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RELATIONSHIP BETWEEN CHANGES IN LIPID WITH AGEING OF CASSAVA ROOTS AND SENESCENCE PARAMETERS

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Key Word Index—*Manihot esculenta*: Euphorbiacea; cassava; relationship; ageing; senescence; lipids; discoloration.

Abstract—The roots of cassava (*Manihot esculenta*) display parenchyma discoloration soon after unearthing. The progressive change in this parameter of senescence was evaluated and its relationship with simultaneous changes in lipid composition in the ageing of the roots were studied in two consecutive years. In total, seven phospholipids were measured, and of these phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and diphosphatidylglycerol showed concerted changes in content and proportion. These changes were correlated with the root discoloration. Overall decreases in total phospholipids and glyceroglycolipids contents, and increases in the ratio of total sterol containing lipids to total phospholipids and to total glyceroglycolipids, suggest alterations of membranes, similar to those occurring in other senescent plant organs. The results indicate a causal relationship between the changes in lipids and the parenchyma discoloration that occur in cassava roots after unearthing. The hypothesis that considers cassava discoloration as a manifestation of senescence, is supported.

INTRODUCTION

Cassava roots develop a discoloration of the parenchyma soon after they are unearthed. The alteration begins at the vascular bundles, and is termed vascular streaking [1]. Passan and Noon [2] pointed out that cassava roots senesce rapidly after harvest, and considered vascular streaking as a senescent reaction. Booth [3] found a close association between mechanical damage and vascular discoloration. Unavoidably, a severe wound is inflicted on the roots of cassava when they are separated from the aerial part of the plant at harvest, and deterioration could be initiated at this point. These two views of the genesis of vascular streaking need not be mutually exclusive of each other.

Tanaka *et al.* [4] pointed out the possibility that upon unearthing the roots of cassava and exposing them to a different environment, membrane disorganization or damage might occur as a response to this treatment. These alterations could cause a loss of compartmentation that would allow the darkening reactions and lead to vascular streaking. An independent indication of the possible implication of lipid in the processes responsible for vascular streaking is their presence among the material occluding the vascular vessels of affected cassava roots [5]. Passan and Noon [2] have demonstrated that vascular streaking is primarily of a physiological nature, though microbial action might result in vascular discoloration.

In several plant organs, concomitant changes occur in lipid composition, membrane structure and membrane function during senescence [6–11]. An early decrease in phospholipid (PL) is common in senescing plant organs [7, 8, 11], and was considered by Beutelman and Kende [7] as a marker of membrane degradation and senescence. Other changes leading to alterations in membrane properties were an increase in the proportion of sterol to PL [8, 11] and variations in the content of neutral lipids [9].

Studies with artificial membranes have shown that the steryl glucoside and esterified steryl glucoside composition [12] and changes in phospholipid head groups [13] affect the physicochemical properties of such membranes. These lipid classes have been demonstrated in cassava roots [14].

RESULTS

The cassava (*Manihot esculenta* Crantz cv. Algodona) roots used in both years (1987 and 1988) of this study followed the usual deterioration pattern for ambient conditions. By the time of the first sample preparation, i.e. one (1987) and two (1988) days from unearthing, and after the zero time analysis, fluorescence was visible in the majority of the pieces cut from whole cassava, which is in agreement with previous work [4]. After four days, the processed samples showed incipient vascular discoloration; this is the stage when vascular discoloration has unmistakably set in. The darkening became moderate by day seven for the 1987 cassava. After nine days, the roots were from moderately to severely affected by vascular streaking. Afterwards the roots started to show signs of microbial damage.

Weight loss was as much as 8.6% over 10 days storage. Due to this loss, and for calculation purposes, fresh weight was corrected to initial fresh weight. Moisture content fell from 71.0 to 63.1% during this period. Throughout the experimental period the total lipid content remained constant at 0.24% (0.02 s.d.) (1987) and 0.31% (0.04 s.d.) (1988).

Seventeen lipid classes were measured in total. The content of PL showed great variations during storage

(Table 1). The levels of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) displayed similar profiles, while that of diphosphatidylglycerol (DPG) followed an opposite course. Phosphatidylinositol (PI) content also showed a profile with sharp changes, unlike those for phosphatidylserine and phosphatidylglycerol. Since it is known that the amounts of macro-components of cassava roots show wide fluctuations from season to season, the contents of PL seem reasonably comparable both within and between the experiments performed in consecutive years. Phosphatidic acid was present in large amounts for most of the time of the second season; given equal conditions this could be due to the larger damage inflicted upon the cassava periderm during unearthing that year. Marriott *et al.* [15] have shown the great influence of periderm damage on the subsequent events in cassava.

The content of glycolipids (Table 2) and neutral lipids (Table 3) were comparable for both years and, with the exception of digalactosyl and monogalactosyldiacylglycerol, which decreased, their level remained more or less constant throughout the experimental period.

When grouping the analysed lipids as total PL, total glyceroglycolipids and total sterol-containing lipids, their contents vary with storage time (Fig. 1). The level of total PL and total glyceroglycolipids decreased. The total PL content dropped 41% during 1987 and 10% in 1988. The level of glyceroglycolipids decreased by 18 and 25% for the years 1987 and 1988 respectively. Sterol-containing lipids showed an initial decrease in their content, and a late recovery to their initial level.

The sum total of the molar contents of the lipids measured, excluding mono and triglycerides, declined 20% for the first year and 13% for the second year.

The ratio total sterol-containing lipids: total PL increased 76 and 14% over the experimental time for 1987 and 1988 respectively. The ratio total sterol-containing lipid:total glyceroglycolipids increased 27 and 28% during the same periods.

Table 1. Changes in phospholipids content with ageing of unearthed cassava roots

Age (days)	Phospholipids (nmol/g initial fr. wt)*						
	PC	PE	PI	PG	DPG	PS	PA
Year 1987							
0	265	152	143	22	20	14	90
2	166	126	53	37	42	39	54
4	79	47	90	37	102	17	111
7	127	72	82	20	66	19	93
9	172	93	52	21	40	5	37
Year 1988							
1	131	66	109	44	37	18	257
3	133	90	52	44	22	16	272
6	71	39	99	46	121	20	201
10	155	104	100	37	89	16	96

*Data are averages of duplicate determinations.

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethalamine; PI, phosphatidylinositol; PG, phosphatidylglycerol, DPG, diphosphatidylglycerol; PS, phosphatidylserine; PA, phosphatidic acid.

The changes in content and proportion of lipids were sharper towards the beginning of the experiment, particularly between harvest day and the next sampling date. It was not possible to prepare any extract on the day of harvest during the second year. This is reflected in the smaller overall changes observed in 1988 and indicates the importance of taking samples as close as practicable to the moment of unearthing of the roots.

DISCUSSION

PL showed considerable changes in content and proportion during the ageing of cassava roots (Table 1). The PL which showed the greatest changes were those present in the greatest amounts, e.g. PC, PE and PI; which are the most ubiquitous in plant membranes [14]. These results contrast with the lack of change in proportion of the PL of flower petals [11], flower buds [7] and bean cotyledons [8] during ageing. The changes in level and proportion of PL (Table 1) look concerted, and are concomitant with the discoloration of cassava parenchyma. It is noteworthy that the major maxima and minima of the PL patterns occurred by the time when vascular streaking was overtly observable. DPG was one of the lipids exhibiting a maxima at this stage. Since DPG is mainly located in the inner mitochondrial membrane, and the darkening reactions involved in vascular streaking are oxidative, this correspondence seems of interest. Hirose *et al.* [16] reported a relationship between respiration profiles and vascular streaking in cassava.

The lipids of cassava are mainly of membrane origin [14], therefore, changes in lipid composition are likely to affect cassava membranes. Michaelson *et al.* [13] found that changes in the proportion of PL head groups affect membrane fluidity, which suggests a possible link between changes in PL composition and the evolution of senescence parameters in cassava.

Considering PL as a group, their content showed a decrease (Fig. 1), comparable to the loss of PL which, for other plant organs, has been correlated with structural and functional alternations in membranes [8], decreases in membrane fluidity [11] and changes in cellular compartmentation [7]. These changes in membranes lead to the senescence of the corresponding plant organs.

The level of TLC-resolved steryl lipids did not show any pronounced trend (Table 3), but total sterol-containing lipid displayed an initial decline in content followed by an increase that brought back the content to its initial value (Fig. 1). The proportion of total sterol-containing lipids to total PL and to total glyceroglycolipids was increased overall. These results are consistent with the capability of steryl lipids to moderate membrane properties, and with known relationships between changes in steryl lipids and senescence of plant organs via alterations in the physicochemical properties of membranes [8, 9, 11, 12].

McKersie and Thompson [9] found that some unidentified neutral lipids induced changes in properties of plant membranes. Among the TLC-resolved neutral lipids of cassava, four were not identified [14], so they were not measured in this study, and no further discussion on their possible effect is warranted.

The changes found in cassava lipids during after harvest evolution were extensive, and should have a deep influence on the underlying biochemical processes, or,

Table 2. Changes in glycolipids content with ageing of unearthed cassava roots

Age (days)	Glycolipids (nmol/g initial fr. wt)*					
	DGDG	MGDG	SG	Cerb	ESG	SL
Year 1987						
0	467	217	116	69	67	16
2	368	175	87	69	23	11
4	316	135	103	96	33	27
7	296	96	136	107	32	7
9	361	123	86	92	43	14
Year 1988						
1	549	171	147	76	21	45
3	553	178	119	104	23	13
6	391	143	143	144	26	40
10	333	157	129	92	82	45

*Data are averages of duplicate determinations.

Abbreviations: DGDG, digalactosydiglyceride; MGDG, monogalactosyldiglyceride; SG. steryl glucoside; Cerb, cerebroside; ESG, esterified steryl glucoside; SL, sulpholipid.

Table 3. Changes in neutral lipids content with ageing of unearthed cassava roots

Age (days)	Neutral lipids (nmol/g initial fr. wt)*			
	TG	MG	FS	ES
Year 1987				
0	677	42	304	54
2	583	42	238	120
4	673	24	197	113
7	646	39	267	161
9	688	49	290	148
Year 1988				
1	514	66	344	99
3	343	56	297	165
6	418	48	293	100
10	410	70	293	128

*Data are averages of duplicate determinations.

Abbreviations: TG, triglycerides; MG, monoglycerides, FS, free sterol; ES, esterified sterol.

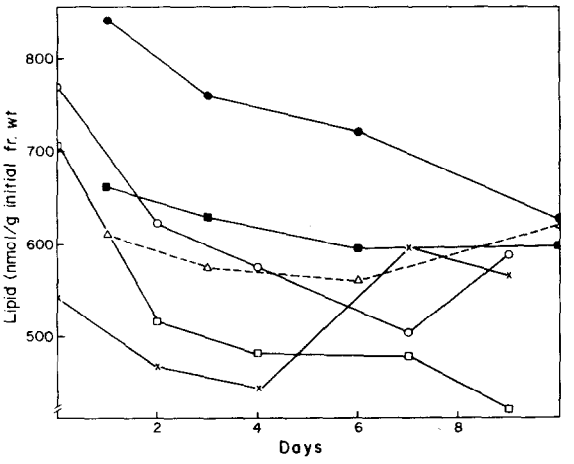

Fig. 1. Changes in the content of total phospholipids, total glyceroglycolipids and total sterol-containing lipids with ageing of unearthed cassava roots. Phospholipids: □ year 1987, ■ year 1988. Glyceroglycolipids: ○ year 1987, ● year 1988. Sterol-containing lipids: × year 1987, △ year 1988.

alternatively, reflect ongoing biochemical and physiological processes. Our results indicate a causal relationship between the changes in lipid classes with ageing of cassava roots and the discoloration of the parenchyma of the roots.

The results presented are in line with literature data about progressive changes in lipids with age of several plant organs, and their simultaneity with the advance of senescence [7, 8, 10]. Therefore, it can be expected that the changes determined in membranes of these plant organs also occur in cassava roots. Changes in cassava lipids with ageing could cause structural alterations in cassava membranes, which might allow reactants and enzymes involved in darkening reactions to come into contact, thus leading to parenchyma discoloration.

The view of vascular streaking of cassava as a phenomenon of senescence is sustained by the results obtained. The signal for the onset of senescence might be the wounds caused by harvest.

EXPERIMENTAL

Cassava roots. Cassava roots (*Manihot esculenta* Crantz, c.v. Algodona) of 10 months of age were purchased on the farm at harvest, in the locality of El Cambur de Paya, Aragua State, Venezuela, in March of both 1987 and 1988, and transported by car to the laboratory in less than 1.5 hr. The 1988 season was drier than the 1987 one, and so as a consequence in 1988 the cassava roots underwent more damage in the periderm during unearthing than these of the previous year.

The roots were kept in the dark under stable conditions: 23.8° (0.96 s.d.) and 59.1% RH (7.38 s.d.) for the 1987 harvest, and 25.0° (0.74 s.d.) and 81.4% HR (3.70 s.d.) for the 1988 season, until samples were taken for analyses at 0, 2, 4, 7 and 9 days and 1, 3, 6 and 10 days for the first and second year experiments. The dry conditions in the second year made unearthing more difficult, and the consequent delay impeded us preparing a zero time extract. Only uninfected cassava pieces were taken for lipid analysis.

Lipid extracts. Three roots were selected per analysis so as to encompass the range of root sizes. Three 2 cm wide discs were cut from the proximal, medial and distal parts of the roots, the cortex was removed and the remaining cylinders cut into small pieces, mixed thoroughly and an aliquot taken for lipid extraction with $CHCl_3$–MeOH by the method of ref. [17]. These extracts were further treated as previously reported [14], and stored at −70° until needed. Total lipid content was calculated from the dry wt of the extracts.

Evaluation of the discoloration. The extent of vascular streaking was evaluated, following the methodology of previous authors [1, 15], by visual estimation of the intensity of discoloration and area affected. Five stages were distinguished: no discoloration, no discoloration plus blue fluorescence under UV light, and incipient, moderate and extensive discoloration.

Lipid analysis. Lipid classes were resolved by TLC on silica gel H and HR. 2D-chromatography with plate activation was required for the separation of PL and glycolipids. The solvent systems $CHCl_3$–MeOH–conc NH_4OH (65:30:4) and $CHCl_3$–MeOH–HOAc–H_2O (170:25:25:6) were used for the first and second dimensions respectively [18]. Neutral lipids were resolved by double irrigation chromatography without plate activation, the solvent system was petrol (60–80°)–Et_2O–HOAc (85:15:1) [19]. Duplicate chromatograms were run for each determination. The separated lipids were stained with I_2 and the bands scraped from the plates for further chemical determinations. Procedures have been extensively described in a previous paper [14].

Chemicals. All the reagents used were of analytical purity. Lipid and other standards were from Sigma Chem. Co., St. Louis, Missouri, and Applied Sci., State College Pennsylvania, U.S.A. Silica gels were from Merck, Darmstadt, F.R.G.

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