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A COMPARATIVE STUDY ON FATTY-ACID COMPOSITION OF SALEP OBTAINED FROM SOME Orchidaceae SPECIES

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Salep is a flour milled from the dried tubers of some wild perennial Orchidaceae species, some of which have double-root tubers [1, 2]. As a raw material of some drugs and food, salep is obtained from tubers of orchids growing naturally in many regions of Turkey. Some species of salep orchids grow only in Turkey. Salep is a well-known indigenous Turkish drink whose main ingredient is also called by the same name (salep powder). Salep is also an ingredient of traditional Kahramanmaras-type of ice cream [3] and is used in the medical treatment of summer diarrhea of babies and children, and for chronic diarrhea in adults [4].

In Turkey salep is obtained from at least 30 species of 8–10 different genera of Orchidaceae [1–7]. Tubers of *Anacamptis pyramidalis, Dactylorhiza romana*, *D. osmanica* var. *osmanica*, *Himantoglossum affine*, *Ophrys fusca*, *Oph. holosericea*, *Oph. mammosa*, *Orchis anatolica*, *O. coriophora*, *O. italica*, *O. mascula* ssp. *pinetorum*, *O. morio*, *O. palustris*, *O. simia*, *O. spitzelii*, *O. tridentata*, and *Serapias vomeracea* ssp. *orientalis* are commonly used in production [8, 9]. For 1 kg of salep, 1000–4000 dried tubers, each of which weighs 0.25–1.00 g, are required [10].

Salep is known to be a valuable source for glucomannan. Glucomannans are natural neutral water-soluble fibers, which help to normalize blood sugar, relieve stress on the pancreas, and prevent blood sugar abnormalities, such as hypoglycemia [2]. The functions of salep depend on the chemical composition, especially the glucomannan level, which varies according to the species. Salep does not have a standard chemical composition [1–11]. Sezik [8] reported that, depending on the species, salep contained 7–61% glucomannan, 1–36% starch, 0.5–1% nitrogenous substances, 0.2–6% ash in the form of dry material, and 6–12% moisture.

According to literature information, the fatty-acid composition of salep has not been investigated. The aim of this study is to determine the fatty-acid compositions and ω6/ω3 ratios of the salep samples obtained from some Orchidaceae spp. grown in Anatolia and to compare species.

The lipid content averages 2.02% in salep samples obtained from Orchidaceae species. The fatty-acid compositions of parts of the salep samples and retention times are presented in Table 1. We identified 32 fatty acids from the salep samples and evaluated their compositions for species. The highest fatty acid ratios are as follows; linoleic acid 18:2ω6 (59.90%) in *O. italica*, palmitic acid 16:0 (33.78%) in *O. anatolica*, oleic acid 18:1 (28.65%) in *O. palustris*, stearic acid 18:0 (14.20%) in *S. vomeracea* ssp. *orientalis*, and myristic acid 14:0 (10.47%) in *O. anatolica*. Linoleic and palmitic acids are the most abundant unsaturated and saturated fatty acids in all parts, respectively. The total SFA (saturated fatty acid) composition of the studied species is assigned between 24.67–61.24%, while the PUFA (polyunsaturated fatty acid) composition is 9.27–66.04%.

Palmitic acid is mostly found in leaf and the major SFA, contributing approximately 47.29–68.83% to the total SFA content. The level of MUFA (monounsaturated fatty acid) depends on the level of oleic acid. The greatest proportion of oleic acid is found in *O. palustris*. EC reported that erucic acid 22:1ω9 in vegetable oils must be at a maximum value of 5.0% for human health [12]. In this study, erucic acid was found to be between 0 and 1.31% in all species.

The long-chain ω3 and ω6 fatty acids are commonly called PUFAs. Long-chain ω3 PUFAs cannot be readily synthesized by the human body and are mostly obtained through the diet, and ratios of ω3/ω6 are considered to be important [13, 14].

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TABLE 1. Fatty-Acid Composition of Tuber Salep Samples Obtained from Orchidaceae Species

Fatty acid	1	2	3	4	5	6	7	8	Rt
8:0	0.63±a	0.69±a	0.00±c	0.00±c	0.00±c	0.34±b	0.00±c	0.00±c	0.89
9:0	0.00±b	0.21±a	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	1.152
10:0	0.00±f	3.10±a	0.00±f	0.91±c	0.00±f	0.49±d	0.00±f	1.12±b	1.283
12:0	2.65±a	0.04±h	0.53±f	1.27±c	0.36±g	1.07±d	0.90±e	2.09±b	1.875
13:0	0.30±c	0.70±b	0.00±d	0.63±b	0.57±b	0.00±d	0.00±d	1.73±a	2.338
14:0	8.63±b	10.47±a	3.83±e	4.74±d	1.68±f	3.37±e	3.83±e	5.42±c	2.874
15:0	1.38±a	0.00±f	0.00±f	0.79±c	0.17±e	0.42±d	0.91±b	0.82±c	3.584
16:0	27.81±c	33.78±a	27.01±cd	26.41±d	16.98±g	23.02±f	29.04±b	25.05±e	4.605
17:0	0.34±b	0.28±bc	0.00±d	0.44±b	0.16±c	0.28±bc	0.72±a	0.74±a	5.854
18:0	12.40±b	11.62±c	6.66±f	7.51±e	4.16±g	8.10±e	10.73±d	14.20±a	7.593
21:0	0.28±cd	0.33±c	1.61±a	0.19±de	0.11±ef	0.16±de	0.00±f	0.56±b	16.757
22:0	0.00±b	0.02±b	0.25±a	0.00±b	0.00±b	0.02±b	0.00±b	0.00±b	21.970
24:0	0.08±c	0.00±c	0.00±c	0.51±b	0.48±b	0.00±c	0.00±c	1.24±a	39.301
ΣSFA	54.50	61.24	39.89	43.40	24.67	37.27	46.13	52.97	
14:1	1.47±b	2.63±a	0.00±e	0.67±c	0.34±d	0.29±d	2.75±a	0.54±c	3.456
15:1	0.38±a	0.00±d	0.00±d	0.42±a	0.00±d	0.24±b	0.11±c	0.19±b	4.037
16:1	3.40±b	2.78±b	6.73±a	1.45±cd	0.00±e	0.30±e	1.21±d	1.88±c	5.157
17:1	0.34±a	0.00±d	0.00±d	0.17±bc	0.10±cd	0.28±ab	0.19±bc	0.26±ab	6.222
18:1	28.65±a	23.77±b	14.44±cd	13.92±d	7.59±f	10.90±e	12.00±e	15.17±c	8.801
20:1	0.55±a	0.32±b	0.00±d	0.00±d	0.00±d	0.02±d	0.15±c	0.00±d	14.649
22:1	0.79±c	0.00±f	0.13±e	1.31±a	1.27±a	1.08±b	0.09±e	0.29±d	24.918
24:1	0.00±a	0.00±b	2.17±b	0.30±b	0.00±b	0.00±b	0.00±b	0.00±b	41.800
ΣMUFA	35.58	29.50	23.47	18.24	9.30	13.11	16.50	18.33	
14:2	0.00±b	0.34±a	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	3.482
16:2	0.81±a	0.64±a	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	5.369
18:2	7.42±g	6.35±h	31.96±c	29.77±d	59.90±a	41.94±b	23.83±e	21.56±f	10.818
18:3	0.81±d	0.47±d	3.22±c	3.63±c	4.69±b	5.38±a	5.35±a	4.34±b	16.149
20:2	0.05±fg	0.13±ef	0.00±g	0.30±d	0.20±de	0.66±c	1.81±a	1.61±b	19.068
20:3	0.26±e	0.29±de	0.29±de	0.76±b	0.40±d	0.37±de	2.13±a	0.55±c	20.886
20:4	0.11±d	0.07±d	0.00±e	0.07±d	0.45±b	0.18±c	0.60±a	0.01±e	23.156
20:5	0.14±de	0.28±cd	0.15±de	0.73±b	0.40±c	1.12±a	1.25±a	0.00±e	29.315
22:2	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	0.00±b	0.65±a	30.717
22:4	0.32±b	0.70±a	0.00±c	0.00±c	0.00±c	0.00±c	0.00±c	0.00±c	36.431
22:5	0.01±c	0.00±c	1.00±b	3.10±a	0.00±c	0.00±c	2.41±a	0.00±c	38.001
ΣPUFA	9.93	9.27	36.62	38.36	66.04	49.65	37.38	28.72	
ω3	1.22	1.04	4.66	8.22	5.49	6.87	11.14	4.89	
ω6	7.90	7.25	31.96	30.14	60.55	42.78	26.24	23.18	
ω3/ω6	0.15	0.14	0.15	0.27	0.09	0.16	0.43	0.21	
ω6/ω3	6.48	6.97	6.86	3.67	11.03	6.23	2.36	4.74	
SFA/PUFA	5.49	6.61	1.09	1.13	0.37	0.75	1.23	1.84	

1 – *O. palustris*; 2 – *O. anatolica;* 3 – *O. coriophora;* 4 – *O. tridentata;* 5 – *O. italica;* 6 – *O. morio;* 7 – *D. osmanica* var. *osmanica;* 8 – *S. vomeracea* ssp. *orientalis.*

Rt: retention time (millivolt signal).

The highest value in the same line is denoted by a, the lowest value is denoted by h, and values between a and h are denoted by other letters. If the value has a denotation of ab, bc, cd, de, ef, fg (or gh), it is found in the range of a and b, b and c, c and d, d and e, e and f, and f and g (or b and c).

a, b, c, d, e, f, g, h values for each sample with different letters in the same fraction are significantly different at $P < 0.05$.

Dyerberg noted that an increase in the ratio of ω3/ω6 PUFA increased the availability of ω3 PUFAs, which are beneficial for human health [15]. The lowest linoleic acid content (59.90%) was found in *O. italica*. Nutritionists suggest that ω3 fatty acids must be in greater amounts in human diet. Therefore, they reported that the ω6/ω3 ratio must be below 4.0 for human health [16]. In the present study, the ω6/ω3 ratio is found to be 3.67% in *O. tridentata* and 2.36% in *D. osmanica* var. *osmanica*.

Collection of Samples. Eight salep samples were collected from the Eastern Mediterranean Region of Anatolia in order to determine the fatty-acid compositions for *O. palustris*, *O. anatolica*, *O. coriophora*, *O. tridentata*, *O. italica*, *O. morio*,

D. osmanica var. *osmanica*, and *S. vomeracea* ssp. *orientalis*. Twenty samples from each of the eight most widespread Orchidaceae species (*O. palustris*, *O. anatolica*, *O. coriophora*, *O. tridentata*, *O. italica*, *O. morio*, *D. osmanica* var. *osmanica*, and *S. vomeracea* ssp. *orientalis*) were collected. Identification of the species of orchids collected was carried out according to the usual procedures, based on methods suggested by Sezik [1], at the Herbarium of the Department of Biology at Selcuk University.

Preparation of the Samples. Salep powder was obtained by the traditional method from the tubers of each species [8–11]. Thus, after washing with cold water, the samples were boiled in milk for 10–15 min; then dried in the shade at 21 ± 2°C for 7–10 days until they had hardened. The tubers were then cut into small fragments, which were ground in a mill.

Fatty-Acid Analysis. Fat extraction was carried out according to the AOAC [17]. Each part of about 5 g was extracted with chloroform–methanol mixture (2:1, v/v) [18]. The fatty acids were converted to their methyl esters using the standard boron trifluoride-methanol method [19]. The resultant fatty acid methyl esters were separated and stored at –20°C. At the beginning of each analysis, the samples were allowed to equilibrate to room temperature and analyzed by gas-liquid chromatography (Shimadzu 15-A), equipped with a dual flame ionization detector and a 1.8 m × 3 mm internal diameter packed glass column containing GP 10% SP-2330 on 100/120 Chromosorb WAW, Cat. No. 11851. Column temperature was 190°C for 31 min, rising progressively at 30°C/min up to 220°C where it was maintained for 10 min at 220°C. Carrier gas was nitrogen (2 mL/min). The injector and detector temperatures were 225 and 245°C, respectively. Conditions were chosen to separate fatty acids of carbon chain length from 8 to 24. The fatty acids were identified by comparison of retention times with known external standard mixtures (Alltech), quantified by a Shimadzu C-R4A integrator, and the results were expressed as percentage distribution of fatty acid methyl esters. Each of the experiments was repeated three times.

Statistical Analysis. Eight salep samples were analyzed for each part of the species in triplicate. The average results of peak area are expressed as means ± SD. Statistical analysis of the percentages of fatty acid was performed by analysis of variance (ANOVA), and comparisons between the mean values were performed using Duncan′s test. Differences between means were reported as significant if $P < 0.05$.

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