Amino Acid Compositions of Three Varieties of Olive Fruit

Athanasios G. Manoukas,* Basilios Mazomenos, and Meropi A. Patrinou

Nitrogen contents and amino acid compositions of olive fruit mesocarp were determined in three Greek varieties (Koroneiki, Throumbolia, and Megaritiki). Nonprotein nitrogen constituted less than 1% of the total nitrogen. All of the common amino acids were present in the free amino acid pool. Arginine, alanine, aspartic acid, glutamic acid, and glycine constituted approximately 60% of the free amino acids. Olive fruit protein contained all of the common amino acids present in other plant proteins. Arginine constituted approximately 25% of all essential amino acids, followed by leucine and valine. Lysine and proline contents were different in all three varieties. Threonine and glycine levels differed only in Megaritiki. All of the other amino acids were present at similar levels.

The qualitative amino acid content of olive fruit, as determined by paper chromatography, has been reported by Narasaki and Katakura (1954) for total amino acids and by Sawas-Dimopoulou and Fytizas (1967) for free amino acids. Apparently no information has been published on the quantitative composition of amino acids in the olive fruit mesocarp.

The amino acid content of olives is of particular importance when considering the nutrition of the larvae of the olive fruit fly (*Dacus oleae*) which in nature feed exclusively in the olive fruit mesocarp. The importance of amino acids in rearing *Dacus oleae* larvae has been emphasized by several investigators (Fytizas and Tzanakakis, 1966a,b; Hagen, 1966; Vakirtzi-Lemonias *et al.*, 1971).

The current work is part of a study of the nutrition and metabolism of the olive fruit fly. Its primary aim was to obtain information on the amino acid composition of olive fruit mosocarp for subsequent nutritional studies with *Dacus oleae*. This paper reports the protein amino acid composition of olive fruit mesocarp collected from three varieties of olive trees. The amino acid composition at two stages of fruit development was investigated in one variety. Data on the free amino acid composition of olive fruit mesocarp were also obtained.

MATERIALS AND METHODS

Plant Material. Olive fruits collected from the Greek varieties Koroneiki, Throumbolia, and Megaritiki of *Oleae europaea* L. were used in this study. A description of the ecology and characteristics of these varieties is given by Anagnostopoulos (1939). Fruits were collected from Koroneiki, located in Crete island on the 13th of November in 1969, from Throumbolia, located in Samos island, on the 26th of November in 1969, and from Megaritiki located in Koropi on the 28th of July and 24th of September in 1970. The same trees were used for both July and September collections. The dates correspond approximately to the period of time during which olive fruits are attacked by *Dacus oleae* (Gmelin). The trees sampled were not irrigated, fertilized, or regularly cultivated. Collection of fruit was made randomly from three to five trees of each variety. An effort was made to obtain olive fruits of the same size and color and presumably of the same stage of development. Olive fruit mesocarp was used in all analyses. The mesocarp samples were stored in a freezer at −20° following delivery of the samples to the laboratory.

Proximate Analysis. Moisture and ash were determined according to the procedures described in the Official Methods of Agricultural Chemists (AOAC, 1955). Nitrogen contents were determined by using a Coleman automated Nitrogen Analyzer Model 29 or a micro-Kjeldahl apparatus. Lipid contents were determined according to the procedure of Folch *et al.* (1957).

Preparation of Amino Acid Samples. Fruit mesocarp samples of approximately 20 g were homogenized in a Virtis 45 homogenizer with 100 ml of a 2:1 chloroform–methanol mixture and the homogenized material was centrifuged. The supernatant was decanted and the residue was resuspended in 100 ml of the same mixture to which 20 ml of water was added. It was again centrifuged and the procedure was repeated two more times. The supernatants from the four extractions were collected and the methanol–water phase was used to determine free nitrogen and amino acids. The lipid free pellet was used for total nitrogen and protein amino acid determinations.

The methanol–water phase (containing nearly 100% of the extractable amino acids) was evaporated to dryness under vacuum at 40° and dissolved in 80% ethanol. Free amino acids were prepared for amino acid analysis by the method of Thomson *et al.* (1959) utilizing 200–400 mesh Dowex 50WX8 ion exchange resin in the H^+ cycle. The amino acid fractions obtained from the ion exchange resin were combined and evaporated to dryness under vacuum at 40°. Because the procedure used in determining amino acids could not separate asparagine and glutamine from threonine and serine, the free amino acid material was dissolved in 20 ml of 6 *N* HCl and hydrolyzed under nitrogen atmosphere at approximately 110° for 4 hr. This hydrolysis resulted in the conversion of asparagine and glutamine into aspartic acid and glutamic acid, respectively.

The lipid free pellet was dried under vacuum at 40°. A small portion of the pellet was used for nitrogen determination and another portion was hydrolyzed with 6 *N* HCl at approximately 110° under nitrogen atmosphere for 24 hr. The hydrolysates were then diluted with water and filtered or centrifuged. An aliquot was dried in a vacuum rotary evaporator. Water was added and the sample was again dried; this was repeated three or four times.

Amino Acid Analysis. To determine the amount of amino acids in each sample, the washed and dried amino acid residues were taken up in an appropriate volume of 0.2 *N* sodium citrate buffer of pH 2.2. One-milliliter aliquots were then used for analysis in a Beckman Model 120C amino acid analyzer. Because tryptophan is destroyed during acid hydrolysis, no determination of this amino acid is reported.

Statistical Analysis. All data, except that for free amino acids, were statistically evaluated according to procedures described by Steel and Torrie (1960). All tests of significance were made at the 0.05 level of probability.

RESULTS AND DISCUSSION

The proximate analyses of the olive fruit mesocarps are presented in Table I and illustrate the proportions of the various components and especially the very low levels of

Greek Atomic Energy Commission, Nuclear Research Center "Democritus," Athens, Greece.

Table I. Proximate Analysis of the Olive Fruit Mesocarp Collected from Three Varieties (g per 100 g Fresh Weight)

Component[a]	Koroneiki[b]	Throum-bolia[c]	Megaritiki[d]	
Moisture	72.5	63.3	77.2	70.7
Lipids	16.8	30.2	4.3	16.1
Ash	1.2	1.2	1.0	1.3
Nitrogen, total	0.32	0.24	0.19	0.20
Nitrogen, free	0.003		0.0004	0.001

[a]Mean of at least three determinations. [b]Collected November 13, 1969. [c]Collected November 26, 1969. [d]Collected July 28, 1970 (first column) and September 24, 1970 (second column).

nonprotein or free nitrogen. The free nitrogen content was considerably higher in Koroneiki than in Megaritiki, which may have been due to differences of the varieties and/or in the time of sampling. Free nitrogen content of Megaritiki collected in September was more than double compared to Megaritiki collected in July, which indicated a difference due to the stage of development of the olive fruit mesocarp. Free nitrogen represented approximately 1.0, 0.2, and 0.5% of the total nitrogen for the November Koroneiki, the July Megaritiki, and the September Megaritiki mesocarp samples, respectively. The coefficient of variation for the mean of free nitrogen values was quite high, ranging from 20 to 50%, contrary to that of all other mean values of Table I, which were within 5 to 10%. Free nitrogen content was not determined in Throumbolia.

The low levels of free nitrogen present required the extraction of large amounts of olive fruit mesocarp for analysis of the free amino acid content. Because of the accumulation of high levels of pigments in the resulting extracts, purification and complete analysis for free amino acids were successful only with the Koroneiki samples. In this case, the higher content of free amino acids (probably associated with the stage of maturity) permitted the satisfactory analysis of two samples. Expressed as percentages of the total free amino acid μmol, the mean values for these samples were: arginine, 9.6; histidine, 0.7; lysine, 1.4; methionine, 1.1; cystine, trace; phenylalanine, 3.0; tyrosine, 2.0; leucine, 5.7; isoleucine, 5.0; threonine, 1.5; valine, 7.9; alanine, 13.1; aspartic acid, 15.6; glutamic acid, 9.9; glycine, 11.1; proline, 4.1; and serine, 8.4. For most of the amino acids individual values varied considerably between the two samples: arginine, alanine, aspartic acid, glutamic acid, and glycine constituted approximately 60% of the free amino acids. Total free amino acid content was quite similar in the two samples analyzed and averaged 1.101 μmol per g of fresh weight. This corresponds to the similarly low content of total free amino acids reported for the leaves of the olive tree (Drossopoulos, 1970). The free amino acid content of the leaves, however, did not include histidine, methionine, cystine, isoleucine, and proline, for which no values were reported. Sawas-Dimopoulou and Fytizas (1967) reported the absence of tyrosine, phenylalanine, leucine, and isoleucine as free amino acids in extracts of Megaritiki olive fruits, which is contrary to the results of the current study. This is probably due to the fact that olive fruits collected in October were used for free amino acid extraction.

A typical chromatogram of a protein hydrolysate sample of olive fruit mesocarp included the 17 amino acids listed in Table II. Three additional compounds were present in most chromatograms. One of them appeared consistently, prior to the appearance of lysine in the basic column. This peak corresponds to one which was also present in hydrolyzed samples of the olive fruit fly, *Dacus oleae* (Manoukas, 1972b). No positive identification of this compound has been made. The other two unidentified peaks appeared just before the appearance of aspartic acid. Cystine was present in some chromatograms but its concentration was in trace amounts. The concentration of ammonia was generally high in all samples.

The amino acid content of olive fruit mesocarp protein in μmol per g of fresh weight is presented in Table II for the Koroneiki and Throumbolia samples and for the samples from the two stages of development in Megaritiki. The data in Table II show that total amino acid concentration is considerably different in the three varieties. This is directly associated with the nitrogen contents of the three varieties (Table I). On the contrary, total nitrogen is similar for the two stages of development in Megaritiki. Table II also shows that aspartic acid, glutamic acid, and arginine were found in the highest concentration in the proteins of all three varieties. These amino acids comprised approximately 30% of the amino acids present. It is clear that arginine, methionine, and threonine were present in the protein hydrolysates of all three varieties examined. This is contrary to the observations of Narasaki and Katakura (1954), who reported that methionine and threonine were not present and that the presence of arginine was uncertain in ripe fruits of the Mission variety.

Table III presents the amino acid composition expressed in g per 16 g of nitrogen (average nitrogen content of protein, 16%). This illustrates the differences in relative concentrations of amino acids among the three varieties. Since no significant differences were found between the two stages of development in each amino acid (Table II), the mean value of all samples analyzed for Megaritiki is presented in Table III. On the basis of statistical analyses for unequal variances and unequal observations, several conclusions can be made. Lysine was the only essential amino acid which differed significantly in all three varieties. The lysine content was 1.85, 5.13, and 7.02 g per 16 g of nitrogen from Throumbolia, Koroneiki, and Megaritiki, respectively. In addition to lysine, threonine is significantly higher in Megaritiki than in Koroneiki and Throumbolia. It will be of interest to know if the differences in lysine and threonine play any important role upon the development of the *Dacus oleae* larvae. Among the nonessential amino acids, glycine in Megaritiki is signifi-

Table II. Amino Acids in Protein Hydrolysates from Three Varieties of Olive Fruit

	μmol/g of fresh weight			
Amino acid	Koroneiki[a]	Throum-bolia[b]	Megaritiki[c]	
Arginine	12.00	7.91	6.30	4.86
Histidine	3.25	2.54	1.26	1.41
Lysine	6.97	1.80	4.11	5.10
Methionine	2.16	1.81	0.84	1.03
Cystine	Trace	Trace	Trace	Trace
Phenylalanine	4.55	2.97	2.09	2.48
Tyrosine	3.33	2.48	1.41	1.54
Leucine	9.97	6.40	4.50	5.68
Isoleucine	5.77	4.01	2.76	3.43
Threonine	5.44	3.76	3.04	3.63
Valine	8.65	5.77	3.81	4.46
Alanine	16.74	14.26	5.00	5.64
Aspartic acid	20.57	14.87	11.60	10.58
Glutamic acid	18.14	15.11	9.44	10.08
Glycine	21.68	20.86	5.05	6.16
Proline	9.32	11.50	2.38	2.89
Serine	7.22	5.90	4.83	5.01
Total	155.76	121.95	68.42	73.98

[a]Mean of three samples collected November 13, 1969. [b]Mean of four samples collected November 26, 1969. [c]Mean of four samples collected July 28, 1970 (figures of first column) and of four samples collected September 24, 1970 (figures of second column).

Table III. Comparison of Amino Acid Composition in the Three Varieties of Olive Fruit

Amino acid	g/16 g of nitrogen ±SEM[a]		
	Koroneiki	Throumbolia	Megaritiki[b]
Arginine	10.17 ± 1.33	9.38 ± 0.30	9.71 ± 0.89
Histidine	2.74 ± 0.91	2.99 ± 0.38	2.34 ± 0.34
Lysine	5.13 ± 0.31	1.85 ± 0.10	7.02 ± 0.57
Methionine	1.29 ± 0.30	1.52 ± 0.37	1.16 ± 0.11
Cystine	Trace	Trace	Trace
Phenylalanine	3.00 ± 0.10	2.76 ± 0.39	3.14 ± 0.20
Tyrosine	2.42 ± 0.35	2.52 ± 0.37	2.22 ± 0.11
Leucine	5.25 ± 1.77	4.71 ± 0.27	5.55 ± 0.37
Isoleucine	3.04 ± 0.83	2.96 ± 0.24	3.28 ± 0.31
Threonine	2.60 ± 0.59	2.51 ± 0.24	3.30 ± 0.29
Valine	4.07 ± 1.11	3.79 ± 0.23	4.03 ± 0.30
Alanine	5.45 ± 0.30	6.49 ± 0.40	3.60 ± 0.16
Aspartic acid	10.99 ± 2.32	11.11 ± 1.73	12.28 ± 0.78
Glutamic acid	10.72 ± 1.61	12.54 ± 0.98	11.85 ± 0.57
Glycine	6.54 ± 1.66	8.79 ± 1.17	3.50 ± 0.38
Proline	4.31 ± 0.62	7.44 ± 1.60	2.70 ± 0.38
Serine	3.05 ± 0.82	3.48 ± 0.53	4.30 ± 0.39

[a]Standard error of the mean of all observations. [b]Mean of samples collected both July and September.

cantly lower than that in Koroneiki and Throumbolia and proline is different in all three varieties. It is not known whether the differences found in the amino acids mentioned above represent any significant differences in the physiology of these varieties. It should be mentioned, however, that these varieties differ in many characteristics, including olive fruit size and olive oil production (Anagnostopoulos, 1939).

The data of this study illustrate clearly the constancy of the protein amino acid composition between the two stages of development of the olive fruit. They also show that the composition does not differ in most amino acids among the three varieties, despite the wide ecological and physiological differences. For most of the amino acids, the composition of the olive fruit is similar to that of several plant products reported by Block and Weiss (1956). Arginine content of olive protein is quite high and similar to that of most oil seed and nut proteins. Conversely, the ratio of glutamic acid to aspartic acid in olive fruit is approximately 1 to 1, which differs from that reported for other plant products (Block and Weiss, 1956). From the results presented it is concluded that the olive fruit mesocarp is not poor in protein quality, as it has been previously reported (Narasaki and Katakura, 1954).

Total protein and nonprotein nitrogen content of the standard artificial diet used in rearing *Dacus oleae* larvae (Tzanakakis *et al.*, 1970; Vakirtzi-Lemonias *et al.*, 1971; unpublished data of this laboratory) was about 4 and 100 times, respectively, the content of olive fruit. This may explain the higher level of nonprotein amino acids found in larvae grown on artificial diets compared to those grown in olive fruit (Manoukas, 1972a). Furthermore, a comparison between the amino acid pattern found in the artificial diet and that of the olive fruit reveals some differences which may have an effect upon the performance of the olive fruit fly. This question is currently being investigated.

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