Chromatographic Identification of Oil and Amino Acid Constituents in Kernels of Some Almond Varieties

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ABSTRACT AND SUMMARY

The physical and chemical properties of the oils of eight almond varieties are presented. Oils of these varieties showed similar qualitative data concerning the lipid classes separated by thin layer-chromatography. Triglycerides comprised the majority of the total lipid extract, while mono- and diglycerides were found in traces. Gas chromatographic analysis of the methyl esters of the fatty acids revealed that the unsaturated fatty acids contribute 70-90% of the total fatty acids present in the samples. The saturated acids, however, were found in a relatively low concentration (7.0-23.6%), and even a lower percentage of arachidic acid is reported. In addition to the five fatty acids identified, another five unknown acids were separated and their retention times were recorded. One of the unknowns, with a retention time of 2.45 min, is believed to be palmitoleic acid. Eleven free amino acids were separated, identified, and quantitatively determined. Aspartate, asparagine, glutamate, and leucine have major existence. Although the presence of hydroxyproline is unusual in plant materials, it was found among the free amino acids in almond kernels.

INTRODUCTION

With the advent of the accelerated interest in growing different varieties of almond in the West Northern Coast of Egypt, and the demand for such crops for the Egyptian market, it was felt that a current survey of the chemical constituents of recently introduced eight sweet almond varieties would be helpful.

Oils and amino acids within the kernel tissue are of fundamental importance for the evaluation and understanding of these varieties. Vaughan (1) reported that oil from almond kernel is extensively used in various cosmetic and pharmaceutical products. Jacobs (2) mentioned that about half of the edible part of sweet almond is a bland fixed oil. Meara (3) stated that the kernel fat from almond grown near Liverpool differed greatly in chemical composition from almond oil normally obtained from warmer countries, i.e., linoleic acid contents was more than double that recorded elsewhere.

The present investigation was carried out to evaluate the biochemical compositions of eight sweet almond varieties.

MATERIALS AND METHODS

The eight almond varieties under investigation, namely, Aboid, Achack, Marcona B 103, Mazzetto, Mollar 200 R, Non-pareil, Zaaff, and Constantini were imported and planted in 1969 at Maruit, Egypt in calcareous soil under open pollination conditions. At the commercial harvesting time of July 28, 1976, fruit samples of equal size were randomly picked in duplicates and air dried at 28 ± 3 C for about 20 days.

Oil Extraction

Two hundred and fifty grams of kernels of each variety were sliced, blended in a Waring Blendor with 750 ml of $CHCl_3$:MeOH (2:1) for 2 min, and kept in the dark. After 24 hr, new solvent was added for further extraction. The combined extractions were filtered over sufficient amounts of anhydrous sodium sulphate. The solvent was removed gently under vacuum, and the oils were transferred to dark bottles and kept in a refrigerator until they were ready for analysis.

Physico-chemical Analysis

The lipid contents of the investigated almond varieties were determined by means of Soxhlet distillation using petroleum ether for 3 hr.

Specific gravity (d^{26}), and free fatty acids were determined according to the AOAC (4) method. The refractive indices (n^{26}), saponification values and iodine values were determined as described by Jacobs (2).

Chromatography

The lipid extracts were passed through a silicic acid column to separate the neutral lipid fraction according to Rouser et al. (5). One gram of lipid extract was applied in about 2 ml diethyl ether to the column containing 10 g of activated silicic acid. The neutral lipids were eluted with 250 ml of diethyl ether.

Thin Layer Chromatography (TLC) Analysis

The neutral lipid fractions eluted from the silicic acid

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TABLE I

The Physical and Chemical Properties of the Oils Produced from Eight Almond Varieties

Variety name	% Oil g/100 g D.W.	Refractive index n^{26}	Density d^{26}	Saponification value	Acid value	Iodine value
1 Abiod	55.5	1.4720	0.9287	188.0	1.4	94.5
2 Achack	57.9	1.4745	0.9311	189.8	1.2	98.8
3 Marcona	58.7	1.4743	0.9310	190.4	1.2	98.1
4 Mazzetto	54.6	1.4738	0.9281	193.8	1.4	95.2
5 Mollar	57.6	1.4747	0.9239	187.5	1.6	100.7
6 Nonpareil	59.3	1.4742	0.9222	194.2	1.2	97.9
7 Zaaff	53.7	1.4728	0.9199	192.5	1.6	94.8
8 Constantini	59.0	1.4744	–	199.5	1.7	97.9

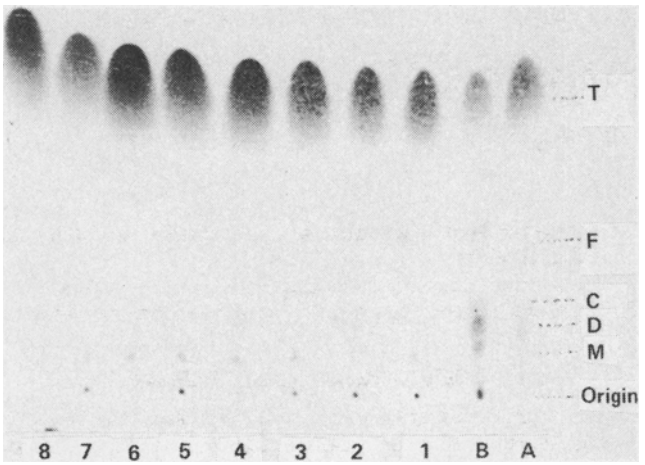

FIG. 1. Thin layer chromatogram of cottonseed oil (A), olive oil (B), and almond varieties (1-8). Solent system:petroleum ether: diethyl ether:acetic acid (80:20:1, v/v). T: triglycerides; F: fatty acids; C:cholesterols; D:diglycerides; M: monoglycerides.

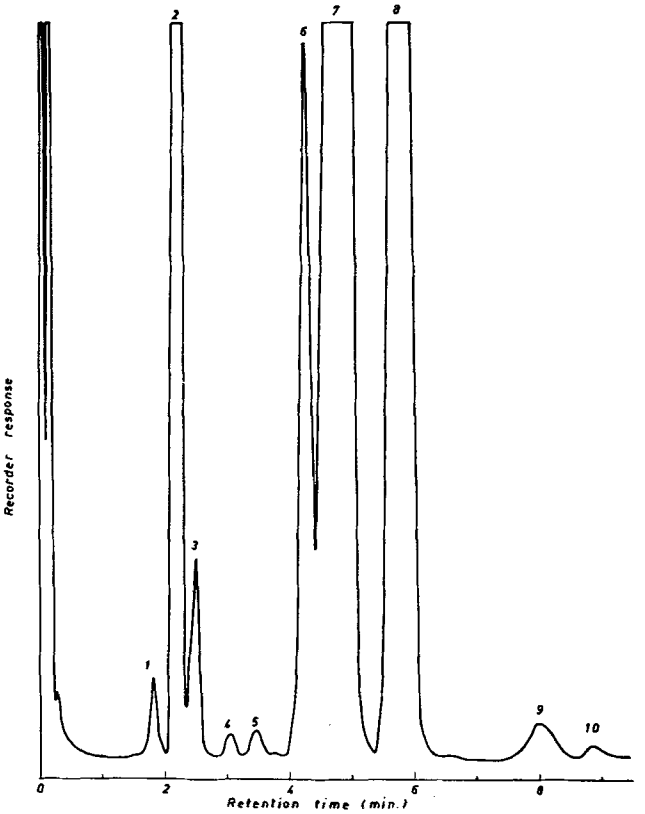

FIG. 2. Gas chromatogram of the fatty acids in the kernel of sweet almond (var. Mazzetto). 1, 3, 4, 5, 10, unknowns; 2, palmitic; 6, stearic; 7, oleic; 8, linoleic; 9, arachidic.

column were subjected to fractionation by means of TLC on activated Silica Gel G plates, 20 x 20 cm. The solvent system used was petroleum ether:diethyl ether:acetic acid (80:20:1, v/v). Iodine vapor was used for the detection of the spots.

Gas Liquid Chromatography (GLC) Analysis

For gas chromatographic analysis, crude methyl esters of the oils were prepared at room temperature, using KOH as a catalyst, similar to the method described by Seelbach and Quackenbush (6). Separation of the fatty acids was then achieved by injecting 2 μl of the methyl ester samples in a Pye Unicom gas chromatograph, Model 104, fitted with flame ionization detector, and a column (150 cm x 4 mm) filled with 10% PEGA on acid washed diatomite (100-120 mesh size). The operating conditions were: N_2 (45 ml/min), H_2 (45 ml/min), detector temperature (220 C); chart speed (2 cm/min).

Amino Acids Determination

Ten-gram samples of sliced almond kernels were extracted with 100 ml of 95% boiling ethanol for 1 hr on a steam bath. The supernatant was decanted and second and third extractions were carried out with 80% and 50% ethanol. Combined ethanolic extracts and plant materials were filtered through a Buchner funnel with several distilled water washings. A volume equivalent to 1 g of sample material from this extract was taken and acidified to pH 1-2, then shaken with 5 g of Dowex 50 W-X8 cation exchange resin (50-100 mesh size) to remove the negative and neutral fractions. The resin was filtered and washed several times with distilled water. The positively charged components including the free amino acids were then extracted from the resin by 3N-NH_4OH followed by water washings. The volume of the combined ammonia extract and washings was reduced to dryness on a water bath at 60 C with a current of air. The amino acids were then separated by one-dimensional paper chromatography with two developments ascendingly. The solvent system was n-butanol:acetic acid:water (4:1:5, v/v). Amino acids were identified on the chromatogram with the aid of authentic amino acids separated under the same conditions. Also, the color and shape of the spots were a helpful guide for such identification. Each sample was spotted in triplicate on the same chromatogram, one of them was sprayed after separation with 0.2% ninhydrin in acetone to locate the position of each amino acid. The individual amino acids separated from the other two spottings were cut and taken in test tubes for quantitative determination by Rosen's (7) method. The same method was also used for the determination of total amino nitrogen.

RESULTS AND DISCUSSION

The physical and chemical properties of the oils produced from almond varieties are presented in Table I. The lipid contents of the investigated varieties ranged between 53.7% (Zaaff) and 59.3% (Non-pareil). It is worth mentioning that although Non-pareil has the highest oil content, it showed the least acid value (1.2). Mollar and Constantini have the highest acid values, 1.6 and 1.7, respectively. The saponification values, the iodine values, and acid values fall within the range reported by other investigators (8,9). The refractive indices of the tested oils of the almond varieties are quite similar (n^{26}) = 1.4720-1.4745.

The actual chromatogram presented in Figure 1 shows the separation of the natural oils produced from the different almond varieties as compared with cottonseed and olive oils. This separation revealed the presence of at least four lipid classes. Triglycerides comprised the majority of the total lipid extract; mono- and diglycerides were found in traces. Almond oil in this respect is quite different from olive oil, since the latter contained a noticeable amount of mono-, diglycerides, cholesterols, and fatty acids. Trace amounts of mono- and diglycerides in almond oil were also found by Heiduschka and Wiesemann (10).

Fatty Acids

A gas chromatographic method was used to study the fatty acid constituents of the oils extracted from the kernels of different almond cultivars. A column of 1% PEGA on washed diatomite was quite suitable. Identification of the acids was carried out with authentic samples

TABLE II

Relative Concentration of Fatty Acids in Kernel of Sweet Almond Varieties Calculated from the Gas Chromatogram

Number	Compound name	Rt min	Relative Concentration %						
			Abiod	Achack	Marcona	Mazzetto	Mollar	Nonpareil	Zaaff
1	Unknown	2.75	1.8	0.9	1.9	0.4	0.6	0.3	0.9
2	Palmitic acid	2.10	13.2	6.3	6.5	11.2	4.3	9.3	10.0
3	Unknown	2.45	3.0	1.5	1.2	1.4	1.2	1.1	0.8
4	Unknown	2.95	0.6	0.2	0.4	0.2	0.3	0.2	0.2
5	Unknown	3.33	1.2	0.4	0.6	0.3	0.2	0.3	0.5
6	Stearic acid	4.20	10.4	3.9	3.1	5.1	2.7	2.6	7.0
7	Oleic acid	4.68	43.3	54.4	53.1	51.5	56.3	57.6	60.5
8	Linoleic acid	5.73	26.3	31.8	32.9	29.3	33.9	28.2	20.1
9	Arachidic acid	8.00	0.1	0.3	0.4	0.5	0.3	0.3	0.1
10	Unknown	8.80	0.1	0.2	0.1	0.3	0.2	0.2	0.0

TABLE III

The Free Amino Acids in the Kernel of the Sweet Almond Varieties

Amino acid	μg/g F.W.							
	Aboid	Achack	Marcona	Mazzetto	Mollar	Nonpareil	Zaaff	Constantini
α-Alanine	69	21	53	26	32	62	24	51
Arginine	26	16	34	8	29	32	13	24
Aspartic acid	310	117	308	113	130	287	99	185
Asparagine	168	49	130	44	84	149	41	128
γ-Amino BA	61	29	48	15	32	52	26	42
Glutamic acid	142	35	104	30	43	112	25	44
Leucine	76	72	61	58	52	69	61	86
Methionine	26	16	51	13	13	14	11	21
Proline	42	12	35	15	15	36	14	28
OH - Proline	82	29	66	35	49	61	29	44
Valine	13	4	23	3	8	18	9	8
Total amino nitrogen	1470	630	1310	540	710	1340	520	980

under the same conditions of GC-separation. The chromatographic pattern of the fatty acids was found to be quite similar in all the varieties tested; therefore, a tracing of only one variety (Mazzetto) is presented in Figure 2. The data presented in Table II are the percentages of relative concentrations of these acids as deduced from GC-analysis. The variety Constantini could not be analyzed by GC for technical reasons.

Ten different fatty acids were separated in all samples, five of which were definitely identified as palmitic, stearic, oleic, linoleic, and arachidic. Components Nos. 1, 3, 4, 5, and 10 in Figure 2, are recorded under unknowns in Table II, and have retention times of 1.75, 2.45, 2.95, 3.33, and 8.80 min, respectively. However, component No. 3 is believed, according to its retention time, to be palmitoleic.

Oleic and linoleic acids contributed the major part of the unsaturated acids present in the kernels of various cultivars, i.e., 60.6% for Abiod and 90.2% for Mollar. On the other hand, those identified saturated ones, palmitic and stearic, existed in a relatively low concentration, i.e., 7.0% for Mollar and 23.6% for Abiod, whereas only a trace amount of arachidic was detected (0.1-0.5%).

The differences among the various cultivars are only quantitative and not qualitative in nature. The cultivars Abiod, Zaaff, and Mazzetto showed the highest percentage of palmitic and stearic acids, which was correlated with the lowest percentage of either oleic or linoleic, when compared with the other cultivars. The results on fatty acids are in good agreement with those reported by Hilditch and Williams (11), Gutfinger et al. (12), and Mehran and Filsoof (13).

Free Amino Acids and Total Amino Nitrogen

Table III summarizes the free amino acids, their approximate concentrations, and the total amino nitrogen in the kernels of eight almond varieties grown in Egypt. Eleven amino acids could be detected in measurable amounts in these samples. These acids were identified by chromatography of authentic samples of amino acids. Aspartate, asparagine, glutamate, and leucine comprise large amounts in these cultivars. Valine, arginine, and methionine, on the other hand, existed only in minor quantities.

The cultivars Abiod, Non-pareil, and Marcona, are rich in free amino acids and total amino nitrogen, i.e., 2.5-3 times as much as those found in Zaaff, Mazzetto, and Achack. The existence of hydroxyproline in almond varieties is unusual in plant materials. However, Joslyn and Stepka (14) reported similar results concerning the major amino acids and the presence of hydroxyproline in plums.

It is clear from the above data concerning the major constituents, the fatty acids, the total amino nitrogen, and the free amino acids, that differences were merely quantitative. The implications of these data of the different almond varieties newly introduced and grown in Maruit, Egypt, remain to be determined.

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