Fatty Acids of Sorghum Leaf and Stem

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Lipides of sorghum leaf and stem were solvent-partitioned into three fractions representing 37, 40, and 23% of the lipide extract. Fatty acid composition was determined by gas chromatography. The major unsaturated acid was linolenic and most was present in the 80% ethyl alcohol fraction. The major saturated acid was palmitic, relatively evenly distributed among the fractions. Fatty acid composition differs markedly from that of sorghum grain, but resembles that of other grasses and nonseed plant lipides.

THE IMPORTANCE of small amounts of certain polyunsaturated fatty acids to the growth and well-being of farm animals has been realized for a considerable time (*4*). The fatty acid intake also affects the quality of the meat produced (*12*). There have been few fatty acid compositional studies carried out for the various forage crops. Fatty acid analyses have been reported for orchard grass (*11*, *14*), rye grass (*14*), mixed pasture (*8*), and more recently for legume-grass silage (*15*).

The present work is a continuation of an earlier investigation (*3*) in which the lipides of sorghum leaf and stem were partitioned between hexane and 90% ethyl alcohol in a 400-transfer countercurrent distribution. The composition of the lipide extract was very complex; nitrogen, phosphorus, sugars, ninhydrin-reacting substances, and pigments were present throughout the distribution. Fatty acid content and distribution for the same lipide extract are now reported. A simpler solvent-partitioning method was devised to yield three fractions having the same partition coefficients as the three main fractions of the 400-tube countercurrent distribution.

Material and Methods

Preparation of Extract. Extract preparation from Atlas sorghum has been described (*3*). Solids in the extract account for 1.66% of the original dry matter of the sorghum leaf and stem.

Solvent Fractionation. The solvent was removed in vacuo from 65 ml. of the original hexane extract, the residue (3.13 grams) was dissolved in 80 ml. of methanol-saturated heptane, and a solvent fractionation was carried out (Figure 1). The efficiency of fractionation was determined by countercurrent distribution. Portions, 100 to 200 mg., of fractions A, B, and C dissolved in hexane (Figure 1), were subjected to 24-transfer countercurrent distributions using a hexane–90% ethyl alcohol (1 to 1) system (Figure 2).

Saponification Number. The method was similar to that of Sims and Stone (*13*). Samples of A, B, or C weighing 40 to 100 mg. were saponified at reflux for 3 to 5 hours, transferred to 100-ml. beakers with ethyl alcohol, and titrated to pH 6.5 read on a pH meter. Saponification numbers are recorded in Table I.

Neutralization Equivalent. Fatty acids for the determination of the neutralization equivalent were obtained from the saponified samples after removing the nonsaponifiables. The portion of the fatty acid fraction extractable by 0.25% sodium carbonate solution and soluble in absolute ethyl alcohol was used for the determination. The acids were titrated to a mixed indicator (1 part of 0.04% aqueous cresol red, 3 parts of 0.04% aqueous thymol blue) end point. The yield of fatty acids and their neutral equivalent values are found in Table II.

Gas Chromatographic Analysis. For gas chromatography, the fatty acids were converted to their methyl esters. A freshly prepared ether solution of diazomethane (*10*) was added dropwise to an ice-cold solution of 5 to 10 mg. of fatty acid in 10 ml. of ether until a yellow color persisted. The excess of diazomethane and the solvent were immediately removed on a warm water bath in a stream of nitrogen. Approximately 3 mg. of the methyl esters were dissolved in 70 μl. of ether, and this solution was injected into the gas chromatographic apparatus by means of a syringe. The instrument used was an Aerograph, Master A-100. A 150-cm. column was packed with Resoflex 446 and operated at 190° C. The helium pressure at the column entrance was 13 p.s.i. A filament current of 220 ma. and a sensitivity of 1 ma. were used. The areas under the individual peaks of the curves were calculated and compared to obtain the relative per-

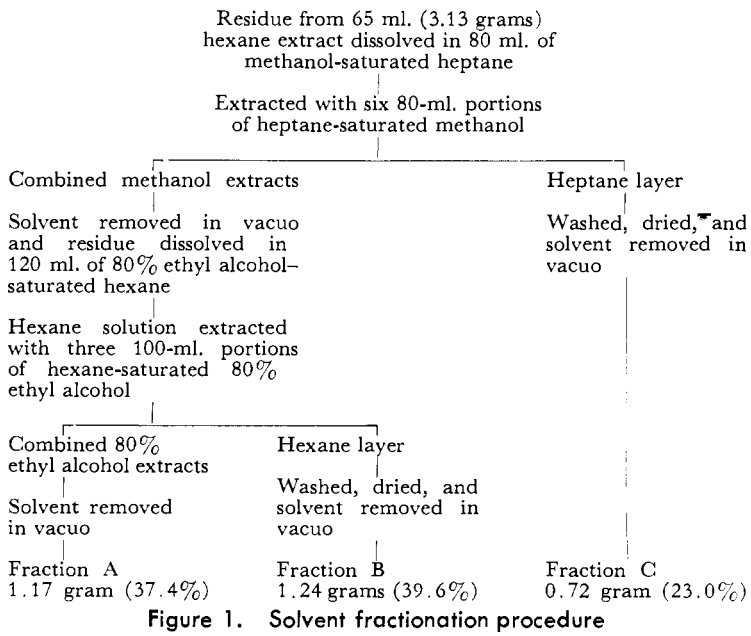

Figure 1. Solvent fractionation procedure

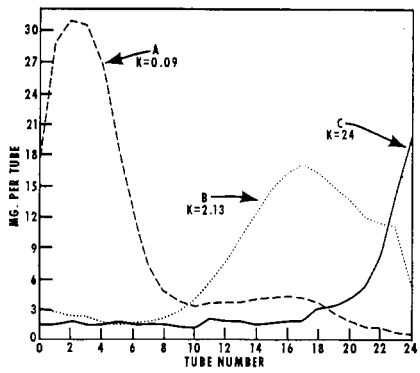

Figure 2. Countercurrent distribution curves

centages of the acids present in fractions A, B, and C (Table II).

Iodine Value. Iodine values were obtained on 3- to 5-mg. samples using the Wijs method (Table II).

Nitrogen and Phosphorus. Approximately 30-mg. samples of A, B, and C were analyzed for nitrogen by a semimicro-Kjeldahl procedure. Colorimetric phosphorus determinations were carried out on 15- to 65-mg. samples according to the method of Fiske and Subbarow (*6*).

Total Fatty Acid Analysis. The original lipide extract was analyzed for fatty acid content after removal of the acetone-insolubles (13%), saponification, and passage of an absolute ethyl alcohol solution of the crude fatty acid fraction through Dacro-Celite (1 to 2). The neutralization equivalent was 285; the iodine value was 149. Approximately 100-mg. samples were subjected to alkali isomerization (*1*). Percentages of component acids found were: saturated, 27.1; oleic, 21.0; linoleic, 12.9; and linolenic, 39.0.

Results and Discussion

A high degree of separation by simple solvent fractionation is indicated by the countercurrent distribution curves of the three fractions (Figure 2). Partition coefficients, K (*16*), were calculated for each fraction and found in

$$K = \frac{\text{tube no.}}{\text{no. of tubes} - \text{tube no.}}$$

good agreement with those of the three main peaks obtained previously with a 400-transfer distribution (*3*).

The low saponification numbers of the individual fractions indicate the absence of major amounts of glyceride material. Fatty acids comprise only 16% of the lipide fraction, accounting for 0.27% of the dry weight of the sorghum. Leaf glycerides have been reported to amount to a little more than 1% of the dry weight of plant leaves (*7*). Sorghum leaves represent about 20% of the dry matter extracted.

The low percentages of nonsaponifiables and fatty acids in the fractions point out that the extract contained a considerable amount of water-soluble material that resulted from the saponifications. Positive ninhydrin and anthrone tests (*3*) indicate the presence of significant amounts of proteinlike and carbohydrate material.

The phosphorus–nitrogen ratio in the heptane-soluble fraction, C, indicates the presence of a large amount of lipide materials having equimolecular amounts of phosphorus and nitrogen. The major portion (56%) of the fatty acids was found in the more polar fraction, A, and linolenic acid comprised 60% of the acids. These findings indicate that the fatty acids of sorghum leaf and stem are derived largely from phosphatides, not triglycerides.

Summation of the acids found in the three fractions by gas chromatography was compared with the values obtained on the original extract by alkali isomerization. Similar values were found for linolenic acid. However, gas chromatography indicated a higher content of saturated acids in the sum of the fractions than was found by alkali isomerization analysis of the whole extract. Lower values were found for oleic acid. The extract used for alkali isomerization had the acetone insolubles removed. Treated with activated carbon to remove pigments that interfered with spectrophotometric determination of polyunsaturated acids may have resulted in fractionation of fatty acids. Consumption of the sample in other analyses prevented direct comparison by gas chromatography. Calculation of the iodine values from the fatty acid composition assigned to the three fractions by gas chromatography gives values close to those determined experimentally. It is believed that the fatty acid composition obtained by gas chromatography for the three fractions gives a better picture of the total fatty acids of sorghum lipides than does the spectrophotometric determination as carried out in the original extract. Agreement of iodine values indicates that the C_{12} and C_{14} acids are saturated.

A striking dissimilarity is presented when the component fatty acids of sorghum leaf and stem lipides are compared with those of sorghum grain (*9*). The chief acid of the leaf and stem is linolenic; this acid is absent from the grain. The leaf and stem are lower in linoleic and oleic acids, and higher in saturates. There is a similarity between the fatty acids of sorghum leaf and stem and of other plants (*2*). Nevertheless, sorghum leaves and stems are lower in linoleic acid, and much higher in saturated acids than other leaf fats. The fatty acid composition of sorghum leaf and stem is more nearly like that of *Brassica oleracea* (cabbage) (*5*).

Table I. Characteristics of Fractions of Sorghum Leaf and Stem Extract

Fraction	Saponification No.	Nonsaponifiables, %	Fatty Acids, %	Nitrogen, %	Phosphorus, %
A	50	12	24.0	0.53	0.11
B	112	26	12.0	0.83	0.01
C	84	25	9.8	0.23	0.54

Table II. Characteristics and Composition of Fatty Acids of Saponified Fractions of Sorghum Leaf and Stem

Fraction	Neutralization Equiv.	Iodine Value	% of Original Extract	Relative Amounts of Fatty Acids						
				C_{12}	C_{14}	Palmitic	Stearic	Oleic	Linoleic	Linolenic
A	297	178	9.0	...	...	22.8	Trace	1.9	15.0	60.3
B	323	108	4.8	12.8	5.6	32.6	2.4	2.9	17.9	25.8
C	283	93	2.3	3.1	5.9	38.4	Trace	7.5	35.0	10.1

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