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 salesp 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.

| 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 °C; injector temperature: 250 °C; 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 °C, standby time, 2 min; 2 °C/min to 210 °C, 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 coriophora (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. 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 docosahexaenoic 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.).
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 linoleic 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%), palmatic 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 speciosum*, 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 fuchsii*. 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

### 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                  |
| o3        | 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                  |
| o6        | 33.81±2.70                     | 27.18±1.39                     | 28.40±2.27                      | 29.55±2.36               | 35.64±2.84                              | 26.18±2.09                     | 34.13±2.72                  |
| o3/o6     | 0.21                           | 1.33                           | 0.50                            | 0.37                     | 0.32                                    | 0.40                          | 0.40                        |
Grammaratophyllum speciosum (Colville, Marks, Pritchard, Custódio, & Machado-Neto, 2016). In our study, a total of 16 fatty acids were determined. As our result of our analysis, it was determined that linoleic acid was higher in O. anatolica, O. mascula subsp. pinetorum and O. spitzeli 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. coriophora, 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. italic and O. morio; PUFA>SFA>MUFA in O. coriophora, O. tridentata, D. osmanica var. osmanica and O. vormacea 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).

REFERENCES

Arditti, J., & Ghani, A. K. A. (2000). Tansley Review No. 110. Numerical and physical properties of orchid seeds and their biological implications. New Phytologist, 143(3), 367-421. doi:10.1046/j.1469-8137.2000.00587.x

Baytop, T. (1984). Türkiye’de Bitkiler ile Tedavi, Geçmişte ve Bugün. (Vol. II. Baskı). Nobel Tıp Kitabevleri: İstanbul

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol, 37(8), 911-917. doi:10.1139/o59-099

Citil, O. B., & Tekinsen, K. K. (2011). A comparative study on fatty-acid composition of salep obtained from some Orchidaceae species. Chemistry of Natural Compounds, 46(6), 943-945. doi:10.1007/s10600-011-9789-4

Colville, L., Marks, T. R., Pritchard, H. W., Custódio, C. C., & Machado-Neto, N. B. (2016). Development of a reliable GC-MS method for fatty acid profiling using direct transesterification of minimal quantities of microscopic orchid seeds. Seed Science Research, 26(1), 84-91. doi:10.1017/S0960258515000367

Dogan, M., & Kayacier, A. (2004). Rheological Properties of Reconstituted Hot Salep Beverage. International Journal of Food Properties, 7(3), 683-691. doi:10.1081/JFP-200033093

Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., & Adıgüzel, N. (2000). Türkiye Bitkileri Kırmızı Kitabı (Vol. Yayın No: 18). Ankara.

Holman, R. T., & Nichols, P. C. (1972). Characterization of the lipids of some orchids. Phytochemistry, 11(1), 333-337. doi:https://doi.org/10.1016/S0031-9422(00)90011-6

Jacicot, B. (1995). El aceite de oliva: alimento medicinal. De aceites y grasas. Oleaginosos. 19, 149-152.

Kreutz, C. A. J. (1998). Die Orchideen der Türkei. Cip-Gegevens Koninklijke Bibliotheek: Den Haag.
Nettleton, J. A., & Exler, J. (1992). Nutrients in Wild and Farmed Fish and Shellfish. Journal of Food Science, 57(2), 257-260. doi:10.1111/j.1365-2621.1992.tb05470.x

Renz, J., & Taubenheim, G. (1984). Orchidaceae. In Flora of Turkey and the East Egean Islands (Davis, P.H., Mill, R., Tan, K. eds.) (Vol. 8). Edinburgh University Press: Edinburgh.

Sandal, G., & Söğüt, Z. (2010). Türkiye Orkideleri (Salepleri). Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 23(2), 109-116.

Saraçoğlu, H., Zengin, G., Akin, M., & Aktümsek, A. (2012). A comparative study on the fatty acid composition of the oils from five Bupleurum species collected from Turkey. Turkish Journal of Biology, 36, 527-532. doi:10.3906/biy-1112-51

Sezik, E. (1984). Orkideleminiz. Sandoz Kültür Yayınları: İstanbul.

Sezik, E. (2002a). Destruction and Conservation of Turkish Orchids. In Ş. Bilge (Ed.), Biodiversity Biomolecular Aspects of Biodiversity and Innovative Utilization (pp. 391-400): Springer, Boston, MA.

Sezik, E. (2002b). Türkiyenin Orkideleri ve Salep. Acta Pharmaceutica Turcica, 44, 151-157. Retrieved from http://www.actapharmasci.com/uploads/pdf/pdf_264.pdf

Sezik, E., & Baykal, T. (1988). Maraş Salebinin Menşei ve Maraş Cıvarıın Orkideleri. TÜBİTAK Projesi, TBAG-664: Ankara.

Stevens, P. F. (2020). Angiosperm Phylogeny Website Version 14. Retrieved from http://www.mobot.org/MOBOT/Research/APweb/welcome.html

Tamer, A., Yildiz, S., Yildiz, N., Kanat, M., Gunduz, H., Tahtaci, M., & Celebi, H. (2006). The prevalence of neuropathy and relationship with risk factors in diabetic patients: a single-center experience. Med Princ Pract, 15(3), 190-194. doi:10.1159/000092180

Telcioglu, A., & Kayacier, A. (2007). The effect of sweeteners and milk type on the rheological properties of reduced calorie salep drink. African Journal of Biotechnology (ISSN: 1684-5315) Vol 6 Num 4, 6.

Turgay, Ö., & Çınar, İ. (2017). Salep: The Name of The Plant, Powder, Hot Beverage, Food Ingredient. KSU. Journal of Engineering Sciences, 20(3), 68-71 doi:https://doi.org/10.17780/ksujes.341382

Woltering, E. J., Van Hout, M., Somhorst, D., & Harren, F. (1993). Roles of pollination and short-chain saturated fatty acids in flower senescence. Plant Growth Regulation, 12(1), 1-10. doi:10.1007/BF00144575