Lipid Classes of Fresh Cassava Roots (*Manihot esculenta* Crantz): Identification and Quantification

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Fresh cassava roots (*Manihot esculenta* Crantz, c.v. Algodona) were processed for lipid analysis within five hr after harvest. The total lipid content was 0.25% fresh weight, and the lipid mixture obtained was complex. Polar lipids plus sterols and steryl esters made up the major part of the extracted lipids (77.9 mol%). Seven phospholipids were identified. Those in higher amount were: phosphatidylcholine, 265.4 nmol/g fresh weight; phosphatidylethanolamine, 151.8 nmol/g fresh weight, and phosphatidylinositol, 143.1 nmol/g fresh weight. Among the six glycolipids identified, digalactosyldiacylglycerol in a content of 333.2 nmol/g fresh weight and monogalactosyldiacylglycerol in a content of 217.1 nmol/g fresh weight were the most abundant. Free sterols were at 304.3 nmol/g fresh weight and triacylglycerol was measured at 444.4 nmol/g fresh weight. Results are discussed in view of recent knowledge of the kind of lipid classes expected in plant material. This work is intended to be the starting point of further research on cassava lipids upon subjecting the roots to conditions leading to its preservation.

Research on cassava lipids is scanty, and published works deal mainly with proximal analysis or only with the component fatty acids; the source material is cassava products more frequently than fresh cassava roots (1-4).

Two papers have been published that report partial qualitative data on lipid classes in cassava flour (5-6). The scopes of these works, fermentation of cassava products and baking quality of cassava flour, are unrelated to the interests of this study.

The importance of lipids in the cause/effect kinds of relationship that underlie the sequence of sensorial, biochemical and physiological events, which supervene on separating an organ from its mother plant or occur during senescence while attached to it, is supported by an increasing number of works that show concomitant changes in the identity and content or proportion of the lipid classes of senescing plant organs before or after harvest.

Lipids are main components of cell membranes, and as such have been related to the responses of plant organs to environmental conditions; thus, chilling injury that besets tropical produce is postulated to arise from changes in membrane lipids (7); the environmental oxygen content has been reported to affect lipid unsaturation (8), and ionizing radiation has a well-known effect on fruit lipids (9).

Substantial changes in the content and proportion of plasma membrane lipid classes as response to a temperature stress condition have recently been demonstrated unmistakably in plants (10). This is a further step from the previously established changes that, at the level of degree of unsaturation and proportion of the constituent fatty acids chains, occur when plants are subjected to stress conditions comparable to those mentioned above.

In cassava, membrane alterations have been proposed as the possible origin of parenchyma darkening (11), which is the primary cause of postharvest losses of fresh cassava roots.

Indications are that for the undertaking of meaningful studies about the effect of stress-preservation conditions on cassava roots some consideration must be given to the lipid component of this organ. Thus, the determination of the component lipid classes of the fresh cassava root seems a prerequisite to fulfill before the named studies about the effects of environmental conditions on cassava lipids or on cassava postharvest physiology can be undertaken. The aim of this work is to determine the profile of lipid classes, in qualitative and quantitative terms, of fresh cassava roots, in order to base on it further research on lipids and postharvest physiology of cassava.

EXPERIMENTAL PROCEDURES

Materials. All the chemicals used were of analytical grade. Lipid standards were obtained from Applied Science Laboratories, State College, Pennsylvania, and Sigma Chemical Co., St. Louis, Missouri. Silica gel G, H and HR were from Merck, Darmstadt, Germany.

Cassava roots. Cassava roots (*Manihot esculenta*) of the variety Algodona were purchased from a producer in El Cambur de Paya, Aragua State, Venezuela. The roots were harvested mid-morning at the usual 10-mo old stage for retail sale, and were transported by car, arriving at the laboratory less than two hr after harvest.

Extracts. After the cortex was removed, the edible portion of cassava roots was processed for lipid extraction. Lipids were extracted by the method of Bligh and Dyer (12) less than five hr from the time of harvest. The chloroform phase was concentrated under reduced pressure at 40 C in a rotatory evaporator, the resulting oily material was made up to a known volume with chloroform/methanol (2:1, v/v) and kept at -70 C until used. Dry weight determination on the extract was used to calculate the total lipid content of cassava root.

Polar lipids TLC. Polar lipids were resolved by two dimensional thin layer chromatography (TLC) on 0.25 mm silica gel G layers activated at 120 C for 45 min. The solvent systems of Lepage (13), chloroform/methanol/water (65:15:2, v/v/v) for the first dimension, and chloroform/acetone/methanol/acetic acid/water (65:20:10:10:3, v/v/v/v/v) for the second dimension; or of Nichols (14), chloroform/methanol/conc. ammonia (65:30:4, v/v/v) for the first dimension, and chloroform/methanol/acetic acid/water (170:25:25:6, v/v/v/v) for the second dimension, were used. The tanks were lined with filter paper and saturated before introducing the plates. A somewhat better separation was obtained

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with the solvents of Nichols (14). The drying procedure between developments was critical to get proper resolution of all spots, and the best results were obtained by drying about seven min under a blower at room temperature (ca. 25 C). The spots were detected with iodine and a series of specific spray reagents for amino groups, phosphate, choline, vicinal OH, sugars, sterols and amido nitrogen (15). Commercial lipid preparations were used as reference. Preparative TLC was performed on 0.5 mm silica gel HR layers. The spots were scraped off the layer, and the lipids were subjected to chemical determinations while on the silica gel or after extraction, depending on the analysis made. All the other conditions were the same as for qualitative TLC.

Neutral lipids TLC. Neutral lipids were resolved by TLC in one dimension, on layers prepared as above but without activation, with the solvent system petroleum ether 60-80/ether/acetic acid (85:15:1, v/v/v) as previously reported (16). Two developments in the same direction were required to separate the lipids completely. Alternatively, the solvent systems used were Freeman and West's (17) ether/benzene/ethanol/acetic acid (40:50:2:0.2, v/v/v/v) for the first development, and ether/hexane (6:94, v/v) for the second development. The chromatograms were visualized with iodine or Rhodamine 6G and with specific spray reagents for sterols, sugar, ester groups, vicinal OH, acids and carbonyl compounds (15).

Preparative 0.5 mm layers were made with silica gel H. The tanks were lined with filter paper and saturated with the solvents.

Analytical methods. Phosphorus analysis was performed on the lipid extracts and on TLC spots containing phospholipids (PL), as previously stated (16). The methods used for sterols and triacylglycerols (TG) have been reported elsewhere (18). Sugars and inositol were determined, without extracting the lipids from the silica gel, by the methods of Devor (19) and Agranoff et al. (20), respectively. For the analysis of nitrogen (21), ester groups (22) and glycerol (18) the lipids were extracted from silica gel with four portions of chloroform/methanol (2:1, v/v); for PL this mixture was water saturated. Alkyldiacylglycerols were determined as a component of the TLC-isolated TG, through methanolysis of the material while on the scraped silica gel, separation of the resulting alkyl ethers by TLC (23), and their spectrophotometric quantification (24).

RESULTS AND DISCUSSION

The method used for lipids extraction (12) is different from the methods followed by previous authors (1-6), but the total content of lipids found, 0.25% of fresh weight (fr. wt.), is within the wide range of reported values.

The lipids isolated from cassava roots are well known in other plant materials (13, 25, 26); thus, it was enough for the identification of the lipids extracted to consider: (a) their resolution by TLC in two different solvent systems (two for polar lipids (13-14) and two for neutral lipids (16-17); (b) comparison with commercial standards and data in the literature; (c) visualizing the TLC plates with specific spray reagents (15), and (d) analyzing chemically the lipids resolved chromatographically (16, 18-24).

The identity and content of the isolated lipids is shown in Tables 1 to 3. In total, 18 lipid classes were identified, and in addition, some minor components were visible on TLC plates developed for polar lipids. Three additional intense bands were found on qualitative layers (or 4 on preparative layers) run for neutral lipids, all of which went unidentified.

Free fatty acids were not found by direct TLC of the lipid extract or by extraction of the lipid solution with aqueous Na_2CO_3, treatment of the acidified aqueous phase with petroleum ether 60-80, followed by TLC and titration of the resulting organic phase.

TABLE 1

Phospholipids Identified in Fresh Cassava Roots (*M. esculenta*) and Their Content[a]

Phospholipids	nmol/g fresh weight	mol%
Phosphatidylcholine	265.4	37.6
Phosphatidylethanolamine	151.8	21.5
Phosphatidylinositol	143.1	20.3
Phosphatidylglycerol	21.9	3.1
Diphosphatidylglycerol	20.4	2.9
Phosphatidylserine	13.8	1.9
Phosphatidic acid	89.6	12.7
Total	706.0	

[a]Data are average of duplicate determinations.

TABLE 2

Glycolipids Identified in Fresh Cassava Roots (*M. esculenta*) and Their Content[a]

Glycolipid	nmol/g fresh weight	mol%
Digalactosyldiacylglycerol	333.2	40.7
Monogalactosyldiacylglycerol	217.1	26.5
Steryl glycoside	116.0	14.2
Cerebroside	69.3	8.5
Esterified steryl glycoside	67.0	8.2
Unidentified glycolipid[b]	16.0	1.9
Total	818.6	

[a]Data are averages of duplicate determinations.
[b]Presumably a sulfolipid.

TABLE 3

Neutral Lipids Identified in Fresh Cassava (*M. esculenta*) and Their Content[a]

Neutral lipid	mol/g fresh weight	mol%
Triacylglycerol	444.4	49.8
Monoacylglycerol	71.8	8.0
Alkyldiacylglycerol	18.5	2.1
Free sterols	304.3	34.1
Esterified sterols	53.6	6.0
Total	892.6	

[a]Data are averages of duplicate determinations.

Alkyl ethers were found by TLC (23) in the methanolysis products (23) of the polar lipids separated as a group from neutral lipids. PL were 96.2% recovered from TLC. The mol ratio for ester groups/glycerol for TG was 3.1, and for monoacylglycerols (MG) 1.1. The phosphorus:nitrogen ratio for PL containing nitrogen, the phosphorus:inositol ratio for phosphatidylinositol (PI) and the phosphorus:vicinal OH ratio for phosphatidyl glycerol (PG), were close to unity.

The calculated mol% contents of the different groups of lipids were as follows: neutral lipids (NL), 36.9; glycolipids (GL), 33.9; PL, 29.2; sterol-containing lipids, 22.4, and nitrogen-containing lipids, 20.7. On an individual basis, the lipid classes with mol% over 10 were: TG, 18.4; digalactosyldiacylglycerol (DGDG), 13.8; free sterols, 12.6, and phosphatidylcholine (PC), 11.0. These four lipid classes represent about half of the total lipid content of cassava root.

The content of TG measured by a digestion method (16) with triolein as standard resulted in a quite higher value (677.1 nmol TG/g fr wt) than the same content obtained through the determination of the glycerol moiety (18), (Table 3). The effect can be due to the length and degree of unsaturation of the component fatty acid chains of TG, which is known to affect the response of the digestion method.

Expressing the results on weight basis would change the relative order of some components, but when the interest is centered on non-storage lipids the mol basis is more convenient than the weight basis.

Because a whole organ (cassava root without cortex) was processed for the isolation of lipids, the resulting lipid mixture could not be otherwise but complex. In this mixture were present lipid classes characteristic of various cell structures (25), e.g., DGDG is abundant in plastid membranes, diphosphatidyldiglycerol is characteristic of mitochondrial inner membrane, etc.

As can be seen in Table 1, within the PL group the lipids at higher levels were PC, phosphatidylethanolamine (PE) and PI, in accordance with the ubiquity of these lipids in plant membranes (25); these three alone constitute ca. 80 mol% of the total PL content.

In GL, monogalactosyldiacylglycerol (MGDG) and DGDG were found in greatest amount; both of them together made up 67 mol% of the total GL. MGDG is the most abundant lipid in thylakoids (25), but because there is not information about the possible presence in cassava roots of chloroplast remnants, as is the case with the potato tuber (26), no suggestion about the possible locus for this very high proportion of MGDG in cassava roots could be advanced.

DGDG is a component of plastid membranes (25), and is thus expected in a reserve root like cassava.

The high content of free sterols found attract attention. A recent study on enriched plant plasma membrane has shown the existence of free sterol in amounts comparable to that of PL in that preparation (10), so the presence of free sterols in such a high amount in our preparation need not be surprising.

The major part of the lipids isolated were lipid classes that usually are associated with membrane structures in the cell. TG, MG and alkyldiacylglycerols, making up together only 22.1 mol% of total lipids, were a minor lipid fraction. The low content of TG, MG and alkyldiacylglycerols is not surprising in an organ that is not specialized in storing fat.

The profile of lipids that emerged is comparable to the profiles reported for turnip roots (13), potato tubers (26) and other produce not cited here.

When compared with the lipids from potato (26), the lipid profile of cassava is similar in component lipid classes. However, several differences are noticeable. The major PL classes are in higher content in potato (412 nmol PC/g fr wt and 204 nmol PE/g fr wt) than in cassava. The opposite is true for the minor PL (e.g., PS was present at 5 nmol/g fr wt). Other noticeable differences are the higher content in cassava of MGDG and DGDG than in potato (92 nmol MGDG/g fr wt and 184 nmol DGDG/g fr wt), and of TG (240 nmol TG/g fr wt, with the greatest difference in lipid content existing between free sterols (20 nmol free sterols/g fr wt in potato).

The lipids identified in cassava flour, PC, sulfolipid, steryl glycoside, MGDG and DGDG (5–6), were confirmed in this work.

Polygalactosyldiacylglycerol (6) was tentatively identified in this work as one of the minor spots in the TLCs run for polar lipids, on grounds of Rf values and a positive reaction for sugars.

The identification by previous authors (5–6) of rather few lipid classes in cassava products might be due to several causes, such as the decomposition of some lipid classes during processing of cassava roots, the incomplete extraction of lipids by the methods followed, or the interest of the authors in specific lipid classes.

The total lipid content of fresh cassava roots indicated they have little nutritional value. Teles et al. (4) reported a good saturated/unsaturated fatty acid balance in fresh cassava, but with no nutritional meaning due to their low content. However, the keeping properties of fresh produce make the difference between them reaching or not reaching the consumer, and lipids seem to play a major role in these post harvest properties of fresh cassava roots.

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