With steadily increasing yield of sweet potatoes and trends toward mechanization, sweet potato may eventually compete with corn as a source of commerical starch. The protein could be retained and used in human diets.

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Received for review November 10, 1977. Accepted January 6, 1978. Paper No. 5414, Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, N.C. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture of North Carolina Agricultural Experiment Station, nor does it imply approval to the exclusion of other products that may be suitable.

Lipid Distributions in Green Leaf Protein Concentrates from Four Tropical Leaves

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Protein concentrates were prepared from the green leaves of four tropical plants: chaya, sorghum × sudan, cassava, and sauropus; and the lipid classes, sterols, and fatty acids of those concentrates were studied. About three-fourths of the green protein lipids were neutral lipids, one-fifth to one-fourth were glycolipids, and less than one-twentieth were phospholipids. After saponification of the total lipids about one-third were fatty acids, less than one-third were nonsaponifiables, and about one-third were "residuals" not extracted by hexane. Sterols were identified as cholesterol, stigmasterol, campesterol, β-sitosterol, and isofucosterol. TLC profiles revealed about 15 neutral lipids and about 11 glycolipids. The distributions of fatty acids in the neutral lipid, glycolipid, and phospholipid fractions were different. The glycolipid fraction was the richest source of linolenic acid. Fatty acid distributions were determined for free fatty acids, monogalactosyl diglycerides, acylated monogalactosyl diglycerides, digalactosyl diglycerides, and acylated sterol glucosides. The presence of acylated galactosyl lipids indicated the presence of glycolipid-hydrolyzing and acyl-transferring enzymes in the expressed leaf juices.

Leaf protein concentrates (LPC's) are currently attracting worldwide attention as a novel protein food to supplement diets which are deficient both in quality and quantity of protein (Pirie, 1971, 1975, 1976; Singh, 1975; Kohler et al., 1976). Extensive research conducted on leaves from temperate zones has resulted in two industrial processes for the preparation of LPC, viz., the VEPEX process (Hollo and Koch, 1970) and the Pro-Xan process (Kohler et al., 1968). Leaves from tropical zones, on the other hand, remain an unexploited source of valuable protein. Although research has been conducted in several tropical countries on leaf proteins (Byers, 1961; Singh, 1964; Nazir and Shah, 1966; Joshi, 1971; Martin et al., 1977; Nagy et al., 1978), no industrial process is currently producing LPC from tropical leaves. Two primary factors for considering leaves indigeneous to tropical regions as LPC sources are: (1) a high potential for good yields of protein because of year-round availability of high amounts of sunlight and the agronomic potential of multiple cropping and (2) a need for production of more protein in developing tropical countries to offset the imbalance between population growth and protein supplies (United Nations World Food Conference, 1974).

The green juice expressed from succulent leaves contain soluble proteins that are coagulable into different fractions by differential heat treatment. Juice heated between 50 and 64 °C yields the first protein coagulum. This green protein curd when pressed and dried is known as "green LPC" or "green-fraction LPC" (Bickoff et al., 1975). Practically all chlorophylls and most of the expressed lipids are coprecipitated with this first protein coagulum (Hudson and Karis, 1973). The coprecipitated lipids, which might range from 10 to 30% by weight of the green LPC (Byers, 1971; Edwards et al., 1975; Vander Zanden, 1974; Pirie, 1975), impart both positive and negative attributes to the LPC. On the positive side, these lipids enhance the nutritional quality of the green LPC by contributing important fatty acids, viz., oleic, linoleic, and linolenic acids (Lima et al., 1965; Hudson and Karis, 1973; Betschart and Kinsella, 1975). Negatively, because more than half the fatty acids in LPC are doubly and triply unsaturated (Buchanan, 1969), they are apt to oxidize during storage (Hudson and Karis, 1976; Hudson and Warwick, 1977). Additionally, reaction of unsaturated fatty acids with amino acid residues during preparation and storage of LPC has been implicated as one cause of decreased nutritive value (measured by enzymatic digestibility) (Henry and Ford, 1965; Pirie, 1966; Shah et al., 1967; Buchanan, 1969).

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0021-8561/78/1426-0701$01.00/0

Most studies on the lipid composition of green LPC's have been limited to fatty acids rather than total lipids (Hudson and Karis, 1973). In this study, we undertook to determine the lipid concentration and composition of the acetone–ethanol extract of green LPC's from tropical leaves. We hope to be able to evaluate the value of LPC-extracted lipids as a potential by-product. We would also like to use LPC lipids to evaluate those agronomic, processing, and storage factors that relate directly to the composition and quality of the LPC preparations.

EXPERIMENTAL SECTION

Samples. Leaf protein concentrates of *Cnidoscolus chayamansa* (chaya), *Manihot esculenta* (cassava), *Sorghum sudanesis* (sorghum × sudan), and *Sauropus androgynus* (sauropus) were prepared according to Telek et al. (1978) from samples harvested at the Mayaguez Institute of Tropical Agriculture, Mayaguez, Puerto Rico. The fresh weights of the leaf samples were: chaya (873 g), cassava (561 g), sorghum × sudan (1230 g), and sauropus (1216 g).

Extraction and Purification of Lipids. The moist, freshly prepared LPC preparations were exhaustively extracted with 10% water in acetone and then with 95% ethanol as follow: The moist LPC was placed in a 250-mL centrifuge tube and varying aliquots of 10% water in acetone were added (the amount varied but the total volume for complete extraction of the LPC was recorded). The mixture was vigorously stirred for ca. 5 min and centrifuged between 1000 and 1500 rpm in an International Centrifuge. The supernatant fluid was poured off and the procedure repeated until the extract appeared devoid of the green chlorophyll color. The final extraction was made with 95% ethanol which removed the acetone from the proteins. The acetone and ethanol extracts were combined and the total volume was measured. One-tenth of this total volume was concentrated in vacuo; the residue was partitioned between $CHCl_3$ and water (Nagy and Nordby, 1970); and the crude lipids were purified through Sephadex according to the method of Wuthier (1966). The remaining extract (nine-tenths) were used for other studies or discarded.

Lipid Class Separation. Separation was accomplished by fractionation on columns (30 cm × 0.9 cm i.d.) containing 10 g of silica gel (60–200 mesh, J. T. Baker Chemical Company, Phillipsburg, N.J.) which had been prewashed with 100 mL of $CHCl_3$. The purified lipid (ca. 100–125 mg) in $CHCl_3$ was added to the gel column. Neutral lipids were eluted first with 200 mL of 0.3% acetic acid in $CHCl_3$, and the glycolipids next with 300 mL of acetone. After elution of both fractions, the silica gel, with adsorbed phospholipids and other polar lipids, was removed quantitatively from the column and placed in a coarse-fritted-glass filter funnel. The adsorbed lipids were removed from the gel by batchwise washing with five 50-mL aliquots of MeOH. The filtrate, which contained polar lipids and silica gel fines, was partitioned between $CHCl_3$ and water to recover the polar lipids. Dry weights of the three lipid fractions (neutral lipids, glycolipid, and the phospholipid and other polar lipid) were determined after vacuum drying under desiccation at room temperature.

Saponification. Samples of the Sephadex-purified lipid were saponified with 5 mL of 6% KOH in 95% ethanol in sealed acylation tubes at 105 °C for 1 h. The nonsaponifiables were extracted into hexane (seven 20-mL). Soaps were next acidified with 20 mL of 4 N HCl and the fatty acids extracted into hexane (three 20-mL). Relative percentages of nonsaponifiables and saponifiables in each of the LPC's were calculated.

Thin-Layer Chromatography (TLC). All analyses were run on nonactivated, 250 μm precoated, 20 × 20 cm silica gel G plates (Analtech, Inc., Wilmington, Del.). In general, plates were developed in tanks lined with filter paper; and the solvent was allowed to run to a height of 17 cm. $CHCl_3$–MeOH (95:5) was used to isolate free fatty acids from the neutral lipid fraction. Acylated monogalactosyl diglycerides (AMGDG), acylated sterol glucosides (ASG), and monogalactosyl diglycerides (MGDG) were separated from other glycolipids with $CHCl_3$–MeOH (95:5 or 85:15) while digalactosyl diglycerides (DGDG) were separated with $CHCl_3$–MeOH (75:25). Absolute $CHCl_3$ resolved the desmethyl and dimethyl sterols from other nonsaponifiables. The TLC-separated lipids, on which fatty acid determination was conducted by GLC, were first sprayed with Rhodamine 6G, next scraped from the plates, and, lastly, esterified directly with BF_3–MeOH or NaOH–BF_3–MeOH (Nagy and Nordby, 1970; Nordby and Nagy, 1971). The fatty acid methyl esters (FAME) were further purified by TLC with hexane–ethyl ether (90:10).

Plates were sprayed with 50% sulfuric acid and placed in a furnace at 200 °C for 3 min for the nonspecific detection of lipids. Sprays used to detect specific moieties within the lipid molecule were Bail's orcinol reagent (Applied Science, 1977) (sugars), Dittmer and Lester's reagent (Dittmer and Lester, 1964) (phosphorus), and 50% H_2SO_4 reagent (heating at 140 °C for 10 min produces specific colors for sugars and sterols) (Nordby and Nagy, 1971).

GLC Analyses. FAME's were determined on a glass column (1.52 m × 4 mm i.d.) packed with 3% SP-1000 (Supelco, Inc., Bellefonte, Pa.) on 100–120 mesh, Gas-Chrom Q. The injection port and detector were at 250 °C, and the helium flow rate was 60 mL/min. The FAME sample was injected on-column and run isothermally at 158 °C. Free desmethyl sterols were run isothermally at 200 °C on the same column. FAME's and sterols were quantitated with an Autolab System IV electronic integrator.

Protein Analyses. Crude protein content of the LPC's was determined by micro-Kjeldahl analysis (AOAC, 1965). True protein was determined by addition of 10% trichloroacetic acid to the leaf extract and analysis of the precipitate (Telek et al., 1978).

RESULTS AND DISCUSSION

The total crude protein contents of dry leaves ranged from about 10 to 30% of the dry matter contents (Table I). The amount of crude proteins expressed from leaves (expressed crude protein is called "green-fraction LPC") ranged from 10.2 to 21.4 g. Based upon the total crude protein contents of the dried leaves, extraction efficiencies for these crude LPC protein fractions ranged from about 26 to 59%. The crude protein values of the green-fraction LPC's included both soluble true protein and nonprotein nitrogen compounds (e.g., peptides and free amino acids). The true protein contents were about 43 to 56% of the crude protein in these four green-fraction LPC's.

The distributions of lipids from acetone–water extractions of the moist, green LPC's are shown in Table II. Saponification of the green protein-extracted lipid showed that about one-third was composed of fatty acids, less than one-third was due to nonsaponifiables, and the remainder or "residual" fraction consisted of compounds that could not be extracted with hexane.

Lipid class separation by silica gel column chromatography showed that about three-fourths of the total lipids were neutral lipids, about one-fifth to one-fourth

Table I. Weights and Protein Contents of Leaves from Four Tropical Plants

Tropical leaves	Fresh wt of leaves, g	Dry matter content, g	Crude protein contents of dry leaves, g	Dried crude LPC protein, g	Dried true LPC protein, g
Chaya	873	170	41.3	15.1	7.2
Sorghum × sudan	1230	180	18.7	11.1	4.7
Cassava	561	102	28.0	10.2	5.7
Sauropus	1216	281[a]	83.7	21.4	10.5

[a] Contains woody stems.

Table II. Lipid Distributions of Four Green Protein Fractions

Tropical leaves	Total extracted lipids from green protein fractions, g	Lipid/crude protein	Total lipid distribution, %			Lipid class distribution, %		
			Nonsaponifiables	Fatty acids	Residuals[a]	Neutral	Glycolipid	Phospholipid
Chaya	2.8	0.19	27.6	33.8	38.6	75.1	23.8	1.1
Sorghum × sudan	2.5	0.23	28.5	35.3	36.2	67.3	30.7	2.1
Cassava	1.2	0.12	25.2	32.0	42.8	74.8	22.3	2.9
Sauropus	4.8	0.22	30.4	34.1	35.5	83.5	16.1	0.5

[a] Includes lipid moieties not extracted by hexane and heptane after saponification and acidification (e.g., glycerol, galactosyl glycerol).

Table III. Percentage Distribution of Desmethyl Sterols in Lipids from Green Protein Fractions

Tropical leaves	Sterol, %				
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol	Isofucosterol
Chaya	1.4	7.4	5.0	69.7	16.5
Sorghum × sudan	2.7	19.7	44.4	33.2	Tr
Cassava	3.4	12.5	6.3	77.8	Tr
Sauropus	2.1	15.6	Tr[a]	45.6	36.7

[a] Tr = trace (<1.0%).

were glycolipids, and less than one-twentieth were phospholipids. The low percentage of phospholipids might be explained by the fact that phospholipids are primarily structural components and are not readily expressed from plant tissue, and the fact that phospholipids are relatively insoluble in acetone, and therefore, would not be readily extracted from the green protein.

We used an acetone–water mixture and 95% ethanol as lipid extractants because a more polar solvent mixture, e.g., chloroform–methanol, would be more expensive to use and recover. Moreover, chloroform–methanol mixtures are toxic and their use in the preparation of food for humans should be avoided. The properties of acetone as an extractant of chlorophyll are well known (Willstatter and Stoll, 1913). Acetone is the recommended solvent for extraction of chlorophylls in plant tissue (AOAC, 1965) and is ideally suited for the extraction of the green color from the green protein fraction. It is one of the least toxic of industrial solvents and is comparable to ethanol in toxicity (Weissberger et al., 1955).

Table III shows the percent distributions of desmethyl sterols in the nonsaponifiable fractions. Major fluctuations were observed for these four tropical leaves. The only sterol to show a relatively constant percentage in all four leaf preparations was cholesterol. The hybrid, sorghum × sudan, exhibited a high percentage of stigmasterol and low percentage of sitosterol when contrasted to the other three plants. Isofucosterol was found in significant amounts in chaya and sauropus but in only trace amounts in sorghum × sudan and cassava. Although the observed sterol compositions might have resulted from the method used for green protein fractionation, they might also reflect

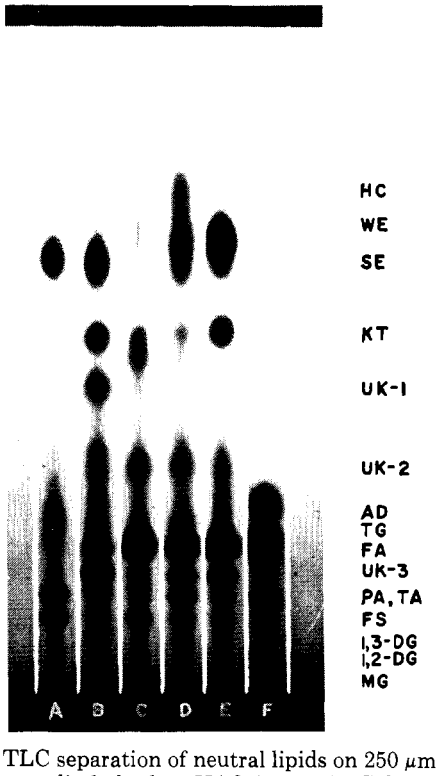

Figure 1. TLC separation of neutral lipids on 250 μm silica gel G with hexane–diethyl ether–HAC (88:10:2). Column identification: (A) reference neutral lipid mixture from citrus juice (Nagy and Nordby, 1970); neutral lipids from green protein fractions of (B) chaya, (C) sorghum × sudan, (D) cassava, and (E) sauropus; (F) standard lipid mixture. Lipid identifications: HC (Hydrocarbons), WE (wax esters), SE (sterol esters), KT (ketones), UK-1, 2,3 (unknowns), AD (long-chain aldehydes), TG (Triglycerides), FA (free fatty acids), PA (primary alcholols), TA (triterpene alcohols), FS (free sterols), 1,3,-DG (1,3-diglycerides), 1,2-DG (1,2-diglycerides), and MG (monoglycerides).

inherent differences among the four plants. Such sterol diversity might be of value in differentiating breeding lines and in plant chemotaxonomy.

Figure 1 shows TLC separations of two standard neutral lipid mixtures (AF) and the neutral lipid fractions from chaya (B), sorghum × sudan (C), cassava (D), and sauropus (E). Of the neutral lipid fractions only the one from

cassava showed an appreciable hydrocarbon content (HC, R_f 0.70). Minor amounts of wax esters (WE, R_f 0.64) were observed for chaya and sorghum × sudan while sauropus showed wax esters as one of the major neutral lipid constituents. Sterol esters (SE, R_f 0.61), noticeably present in chaya, cassava, and sauropus, were present only in trace amounts in sorghum × sudan. Ketones (KT, R_f 0.50) were observed in all leaf preparations. An uncharacterized lipid group (UK-1, R_f 0.42) that migrated below the ketones was found in chaya. A second uncharacterized lipid group (UK-2, R_f 0.32) that showed a higher R_f value than the standard, hexadecanal (AD, R_f 0.26), was observed in all four leaf preparations. Minor amounts of triglyceride (TG, R_f 0.24) occurred in all preparations. Free fatty acids (FA, R_f 0.21) were major constituents in sorghum × sudan, cassava, and sauropus, but were present in smaller amounts in chaya. A major lipid group (UK-3, R_f 0.17) that migrated below the free fatty acids has been tentatively identified as secondary alcohols. Isolation of UK-3 by preparative TLC, followed by GLC analysis, revealed a series of compounds. When chromatographed in benzene–$CHCl_3$ (70:30), UK-3 showed an R_f value similar to that reported by Holloway and Challen (1966) for secondary alcohols. Linear or primary alcohols (PA, R_f 0.13) occurred in all neutral lipid fractions in small amounts. Triterpene alcohols (TA, R_f 0.13) migrated with the primary alcohols (no visible separation). Free sterols (FS, R_f 0.10), 1,3-diglycerides (1,3-DG, R_f 0.08), 1,2-diglycerides (1,2-DG, R_f 0.05), and monoglycerides (MG, R_f 0.02) occurred in all samples in small amounts. A majority of chlorophylls and other pigmented materials were separated with the neutral lipids. Small amounts of pigmented materials were also found in the glycolipid fraction. TLC separation of the neutral lipid fraction in hexane–diethyl ether–HAC (88:10:2) (Figure 1) showed that the chlorophylls remained at the origin while other pigmented materials showed limited migration (R_f 0.02 to 0.12). An insignificant amount of yellow carotenoid pigments migrated with the hydrocarbons (R_f 0.70).

Figure 2 shows a reference glycolipid mixture (A) obtained from citrus vesicular lipids (Nordby et al., 1976), glycolipid fractions from chaya (B), sorghum × sudan (C), cassava (D), and sauropus (E), and oleic acid and cholesterol standard (F). In silica gel column chromatography, free fatty acids are generally eluted with glycolipids; but they can be separated with the neutral lipid fraction by elution of the silica gel column with $CHCl_3$ containing small amounts of acetic acid (Nagy et al., 1975).

Sastry and Kates (1964a) observed that homogenization of plant tissues caused enzymatic alterations of lipids with the concomitant formation of deacylated phospholipids and glycolipids. In another alteration, Heinz (1967) showed that during homogenization acyl transfer reactions from DGDG to MGDG caused the formation of acyl galactosyl diglycerides. Heinz et al. (1974) separated the acyl galactosyl diglycerides by TLC with $CHCl_3$–MeOH (85:15) and showed that this lipid group migrated much further than acyl sterol glucosides (ASG).

In Figure 2, two major glycolipids occurred in the leaf preparations which had TLC migratory properties similar to that of the acyl galactosyl diglycerides isolated by Critchley and Heinz (1973). When separated by TLC, sprayed with sulfuric acid, and heated to 140 °C, this lipid showed a reddish-brown color similar to that of MGDG and DGDG. Since this lipid appeared to be the major glycolipid in chaya (B) and occurred in appreciable amounts in the other three leaves, we examined a series of solvent systems varying in gradient polarity for their

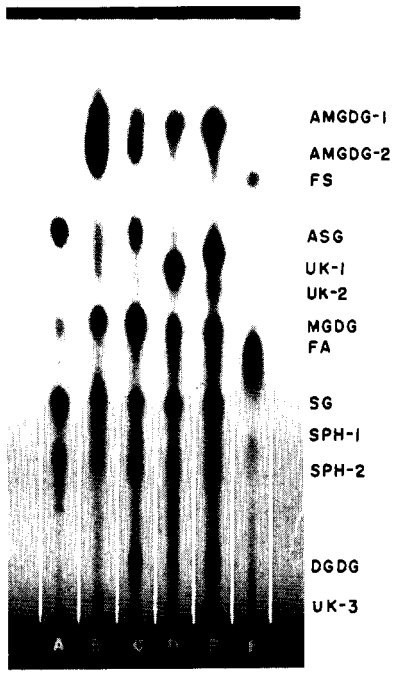

Figure 2. TLC separation of glycolipids on 250 μm silica gel G with $CHCl_3$–MeOH (85:15). Column identification: (A) reference glycolipid mixture from citrus vesicular lipids (Nordby et al., 1976); glycolipids from green protein fractions of (B) chaya, (C) sorghum × sudan, (D) cassava, and (E) sauropus; (F) standard free fatty acids and cholesterol. Lipid identifications: AMGDG-1,2 (acylated monogalactosyl diglycerides), FS (free sterols), ASG (acylated sterol glucosides, UK-1,2,3 (unknown), MGDG (monogalactosyl diglyceride), FA (free fatty acids), SG (sterol glucosides), SPH (sphingolipids), and DGDG (digalactosyl diglycerides).

ability to resolve this lipid group from others. Of the solvents tested, $CHCl_3$–MeOH (95:5) proved to be the best and this solvent resolved the acylated galactosyl diglyceride fraction into two distinct components (AMGDG-1, AMGDG-2). AMGDG-1 and -2 were found in about equal amounts (measured by charring intensity) in chaya (B). AMGDG-1 predominated in cassava (D) and sauropus (E), whereas AMGDG-2 predominated in sorghum × sudan (C).

Acylated sterol glucosides (ASG, R_f 0.64) were found in sorghum × sudan and sauropus in appreciable amounts but were only minor components in chaya and cassava. In cassava, a major glycolipid (UK-1, R_f 0.58) was observed which migrated below ASG. This lipid (UK-1) turned purple to black when sprayed with sulfuric acid. This was in contrast to the rose-violet color produced when ASG was sprayed with sulfuric acid. ASG, UK-1, and UK-2 gave the characteristic blue color of carbohydrates when sprayed with Bail's reagent. UK-1 and UK-2 had TLC migratory patterns similar to the pattern for acyl galactosyl monoglycerides reported by Critchley and Heinz (1973) and by Heinz et al. (1974). Monogalactosyl diglycerides (MGDG, R_f 0.49) were major components in all four plant preparations; the most prevalent concentration was highest in sorghum × sudan.

Sterol glucosides (SG, R_f 0.37) were found in about equal amounts in the four plants. Two sphingolipids (SPH-1, R_f 0.31; SPH-2, R_f 0.25) were observed in sorghum × sudan but only SPH-2 was present in cassava and sauropus. Heinz et al. (1974) reported the presence of monogalactosyl monoglyceride (MGMG) and acyl digalactosyl diglyceride (ADGDG) in spinach preparations. Although we did not find these two lipids in our preparations, they might have been present but overlapped by the sterol glucosides and the sphingolipids.

Table IV. Relative Percent Fatty Acid Distributions in Total Lipids and Lipid Classes from Green Protein Fractions[a]

Lipid fraction	Fatty acid 16:0[b]	18:0	18:1	18:2	18:3
Total lipid					
Chaya	25.1	2.5	2.9	7.3	62.2
Sorghum × sudan	31.5	1.7	3.7	13.0	50.1
Cassava	33.7	3.3	4.9	8.7	49.4
Sauropus	30.0	1.3	5.1	13.0	50.6
Neutral lipid					
Chaya	37.8	4.2	5.2	13.5	39.3
Sorghum × sudan	42.2	2.1	5.3	16.0	34.4
Cassava	42.7	4.1	6.1	10.7	36.4
Sauropus	30.8	1.6	5.1	12.3	50.1
Glycolipid					
Chaya	12.1	1.7	1.1	3.2	81.9
Sorghum × sudan	13.4	1.3	1.8	8.3	75.2
Cassava	16.2	1.8	2.4	4.2	75.4
Sauropus	14.5	0.8	7.2	20.6	56.9
Phospholipids					
Chaya	34.1	5.6	6.0	4.7	49.6
Sorghum × sudan	31.5	2.8	4.2	12.9	48.6
Cassava	26.3	4.2	6.3	8.2	55.0
Sauropus	31.0	3.7	9.1	10.1	46.1

[a] Percent by weight of each fatty acid ester calculated from area of each peak. [b] No. of carbons: no. of double bonds.

Noticeable amounts of digalactosyl diglycerides (DGDG, R_f 0.11) were found in sorghum × sudan and sauropus, minor amounts in cassava, and virtually none in chaya. An uncharacterized lipid (UK-3, R_f 0.04) was observed in minor amounts in all leaf preparations. Although not completely identified, this unknown lipid had TLC R_f values similar to those for 6-sulfoquinovosyl diglyceride (Hitchcock and Nichols, 1971). In photosynthetic tissues, 6-sulfoquinovosyl diglyceride is localized in the chloroplast's membrane and lamellae (Douce et al., 1973) and would be expressed from leaf tissue during maceration.

Fatty acid distributions in total lipids and lipid classes are shown in Table IV for the green protein fractions of four plants. The predominant unsaturated fatty acid in the total lipid fractions of all leaf preparations was linolenic acid. The percentage order for the total lipids was: linolenic acid (18:3) > palmitic acid (16:0) > linoleic acid (18:2) > oleic acid (18:1) > stearic acid (18:0). The distributions of fatty acids in the total lipid fractions were about similar for sorghum × sudan, cassava, and sauropus but were noticeably different for chaya.

The fatty acids of the neutral lipid fractions of chaya, sorghum × sudan, and cassava, in contrast to their respective total lipids, showed major differences in the levels of 16:0 and 18:3. In sorghum × sudan and cassava, the amounts of 16:0 were greater than 18:3, while chaya and sauropus showed a reverse pattern for these two acids. In sauropus, the total lipid and neutral lipid patterns appeared similar.

The glycolipid fractions of chaya, sorghum × sudan, and cassava showed a high concentration of 18:3. Fatty acids were distributed in the following descending order: 18:3 > 16:0 > 18:2 > 18:1 > 18:0. The high percentage of 18:3 in the glycolipid fraction of leaves agrees with results of other workers (Allen et al., 1964; Sastry and Kates, 1964b; Tullock et al., 1973; Nordby et al., 1976). In contrast to the other three leaf preparations, the sauropus preparation showed a noticeably high percentage of 18:2.

The fatty acid distribution of the phospholipid fraction showed the following order: 18:3 > 16:0 > 18:2 > 18:1 > 18:0. Because phospholipids were minor components of the green protein fractions (0.5–2.9%, Table I), they would not be expected to contribute significant amounts of essential fatty acids.

The fatty acid distributions in the major lipids of the neutral lipid fraction (FA) and in the glycolipid fraction (MGDG, AMGDG, ASG, DGDG) were analyzed (Table V). Because the major lipids in each leaf preparation were different, only those present in particularly high concentrations were subjected to fatty acid analysis. Free fatty acids were the predominate lipid in the neutral lipid fraction. The free fatty acid distributions in the four leaf preparations were noticeably similar except for low amounts of 18:2 in sauropus and low amounts of 18:3 in chaya. In contrast to the neutral lipid fraction, the free fatty acid group showed lower percentages for 16:0.

The monogalactosyl-containing lipids of the glycolipid fractions all showed high contents of 18:3. This fatty acid ranged from 70.1% in MGDG for sorghum × sudan to 85.8% in AMGDG for chaya. The fatty acid patterns of MGDG were similar in chaya and cassava but differed in sorghum × sudan (lower 18:3; higher 18:2 and 16:0) and sauropus (lower 18:3; higher 18:2). In contrast to the monogalactosyl lipids, the digalactosyl lipid (DGDG) of sorghum × sudan showed higher contents of 16:0 and lower contents of 18:3. This observation is in agreement with the results of Nichols et al. (1967) for MGDG and DGDG

Table V. Relative Percent Fatty Acid Distributions in Specific Lipids from Green Protein Fractions

Plant lipid	Fatty acid 16:0	18:0	18:1	18:2	18:3
Chaya					
Free fatty acid	29.8	5.2	7.7	20.3	37.0
Monogalactosyl diglyceride	11.4	2.8	3.0	2.8	80.0
Acylated monogalactosyl diglyceride	9.2	0.3	2.1	2.6	85.8
Acylated sterol glucoside	32.6	2.5	5.3	15.1	44.5
Sorghum × sudan					
Free fatty acid	28.4	2.5	6.7	18.9	43.5
Monogalactosyl diglyceride	15.8	1.2	3.2	9.7	70.1
Acylated monogalactosyl diglyceride	9.5	1.1	1.6	7.1	80.7
Digalactosyl diglyceride	19.5	2.1	2.8	10.1	65.5
Cassava					
Free fatty acid	26.9	2.5	5.4	18.0	47.2
Monogalactosyl diglyceride	10.1	2.4	3.2	2.6	81.7
Acylated sterol glucoside	29.1	3.6	6.7	13.6	47.2
Sauropus					
Free fatty acid	26.9	3.1	8.3	13.8	47.9
Monogalactosyl diglyceride	9.2	0.6	3.6	11.8	74.8
Acylated monogalactosyl diglyceride	10.9	1.2	4.0	10.2	73.7
Acylated sterol glucoside	11.2	1.0	12.6	36.9	38.3

in castor leaf lipids. The ASG lipids showed 18:3 percentages of about 38 to 47% which were about 30 percentage points lower than in the galactosyl lipids. The ASG lipids of chaya and cassava were similar but differed from the ASG lipid of sauropus (lower 16:0; higher 18:2).

CONCLUSION

The lipid composition of leaf protein preparations is influenced by agronomic factors and by processing and storage conditions (Hudson and Warwick, 1977). The type leaf, stage of maturity, climate, seasonal changes (resulting from different harvesting periods during the year for perennial leaves and grasses), and other factors (irrigation, fertilization) influence the distributions of lipids and of fatty acids (Hawke, 1963; Hudson and Karis, 1973). Processing conditions involving pulping, maceration, and precipitation techniques would also be expected to influence the lipid patterns of the green protein factions (Pirie, 1971). The presence of large amounts of free fatty acids and acylated galactose-containing lipids in our green protein preparations indicate the presence of highly active glycolipid-hydrolyzing enzymes (Sastry and Kates, 1964a; Galliard, 1970; Heinz, 1973) and acyl-transferring enzymes (Heinz et al., 1974). Figure 2 shows a predominance of acylated galactosyl lipids. Heinz et al. (1974) showed that these acylated derivatives possess increased stability against further enzymatic attack. Destruction of cellular compartmentation during maceration results in enzymatic alterations of not only the glycolipids but phospholipids as well (Galliard, 1970).

Expressed juice which is generally about pH 5.5–7.0 (Garcha et al., 1970) is susceptible to enhanced enzymatic activity unless heat is applied quickly to inactivate the enzymes. The amounts of free fatty acids and acylated glycosyl lipids in a leaf protein preparation might serve as an index of the preparational time. We postulate that the amounts of these lipids will depend upon the time interval between maceration of the plant tissue and the application of heat to coagulate the proteins within the expressed leaf juice. In the production of green LPC, heat inactivation of enzymes directly after maceration of the leaves should minimize formation of lipid artifacts.

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