of these, palmitic acid (16:0), oleic acid (18:1n-9) and linoleic acid (18:2n-6) were found as major fatty acids. Other fatty acids, myristic, pentadecanoic, stearic, linolenic, arachidonic and docosapentaenoic acid were detected in trace amounts. When the percentage distribution of palmitic acid was considered, it was observed that it ranged between 16.76% and 25.32%, and the highest one was in Anacamptis collina. It was also found that the distribution of stearic acid was similar among species. Oleic acid exhibited the highest distribution as percentage in Orchis simia (27.33%), Orchis mascula subsp. pinetorum (25.16%) and Orchis spitzelii (25.66%). Linoleic acid showed different distribution among species. The lowest percentage was found in Orchis tridentata (15.13%), while the highest one was in Orchis mascula subsp. pinetorum (34.02%). This study carried out on fatty acids is a part of the study on the use of natural bioactive products and fatty acids, hoping to be used in industrial applications.

Keywords: Orchidaceae, Types of Orchids, Fatty Acids

1. INTRODUCTION

There are 880 genera and 21,000-26,000 species of orchids worldwide (Stevens, 2020). Turkey is one of the richest countries in terms of orchids with 24 genera and 128 species (Sandal & Söğüt, 2010). Recent studies have shown that around 150 species grow in Turkey (Kreutz, 1998; Renz & Taubenheim, 1984; Sezik, 2002b). Furthermore, middle generation orchids are registered in a book entitled “Red Data Book” and are under protection (Ekim et al., 2000). It was determined that salep was obtained from 117 species belonging to *Ophrys*, *Orchis*, *Himantoglossum*, *Serapias*, *Anacamptis*, *Comperia*, *Barlia*, *Aceras* and *Neotinea* genus in the Anatolian region (Sezik, 2002a, 2002b). It is also reported that approximately 40 million orchids have been destroyed due to the saleps obtained (Sezik, 2002b).

Salep has been used as a traditional beverage, food additive and medicine in the society since ancient times. It is also reported to be consumed as an aphrodisiac in Europe and especially in Eastern countries (Baytop, 1984; Sezik, 1984). Today, it is said to have a breast softening and expectorant effect. In addition to these, it is used as the raw material of salep and ice cream, as nutrition. The composition of the salep varies depending on its geographical location, the period in which it was collected and the type. The main component that determines the effect and quality of salep is glucomannan. Studies have indicated that salep contains approximately 16-55% glucomannan, 2.7% starch, 12% moisture and 2.4% mineral substance. In another study, it was found that it contains 48% mucilage, 1% sugar, 2.7% starch, 5% nitrogenous substance and a trace amount of essential oil in the fresh state (Dogan & Kayacier, 2004; Sezik, 1984; Sezik & Baykal, 1988; Tamer et al., 2006; Telcioglu & Kayacier, 2007).

Salep samples from some *Orchidaceae* species contains 2.02% lipid (Turgay & Çınar, 2017), while *Cycnoches ventricosum var.* contains 32% lipid (Arditti & Ghani, 2000). Out of the fatty acids, it was found to be rich in oleic acid (28.65%) out of monounsaturated fatty acids and in linoleic acid (31.96%) out of polyunsaturated ones (Citil & Tekinsen, 2011). Fatty acids are the compounds that have many important biochemical functions, such as energetic, metabolic and structural activities (Saraçoğlu, Zengin, Akin, & Aktümsek, 2012). In particular, carboxylic and short-chain fatty acids have been shown to play a role in the induction of seed rest and have growth-inhibiting properties (Woltering, Van Hout, Somhorst, & Harren, 1993). Furthermore, fats containing oleic acid have been reported to contribute to the reduction of cholesterol and cardiovascular diseases (Jacocot, 1995). In addition to these, it was found to have an important effect on protection from many diseases such as depression, migraine, joint rheumatism, diabetes, some types of allergies and cancer (Nettleton & Exler, 1992). This study aims to determine the fatty acid distribution in the seeds of some *Orchidaceae* species grown in the province of Van in the Eastern Anatolia region.

2. MATERIALS AND METHODS

2.1. SEED SAMPLES

Orchid seeds were from the seeds of dried samples collected from the villages of Gevaş district of Van province between 1995 and 2005 and preserved in Van herbarium (VANF). Information for the seed samples used in the study is given in Table 1.

Table 1. The type, the region of collection and herbarium numbers of seeds used in the study,

Type	Habitat	Herbarium No:
Orchis anatolica	Van-Gevaş İnkaya Village	(VANF 4354)
Orchis tridentata	Van-Gevaş Altınsaç, İnkaya Village	(VANF 4334), (VANF 4353)
Anacamptis collina	Van-Gevaş Altınsaç, İnkaya Village	(VANF 4309), (VANF 4349)
Orchis simia	Van-Gevaş Altınsaç Village	(VANF 4304)
Orchis mascula subsp. *pinetorum*	Van-Gevaş Altınsaç, İnkaya Village	(VANF 4287), (VANF 4351)
Anacamptis coriophora	Van-Gevaş Altınsaç Village	(VANF 4420)
Orchis spitzelii	Van-Gevaş Altınsaç, İnkaya Village	(VANF 4289), (VANF 4423)

2.2. TOTAL LIPID EXTRACTION AND CONVERSION OF FATTY ACIDS TO THEIR METHYL ESTERS

Seeds of plant samples were kept in chloroform/methanol (2:1) for 48 h in a dark environment and then filtered with homogenate Whatman No: 1 filter paper according to Bligh (1959). Fatty acid methyl esters of the extracts for the samples were obtained.

Fatty acids of fat samples converted into methyl esters were analysed using a SHIMADZU GC 2010 PLUS model Gas Chromatography instrument, flame ionization detector (FID) and DB–23 (Bonded 50 % cyanopropyl) (J & W Scientific, Folsom, CA, USA) capillary column (30m x 0.25mm inner diameter x 0.25μm film thickness). Detector temperature: 250 ºC; injector temperature: 250 ºC; injection: Split-model 1/40. Gas flow rates: carrier gas: helium 0.5 ml/min for 30 m column; hydrogen: 30 ml / min; dry air: 400 ml/min. Column (oven) temperature: 170 ºC, standby time, 2 min; 2 ºC/min to 210 ºC, standby time 20 min; total analysis time: 42 min. In the diagnosis of fatty acids, a mixture of methyl esters of fatty acids (Sigma-Aldrich Chemicals) was used as standard. Chromatograms for methyl esters of fatty acids and total amounts of fatty acids were obtained using the GC Solution (Version 2.4) computer software. The peaks in the chromatogram of the analysed samples were identified by comparing the retention times of the methyl esters of all fatty acids in the standard. The results are given as qualitative value as % fatty acid. Percentages of fatty acid were compared with SPSS 16 computer program by using one-way analysis of variance (ANOVA). The differences were determined by the TUKEY HSD test. As a result of the statistics, the differences were accepted to be significant when the data was at $P < 0.05$ level.

3. RESULTS

As a result of the analysis, a total of 16 fatty acids were detected. Out of saturated fatty acids, 16:0; out of monounsaturated fatty acids, 18:1n-9; and out of polyunsaturated ones, 18:2n-6 were found to be major fatty acids (Table 2.). Out of saturated fatty acids, amounts of 14:0 were found similar and 3 times more than that of the others in *Orchis anatolica* (2.65%), *Orchis tridentata* (2.71%) and *Anacamptis coriophora* (3.19%). When percent distribution of palmitic acid was considered, it was observed that it ranged between 16.76% and 25.32% and it was the highest in *Anacamptis collina*. Stearic acid was also found to show similar distribution among species. A similar percent distribution was determined in total SFA as well depending on palmitic acid (Table 2.).

When the distribution of monounsaturated fatty acids was considered, it was observed that the major fatty acid is oleic acid. This fatty acid showed the highest distribution as percentage in *Orchis simia* (% 27.33%), *Orchis mascula* subsp. *Pinetorum* (25.16%) and *Orchis spitzelii* (25.66%). In other species, this fatty acid was found to be similar and lower. Palmitoleic acid, which is the other monounsaturated fatty acid, was determined to be at the lowest percent in *Orchis simia* (2.38%), while it was at the highest percentage in *Orchis tridentata* (11.48%) and *Anacamptis coriphora* (12.77%). In other species, their percentages were close to each other. Percent distribution of ∑MUFA was found to be close to each other (Table 2.), depending on oleic and palmitoleic acid.

When the distribution of polyunsaturated fatty acid was considered, it was observed that the major fatty acid was linoleic acid. The other fatty acids were in trace amounts. This fatty acid showed different distribution among species as well. It was the lowest in *Orchis tridentata* (15.13%), whereas *Orchis mascula* subsp. *pinetorum* (34.02%) contained the highest percent. It was close to each other in other species. The other polyunsaturated one, 18:3n-3 (Linolenic acid) was found to be six times more than the others as percentage in *Anacamptis collina*. Amount of Eicosapentaenoic acid (EPA) was the highest in *Orchis tridentata* (7.95%) and *Orchis spitzelii* (5.37%), while the other species exhibited a lower and similar distribution. The amount of docosahexaenonic asit (DHA) was the highest in *Orchis tridentata* (9.82%). This ratio was found to be between 3.09% and 5.25% and close to each other in the other species. The amount of total PUFA was the highest in *Orchis spitzelii* (47.81%), while it was the lowest in *Anacamptis coriophora* (36.75%), depending on 18:2n-6, 20:5n-3 and 22:6n-3 (Table 2.).

Table 2. Fatty acid distribution of orchids species

Fatty acid	*Orchis anatolica* (Mean±S.D) *	*Orchis tridentata* (Mean±S.D)	*Anacamptis collina* (Mean±S.D)	*Orchis simia* (Mean±S.D)	*Orchis mascula* subsp *pinetorum* (Mean±S.D)	*Anacamptis coriophora* (Mean±S.D)	*Orchis spitzelii* (Mean±S.D)
14:0§	2.65±0.21	2.71±0.22	0.92±0.07	0.57±0.05	0.75±0.06	3.19±0.25	0.89±0.07
15:0	0.38±0.03	0.39±0.03	0.51±0.04	1.34±0.11	0.27±0.02	0.42±0.03	0.33±0.03
16:0	20.26±1.62	17.70±1.41	25.32±2.02	23.48±1.87	17.37±1.39	20.22±1.61	16.76±1.34
17:0	0.08±0.01	0.38±0.03	0.11±0.01	0.06±0.00	0.10±0.01	0.28±0.02	0.13±0.01
18:0	3.16±0.25	4.42±0.35	3.30±0.26	4.16±0.33	3.32±0.27	3.94±0.31	3.48±0.28
∑S.F.A	26.53±2.12	25.60±2.04	30.16±2.41	29.61±2.36	21.82±1.74	28.05±2.24	21.58±1.72
16:1n-7	7.87±0.63	11.48±0.92	5.04±0.40	2.38±0.19	5.54±0.44	12.77±1.02	4.66±0.37
18:1n-9	24.68±1.97	21.99±1.75	22.02±1.76	27.33±2.18	25.16±2.01	22.18±1.77	25.66±2.05
20:1n-9	0.11±0.01	0.26±0.02	0.17±0.01	0.26±0.02	0.31±0.02	0.24±0.02	0.28±0.02
∑M.U.F.A	32.66±2.61	33.73±2.69	27.24±2.17	29.97±2.39	31.00±2.47	35.19±2.81	30.61±2.44
18:2n-6	32.50±2.59	15.13±1.21	27.29±2.18	28.75±2.29	34.02±2.71	24.97±1.99	32.55±2.60
18:3n-3	0.63±0.05	1.00±0.08	6.82±0.54	1.09±0.09	0.66±0.05	0.94±0.07	0.66±0.05
20:2n-6	0.07±0.01	0.28±0.02	0.16±0.01	0.05±0.00	0.19±0.02	0.19±0.02	0.10±0.01
20:3n-6	0.12±0.01	0.53±0.04	0.26±0.02	0.15±0.01	0.33±0.03	0.26±0.02	0.17±0.01
20:4n-6	1.12±0.09	1.54±0.12	0.70±0.06	0.60±0.05	1.10±0.09	0.76±0.06	1.31±0.10
20:5n-3	2.52±0.20	7.95±0.63	2.83±0.23	3.62±0.29	3.53±0.28	3.33±0.27	5.37±0.43
22:5n-3	0.76±0.06	4.42±0.35	0.97±0.08	1.12±0.09	2.10±0.17	1.71±0.14	2.45±0.20
22:6n-3	3.09±0.25	9.82±0.78	3.58±0.29	5.05±0.40	5.25±0.42	4.60±0.37	5.21±0.42
∑P.U.F.A	40.81±3.26	40.67±3.24	42.60±3.40	40.43±3.23	47.18±3.76	36.75±2.93	47.81±3.81
ω3	7.00±0.56	23.19±1.85	14.19±1.13	10.88±0.87	11.55±0.92	10.57±0.84	13.68±1.09
ω6	33.81±2.70	17.48±1.39	28.40±2.27	29.55±2.36	35.64±2.84	26.18±2.09	34.13±2.72
ω3/ω6	0.21	1.33	0.50	0.37	0.32	0.40	0.40

* Each data is the average of three repeats. Three injections were performed per repetition.
§ The data determined with the same letters in each line are not different from each other in the probability level P> 0.05.
S.D.: Standard deviation, S.F.A.: Saturated Fatty Acids, M.U.F.A.: Monounsaturated Fatty Acids, P.U.F.A.: Polyunsaturated Fatty Acids.

As a result of the analysis, amount of linoleic acid, which is the major fatty acid, (18:2n-6) was 15.41- 34.33%; the amount of EPA and DHA were found to be slightly higher than the others in *Orchis tridentata* and that of linolenic acid (18:3n-3) was also slightly higher in *Anacamptis collina*. The ratio of total n-3 was between 7.00-23.19%, that of ∑n-6 was between 17.48-35.64% and that of n-3/n-6 was found to be between 0.21-1.33. The highest fatty acid was PUFA, then MUFA and the lowest one was SFA in *Orchis anatolica*, *Orchis tridentata*, *Orchis mascula* subsp. *pinetorum* and *Orchis spitzelii*; the highest one was PUFA, then SFA and the lowest one was MUFA in *Anacamptis collina* and *Anacamptis coriophora*; the highest one was PUFA, while the ratios of SFA and MUFA were similar in *Orchis simia* (Table 2.).

4. DISCUSSION AND CONCLUSION

In a study carried out by Citil and Tekinsen (2011) , fatty acids of saleps obtained from *O. palustris*, *O. anatolica*, *O. coriophora*, *O. tridentata*, *O. italica*, *O. morio*, *D. osmanica* var. *osmanica*, *S. vomeracea* ssp. *orientalis* were analyzed. As a result of analysis carried out on eight different wild orchid species from which salep was obtained, a total of 32 fatty acids were determined, and linoleic acid was found mostly in *O. italica* (59.90%), palmitic acid in *O. anatolica* (33.78%), oleic acid in *O. palustris* (28.65%), stearic acid in *S. vomeracea ssp. orientalis* (14.20%) and myristic acid was found mostly in *O. anatolica* (10.47%). In another study, as a result of analysis carried out on *Dactylorhiza fuchsii* and *Grammatophyllum specios*, a total of eight fatty acids were determined. 16:0 was found to be 14.25%; 18:1n-9 was 13.73%; and 18:2n-6 was 69.82% on average in *Dactylorhiza fuchsia*. In the same study, 16:0 was found as 9.37%; 18:1n-9 was 5.08%; and 18:2n-6 was 81.21% on average in

Grammatophyllum speciosum (Colville, Marks, Pritchard, Custódio, & Machado-Neto, 2016). In our study, a total of 16 fatty acids were determined. As a result of our analysis, it was determined that linoleic acid was higher in *O. anatolica*, *O. mascula subsp pinetorum* and *O. spitzelii* compared to the other species, however it was lower than that found (*O. italic* 59.90%; *O. morio* 41.94%) by Citil and Tekinsen (2011). This is because the analysis was carried out on the seed in our study. In our study, the percentage of palmitic acid was the highest in *Anacamptis collina*, not in *O. anatolica*, and this value was lower than that found by Citil and Tekinsen (2011). Oleic acid in our study was found to be higher in *Orchis simia*. The amount of stearic acid was not different among species and was at a similar rate. On the other hand, myristic acid was the highest among analyzed ones in *O. anatolica*, *O. tridentata* and *A. coriphora*, however, it was at a lower percentage compared to that found by Citil and Tekinsen (2011). As in the study of Colville et al. (2016), 18:2n-6 was found as the highest percentage in all species, however, it was lower than that study. It is thought that this difference is due to the difference among the species.

In their analysis, Citil and Tekinsen (2011) found 16:0, 18:1n-9 and 18:2n-6; in the study carried out by Holman et al. (1972) found 16:0, 18:2n-6 and 18:3n-3; Louise et al. (2015) found 16:0, 18:1n-9 and 18:2n-6 as major fatty acids. Furthermore, unlike most plants, they found the existence of fatty acids with 20 carbons in both studies. Similar major fatty acids were found in our study. In the analysis carried out on the samples of salep, order of fatty acids was found to be SFA>MUFA>PUFA in *O. palustris* and *O. anatolica*; PUFA>SFA>MUFA in *O. italica* and *O. morio*; PUFA>SFA>MUFA in *O. coriophora*, *O. tridentata*, *D. osmanica var. osmanica* and *O. vomeracea ssp. orientalis* (Citil & Tekinsen, 2011). In our study, we found that the distribution among species differed similarly. In the study conducted on eight wild orchid species in Salep samples, the percentage of omega-3 was in the range of 1.04-8.22%. They found that the distribution of omega-6 fatty acids changed between 7.25-60.55%, depending on the species. They determined the ratio of Omega-3/Omega-6 was in the range of 0.09-0.43. In the study, they reported that the ratio of Omega-6 to Omega-3 was below 4 in *O. tridentata* and *D. osmanica var. osmanica* species reported that they are in *osmanica* species (Citil & Tekinsen, 2011). In our study, it was found that the ratio of n-3/n-6 was similar in the other species except for *Orchis tridentata* (1.33).

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