Fatty Acids of Spinach Chloroplasts [1]

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The photosynthetic apparatus of higher plants consists of discrete cytoplasmic organelles, the chloroplasts, which contain proteins, lipids, pigments, water, and other substances. Relatively little is known, apparently, concerning the composition of the lipid fraction of the chloroplast. Menke (8), studying chloroplasts of spinach, *Spinacia oleracea,* found that essentially all the lipids of the leaf were located in the chloroplasts. The chloroplasts had an average lipid content of 30.9 % of the dry weight, while the cytoplasm had a lipid content of only 0.5 %. For this reason, a number of earlier studies of the lipid content and composition of green leaves as a whole are pertinent to the present problem.

Chibnall and Channon (3), studying the fatty acids of leaves of cabbage, *Brassica oleracea,* found that approximately 10.7 % of the fatty acid fraction consisted of saturated acids, and 89.3 % of unsaturated acids. The saturated fraction was composed of 70 % palmitic acid and 30 % stearic acid. The unsaturated fraction included large quantities of linoleic and linolenic acids. Speer et al. (13) examined the lipids of spinach leaves, finding that 53 % of the fatty acids occurred in the free form, with 47 % as glycerides. The saturated acids consisted chiefly of palmitic and stearic acids. Oleic acid made up 26.3 %, linoleic acid 34.7 %, and linolenic acid 12.7 % of the unsaturated lipid fraction.

The fatty acids of the forage grasses *Dactylis glomerata* and *Lolium perenne* were examined by Smith and Chibnall (12). Saturated acids composed 11 % of the total in Dactylis and 12 % in Lolium; the corresponding figures for oleic acid were 16 and 22 %, for linoleic acid 31 and 26 %, and for linolenic acid 42 and 40 %, respectively.

According to Chibnall (2), cabbage leaves contain 17.5 % glycerides, 12.3 % waxes, 4.5 % sterols, and 18.4 % phospholipids. Comparable figures for cocksfoot grass, *Dactylis glomerata,* were 38.0 % glycerides, 23.5 % waxes, 3.0 % sterols, and 1.5 % phospholipids.

Menke (9) isolated chloroplasts from spinach leaves by centrifugation; he found that their lipid content was approximately 37 %. The ether-soluble fraction of the chloroplasts is a complex mixture of pigments, fatty acids, glycerides, phosphatides, and sterols. Bot (1) isolated grana from chloroplasts of *Lathyrus odoratus* and *Spinacia oleracea.* These were shown to have an ether-soluble fraction, excluding chlorophyll, of 18 to 30 % of the dry weight in sweet pea and 26 to 32 % in spinach. Comar (4) determined the lipid content of spinach chloroplasts as 34 %.

Menke and Jacob (10) extracted isolated chloroplasts of spinach with ether or ether-alcohol mixtures. The extracts were fractionated into a larger acetone-soluble fraction, making up about 80 % of the total, and containing the pigments, triglycerides and sterols, and an acetone-insoluble fraction including phosphatides and waxes. The pigment-free portion of the acetone-soluble fraction consisted of 5.1 % glycerol, 42.2 % fatty acids, 49.0 % triglycerides, and 2.1 % sterols.

Sisakyan and Smirnov (11) found that fatty acids composed 57.8 % of the dry weight of the chloroplasts of sugar beet, 48.8 % in sunflower, and 60.0 % in red clover. Isolated chloroplasts of sunflower incorporated acetate-C^{14} into higher fatty acids in either light or darkness, but bean chloroplasts did not. In manometric experiments, chloroplasts of bean oxidized palmitic, oleic, linoleic, and linolenic acids. Therefore, the chloroplasts are metabolically active in both fatty acid synthesis and oxidation.

Zill and Harmon (14) investigated the chloroplast lipids of spinach by the technique of chromatography on silicic acid columns. Of the crude whole spinach extract 24 % was represented by two monoglycerides, which were not specifically identified. Further studies of chloroplast lipids by these workers are in progress (personal communication).

Debuch (5) has recently analyzed the fatty acid composition of chloroplasts of *Antirrhinum majus,* a plastid mutant of this species, and *Allium porrum* by means of gas chromatography. C_{16} fatty acids composed 10 to 20 % and C_{18} fatty acids composed 80 to 90 % of the total. Linolenic acid was the component present in greatest amount (44.1–71.3 % of the total), linoleic acid (15.0–29.2 %) and palmitic acid (9.5–19.7 %) were the other principal constituents.

The studies reported in this paper were designed to determine the fatty acid composition of chloroplasts obtained from spinach leaves.

Materials & Methods

Fresh spinach leaves obtained from a local distributor were cut into small pieces and homogenized

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for 30 seconds in 0.35 M sodium chloride in a Waring blendor. The homogenate was filtered through cheesecloth and centrifuged for 10 minutes at approximately 100 × *g*. The precipitate was discarded. The combined supernatants of three batches treated in this manner were centrifuged for 10 minutes at 600 × *g*. The supernatant was discarded and the residue was suspended in a minimal volume of 0.35 M NaCl. The operations described above were all performed at 4 C.

To the chloroplast suspension were added 25 ml of 10 % KOH in 90 % ethanol. The mixture was refluxed under nitrogen for 1 hour. Non-saponifiable matter was removed by multiple extractions of the alkaline solution with petroleum ether, and the fatty acids were extracted from the acidified solution by use of petroleum ether. Anhydrous sodium sulfate was used for drying the extract. Aliquots of the extract were treated under nitrogen with diazomethane in order to esterify the fatty acids.

Fatty acid composition was determined by gas-liquid chromatography of the methyl esters in a Barber-Colman instrument equipped with an ionization detector. The column packing was 12.5 % by weight of ethylene glycol-succinate polyester coated on acid-washed 80 to 100 mesh chromosorb W. The column length was 6 feet, and column temperature was 185 C. Argon was used as the carrier gas; the gas flow rate was 160 ml/minute. The amount of each component present in the chromatographed sample was determined by integration of the area under the curve. Identification of the major components was done by comparing retention times with those obtained with pure standards. A portion of the methyl esters was hydrogenated using platinum oxide as catalyst (Farquhar et al., 6). The hydrogenated fatty acids were then analyzed by gas-liquid chromatography as described above.

Polyunsaturated fatty acids were also determined by alkaline isomerization and subsequent ultraviolet spectrophotometry (Holman, 7).

Results

The total fatty acid composition of chloroplasts obtained from spinach leaves as determined by gas-liquid chromatography is given in table I. The fatty acid present in largest quantity (69 % of the total fatty acids) was an 18-carbon fatty acid with a retention time similar to that of linolenic acid. By alkaline isomerization and subsequent ultraviolet spectrophotometry, this material was shown to be a triene. It is assumed that the acid is linolenic acid, but the position of the double bonds was not investigated. Palmitic acid was the only saturated fatty acid present in substantial quantities. An unsaturated fatty acid with relative retention time similar to that expected for a 16-carbon triene was present in amounts equal to about 11 % of the total. Linoleic acid, palmitoleic acid, and oleic acid were present, and five other fatty acids were detected in trace quantities.

Table I

Fatty Acid Composition of Chloroplasts of Spinach

Fatty acid (C atoms: double bonds)		% of Total fatty acids
10:0	(Capric)	Trace
12:1		Trace
14:0	(Myristic)	Trace
16:0	(Palmitic)	11.2
16:1	(Palmitoleic)	3.5
16:2*		Trace
16:3*		10.8
18:0	(Stearic)	Trace
18:1	(Oleic)	1.1
18:2	(Linoleic)	4.6
18:3	(Linolenic)	68.9

* Tentative identification by relative retention time.

To determine more definitely the ratio of 16-carbon to 18-carbon fatty acids in the chloroplasts, an aliquot of the total fatty acids was hydrogenated, using platinum oxide as catalyst; the hydrogenated sample was analyzed by gas-liquid chromatography. Two major components were obtained corresponding to palmitic acid (24 % of total) and stearic acid (76 % of total). Minor peaks (too small for calculation of area) were also seen with retention times corresponding to capric, lauric, and myristic acids. The sum of the areas of individual 16-carbon fatty acids (table I) is 25.5 %, which agrees satisfactorily with the figure (24 %) obtained after hydrogenation. Satisfactory agreement was obtained also for the 18-carbon fatty acids (74.6 % versus 76 %).

Discussion

The present findings indicate that the complex lipid fraction of spinach chloroplasts includes at least 11 different fatty acids. Unsaturated fatty acids make up 89 % of the total fatty acids, with linolenic acid alone composing some 69 %. Fatty acids of the 18-carbon series make up 75 % of the total; the remainder is almost entirely composed of 16-carbon acids, although C-10, C-12, and C-14 acids were detected in small amounts.

These results are closely comparable to those of Debuch (5), differing principally in that spinach contained considerably more of a 16-carbon trienoic acid and considerably less linoleic acid than the chloroplasts which she examined.

Since photosynthesis is now thought to involve the participation of oriented molecules of chlorophyll and other pigments at a lipid-protein interface, we hope that this analysis of the lipid fraction of the chloroplast may ultimately contribute to an increased understanding of photosynthesis.

Summary

The total fatty acid composition of chloroplasts of spinach has been determined by gas-liquid chromatography. About 89 % of the total fatty acids are un-

saturated; approximately 75 % of the fatty acid fraction is composed of acids of the 18-carbon series. Linolenic acid is the predominant unsaturated fatty acid present and palmitic acid is the predominant saturated acid. About 11 % of the total fatty acids is present as an unsaturated component tentatively identified as a 16-carbon trienoic acid. Analytical results after hydrogenation of the samples are in good agreement with the data presented.

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Condensing Enzyme from Higher Plants [1, 2]

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Condensing enzyme, which couples acetate and oxaloacetate to form citrate, has been extracted from a variety of animal tissues, yeast, and bacteria (12). The enzyme was obtained in crystalline form from pig heart by Ochoa et al. (10) in 1951. The presence of condensing enzyme in the tissues of higher plants, however, has not been directly demonstrated.

Brummond and Burris (1) demonstrated that the rate at which mitochondrial preparations from cotyledons of lupine seedlings oxidized malate and pyruvate in combination was greater than the sum of their rates singly. They also reported that when pyruvate-2-C^{14} is oxidized in the presence of L-malate, the specific activity of the citrate produced is almost identical to that of the pyruvate used. Walker and Beevers (15) reported that a particulate fraction from castor bean endosperm oxidized neither oxaloacetate nor pyruvate alone and that oxygen uptake in the presence of a combination of these substrates proceeds at its maximum rate from the outset only when CoA, cocarboxylase, ATP, and DPN are present. Vickery and Zelitch (14) supplied pyruvate-2-C^{14} to cultures of tobacco leaves and found that the citrate formed possessed the same specific activity as the pyruvate used.

The available evidence strongly suggests, therefore, that the synthesis of citrate in plant tissues proceeds by the reaction of an enzyme system analogous to the condensing enzyme of Stern and Ochoa (13). Although Brummond and Burris (2) demonstrated the presence of most of the enzymes of the citric acid cycle in the green leaves of young lupine plants, the evidence for the presence of condensing enzyme was inconclusive. In this paper we describe the preparation from various plant tissues of enzyme extracts which possess condensing enzyme activity. The en-

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