Membrane Lipid Metabolism, Cell Permeability, and Ultrastructural Changes in Lightly Processed Carrots

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ABSTRACT

We monitored changes in phospholipid (PL), steryl lipid, and glycolipid classes, cell permeability, and ultrastructure in wound-stressed tissues (shreds and disks) of carrots (*Daucus carota* L. 'Apache'), stored up to 10 days at 10°C and 95% RH. Total PL rose 47% ten days after shredding, with phosphatidylcholine decreasing and phosphatidic acid increasing in relative abundance. Acylated sterol glycoside doubled after 2 days. Leakage of UV-absorbing metabolites from disks increased by 45% between 1 and 3 days storage. Extensive, storage-dependent accumulation of endoplasmic reticulum and attached ribosomes within vascular parenchyma cells occurred 10 days after wounding. Thus net synthesis of membrane lipid components occurred together with increases in permeability and the accumulation of phosphatidic acid. Membrane degradation and repair processes likely coexist during prolonged storage of lightly processed carrots.

Key Words: membrane repair, senescence, phospholipids, glycolipids, endoplasmic reticulum

INTRODUCTION

CONSUMER DEMAND for lightly processed or precut vegetables has increased, but limited shelf life has slowed development of new markets (Bolin and Huxsoll, 1989). Lightly processed tissues experience injury upon cutting or slicing and thus are more perishable and senescence-prone than the intact organs from which they were obtained (Huxsoll et al., 1988). Tissues of quiescent plant storage organs (fleshy roots, tubers, etc.) become highly activated after cutting or slicing and incubation in a moist environment (Kahl, 1974). Wounding likely leads to rupture of membranes and activation of membrane lipid catabolism (Kahl, 1982), the extent of which is species-related. For example, in potato tubers, up to 30% of membrane phospholipid (PL) is lost within 2 hr of cutting whereas in carrot roots, no measurable PL breakdown occurs (Theologis and Laties, 1980), despite the considerable disorganization of cells which may occur at the cut surface (Tatsumi et al., 1991).

Wounding also initiates a complex series of slower metabolic changes which are related to *de novo* synthesis of proteins and which comprise cellular "repair" processes. The biosynthesis of new membranes is an important component of this response. In potato, a marked increase in lipid synthesizing ability in wounded tuber tissue within hours of excision (Tang and Castelfranco, 1968) is associated with induction of key enzymes in PL synthesis (Kahl, 1983) as well as fatty acid synthesis (Willemot and Stumpf, 1967). Ultrastructural changes documented in such tissues are consistent with increased protein synthesis, membrane repair, and secretory phenomena. These include development of endoplasmic reticulum (particularly lamellae with bound ribosomes), appearance of polyribosomes, nuclear enlargement, and increased number of well-developed dictyosomes (Leaver and Key, 1967; Sparkuhl et al., 1976; Barckhausen, 1978).

Many reports have been published describing metabolic and ultrastructural changes within cells of wounded storage organs (Kahl, 1974, 1982, and 1983; Barckhausen, 1978; Mazliak and Kader, 1978; Stanley, 1991). Most studies have monitored changes in excised tissue segments (often from potato tuber) up to 24 hr after wounding. However, very little is known about membrane lipid metabolism and ultrastructural modifications of lightly processed organs which must necessarily withstand more prolonged storage (e.g., days). This may be because interest in lightly processed foods has emerged only recently (King and Bolin, 1989).

Better understanding of membrane lipid metabolism in cells is needed in order to determine how senescence is regulated, particularly in lightly processed fruits and vegetables. Our objective was to monitor levels of membrane lipids and examine permeability and ultrastructural changes of stored, lightly processed carrots.

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MATERIALS & METHODS

Test material and experimental conditions

Whole 'Apache' carrots were obtained from a wholesale distributor, and after peeling, 5-cm root sections were shredded using a food processor. Shredded samples (25g fresh weight) were either frozen immediately in liquid N_2 and kept sealed under N_2 gas at −80°C (0 days storage), or stored to simulate conditions in retail markets. During storage, shreds were placed on layered plastic grids within a 10-L plastic container (≈300g total fresh weight) covered with a polyethylene bag and aerated with humidified air at 15 mL/min. The container was stored in a controlled room at 95% RH and 10°C. After 2, 5, and 10 days, samples were frozen and stored at −80°C as described.

For cell permeability evaluation (see below), carrot disks were placed atop 2.5 cm^2-wide plastic cups and stored within sealed 1-L glass jars (≈6.5g total fresh weight tissue/jar) aerated at 3–5 mL/min.

Sample preparation and lipid analyses

Frozen tissue was lyophilized, ground, and a 200-mg dried subsample was homogenized in $CHCl_3$:MeOH (2:1) with three 15-s bursts of a Polytron homogenizer. Homogenates were filtered through a sintered glass funnel and the residue was re-extracted with $CHCl_3$:MeOH (2:1). Combined extracts were washed sequentially with 0.85% (w/v) NaCl and MeOH:H_2O (1:1). The $CHCl_3$ phase containing total lipids was evaporated to dryness under N_2 and redissolved in 1 mL $CHCl_3$. The total lipid extract was passed through a silica Sep-Pak cartridge (Waters, Milford, MA) to sequentially elute neutral lipid (NL), glycolipid (GL), and phospholipid (PL) fractions after modifying the method of Glass (1990). Only a single cartridge was used per sample and it was preconditioned with 3 mL $CHCl_3$. Four mL $CHCl_3$ eluted pigments and steryl esters (the latter were typically below detection limits); 8 mL $CHCl_3$:Me_2CO (9:1) eluted free sterols (FS) and pigments of greater polarity; 8 mL Me_2CO eluted glycolipids (GLs), including acylated sterol glycoside (ASG), sterol glycoside (SG), and monogalactosyldiacylglycerol (MGDG); finally, phospholipids (PLs) and digalactosyldiacylglycerol (DGDG) were eluted with 12 mL MeOH:H_2O (9:1). Each collected fraction was evaporated to dryness under N_2, redissolved in 1 mL $CHCl_3$ for NL and GL, or 1 mL $CHCl_3$:MeOH (1:1) for PL, sealed under N_2, and stored at −80°C until time of analyses.

Table 1—Phospholipid concentrations (mg/100g dry wt) in 'Apache' carrot shreds during 10 days storage at 10°C and 95% RH[x]

Storage (days)	Phospholipid class[y]					
	PC	PE	PI	PA	LPC	Total
0	423 ± 27	210 ± 15	75 ± 17	76 ± 24	9 ± 0.2	793 ± 83
2	464 ± 11	226 ± 6	71 ± 3	79 ± 7	6 ± 0.1	847 ± 23
5	505 ± 29	247 ± 9	84 ± 7	114 ± 15	8 ± 0.5	958 ± 60
10	522 ± 18	266 ± 11	94 ± 8	151 ± 10	7 ± 0.7	1039 ± 47

[x] Each value is the mean ± standard deviation of three sample replicates.

[y] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatidic acid; LPC, lysophosphatidylcholine. Phosphatidylglycerol and lysophosphatidylethanolamine were below detection limits.

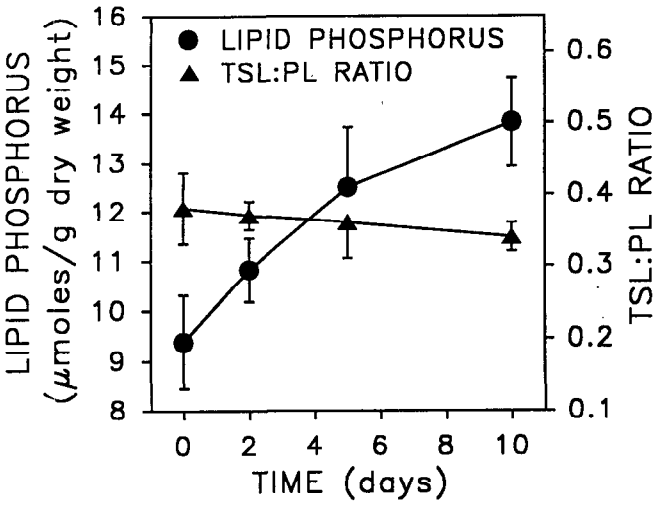

Fig. 1—Changes in total lipid-P and in the mole ratio of total steryl lipid:phospholipid (TSL:PL) in 'Apache' carrot shreds during 10 days storage at 10°C and 95% RH. Each point is the mean ± standard deviation of three sample replicates. Molar values for steryl lipids (moles/g dry weight of tissue) were calculated from data in Table 4 using the following molecular weights: FS = 410; ASG = 825; and SG = 572. Molar values for total PL (moles/g dry weight) were obtained by phosphate assay.

Free sterols (FS) in the NL fraction were isolated and quantified by GLC (Whitaker and Lusby, 1989) using lathosterol (cholest-7-en-3β-ol; Sigma, St. Louis, MO) as an internal standard (10 μg added to total lipid extract). Total lipid phosphorus (lipid-P) was determined on duplicate, 20-μl aliquots taken from the final PL fraction using the method of Ames (1966).

Component classes in GL and PL fractions were resolved within separate samples (injections) by normal phase HPLC using a 10 cm × 3 mm ChromSep LiChrosorb Si 60 (5-μm) silica cartridge system (Chrompack, Raritan, NJ). The HPLC instrument was equipped with a WISP 712 programmable injector, 600E quaternary solvent delivery system/gradient controller, and Maxima 820 software with personal computer to determine peak areas and automate analyses (all hardware from Waters, Milford, MA; software from Dynamic Solutions, Millipore Corp., Ventura, CA).

Prior to injection, aliquots of GL and PL fractions were taken to dryness, redissolved in HPLC mobile phase (1:1 mixture of 2-propanol:hexane for GL, or 58:40:2 mixture of 2-propanol:hexane:H_2O for PL), and passed through a 0.2-μm PTFE membrane filter (Gelman Sciences, Ann Arbor, MI) using a gas-tight syringe. The syringe and filter were flushed twice with mobile phase to recover held-up sample volume, and the combined filtrates were dried and again redissolved in a known volume (250–500 μL) of the same solvent. The injection volume (100 μL) represented 14% or 20% of the total PL or GL fraction, respectively.

GL and PL components were detected using a Varex IIA evaporative light scattering detector (Varex Corp., Burtonsville, MD) with N_2 flow rate at 45 mm (2.5 L/min) and the drift tube temperature at 90°C. Individual GL and PL classes were quantified by external standardization using calibration curves generated using authentic standards. MGDG, DGDG, SG, and ASG standards were purchased from Matreya (Pleasant Gap, PA). PL standards were obtained from Sigma (St. Louis, MO). HPLC grade solvents were obtained from Mallinckrodt Specialty Chemicals, Inc. (Paris, KY).

The mobile phase was similar to that used by Moreau et al. (1990) and Letter (1992) with modifications in elution times and flow rate. PLs (including the GL DGDG) were eluted with a mobile phase of 2-propanol:hexane:H_2O using a logarithmic gradient from 58:40:2 to 52:40:8 in 20 min, a hold for 40 min, a reverse linear gradient to the starting solvent mixture in 15 min, and a 40-min hold for re-equilibration. GLs were eluted using linear gradients (2-propanol:hexane without H_2O) from 5:95 to 20:80 in 15 min, to 40:60 in 10 min, a 25-min hold, a reverse to the starting solvent mixture in 5 min, and a 40-min hold for re-equilibration. The flow rate was 0.5 mL/min throughout all analyses. Lipid class data represent the mean ± standard deviation of three sample replicates, each derived from two roots.

Cell permeability

In a second experiment, carrot slices were prepared by first using the food processor (equipped with single blade), then disks (7 mm wide × 4 mm thick) were obtained from secondary vascular tissue of the slices using a cork borer. Disks (averaging 180 ± 10 mg fresh weight) were randomized, then immersed for 15 min in solutions containing deionized water or $CaCl_2$ (45 or 90 mM) on an orbital shaker. An earlier study showed that exposure to water did not increase cell permeability from carrot tissue when compared to hypertonic solutions (Simon, 1977). The pH of all solutions ranged from 5.8 to 6.0. Immediately following treatment, disks were placed in a salad spinner and spun for 30 sec at 200 rpm to remove residual treatment solution. Tissues were then held in storage for 1, 3, or 5 days. Each treatment was placed in a storage jar and was composed of four, 3-disk replicates per storage interval.

Following storage, the leakage of UV-absorbing solutes was monitored using methods reported by Picchioni et al., (1991) with slight modifications. Three disks per treatment were removed from storage and incubated in 7.5 mL deionized water on an orbital shaker for 4 hr at 25°C. Three-ml volumes of incubation medium were centrifuged at 1300× g for 10 min, and leakage was expressed as absorbance of the clarified solution measured at 260 nm (A_{260}) using a Shimadzu UV-160 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Transmission electron microscopy (TEM)

In a third experiment, carrot shreds were prepared and stored as described above. Samples (2 to 3 mm^3) of carrot were randomly taken from shreds that were freshly prepared and from others that had been stored for 10 days. The excised samples were chemically fixed with 2.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.2 for 5 hr, washed in cacodylate buffer, and postfixed overnight in 1% OsO_4. After dehydration in an alcohol series, samples were embedded in Spurr's resin as described by Roland and Vian (1991). Ultrathin sections were cut with a diamond knife, stained with 1% uranyl acetate for 10 min and 2% lead citrate for 2 min, and then observed with a Hitachi H500H transmission electron microscope operating at 75 keV.

RESULTS & DISCUSSION

Lipid composition and cell permeability

PL composition of carrot roots showed a pattern typical of higher plants in that phosphatidylcholine (PC) was the dominant component followed by phosphatidylethanolamine (PE) (Table 1). The total PL mass in each sample was calculated both by lipid-P determination (using average MW of 750) and by summation of individual PL classes determined by integration of HPLC peaks. Total PL values by the 2 methods were in good agreement (lipid-P values = 96% ± 5% of HPLC values). Total lipid-P increased by 15%, 33%, and 47% within

Table 2—Distribution of individual PL classes expressed as the weight percent of total PL in 'Apache' carrot shreds during 10 days storage at 10°C and 95% RH[z]

Storage (days)	Phospholipid class[z]				
	PC	PE	PI	PA	LPC
0	54 ± 2	27 ± 1	9 ± 1.1	9 ± 2	1.0 ± 0.1
2	55 ± 1	27 ± 0.3	8 ± 0.1	9 ± 1	0.7 ± 0.01
5	53 ± 0.4	26 ± 1	9 ± 0.3	12 ± 1	0.8 ± 0.1
10	50 ± 1	26 ± 0.1	9 ± 0.3	15 ± 0.5	0.7 ± 0.04

[z] Each value is the mean ± standard deviation of three sample replicates. For abbreviations see Table I.

Table 3—Leakage of solutes from 'Apache' carrot disks during 5 days storage at 10°C and 95% RH[z]

$CaCl_2$ conc (mM)	Time in storage (days)		
	1	3	5
0	0.029 ± 0.003	0.042 ± 0.002	0.036 ± 0.008
45	0.031 ± 0.003	0.030 ± 0.005	0.025 ± 0.002
90	0.032 ± 0.001	0.030 ± 0.004	0.024 ± 0.003

[z] Each value is the mean ± standard deviation of 4, three-disk replicates. Prior to storage, disks were pretreated for 10 min with water (0 mM $CaCl_2$) or with Ca-containing solutions (all at pH 5.8–6.0). Following storage, leakage was measured and expressed as UV absorbance of released solutes (A_{260}). Data are the A_{260} values obtained following a 4-hr incubation period in water at 25°C.

Table 4—Steryl lipid concentrations (mg/100g dry weight) in 'Apache' carrot shreds during 10 days storage at 10°C and 95% RH[z]

Storage (days)	Steryl lipid[y]			
	FS	ASG	SG	Total
0	110 ± 5	49 ± 3	14 ± 0.3	174 ± 5
2	107 ± 7	98 ± 14	14 ± 1.0	219 ± 8
5	117 ± 2	115 ± 9	14 ± 0.7	246 ± 11
10	121 ± 4	119 ± 9	15 ± 2.5	255 ± 14

[z] Each value is the mean ± standard deviation of three sample replicates.
[y] Abbreviations: FS, free sterol; ASG, acylated sterol glycoside; SG, sterol glycoside.

2, 5, and 10 days of storage, respectively (Fig. 1). All PLs except lysophosphatidylcholine (LPC) increased in concentration from 0 to 10 days. PC, PE, and phosphatidylinositol (PI) rose 23% to 27% by 10 days, whereas phosphatidic acid (PA) increased 99% by day 10. Thus, PA increased at the highest rate and represented a significantly greater proportion of the total PL fraction after 5 and 10 days storage.

The proportions of individual PL classes (from Table 1) varied little during storage, except that the relative amounts of PC and PA changed inversely over time (Table 2). PA is generally regarded as a membrane degradation product resulting from action of phospholipase-D (Larsson et al., 1990). This enzyme affects membrane-bound PLs in disrupted or senescing plant tissue (Galliard et al., 1976; Chéour et al., 1992) and can be specific for PC over other PLs (Mounts and Nash, 1990). Phospholipase-D activity in carrot storage root extracts ranked relatively high in a survey among many plant species and organs (Quarles and Dawson, 1969). Thus, the inverse changes in proportions of PC and PA (decrease in PC offset by increase in PA) probably reflects PL catabolism.

Such an accumulation of PA (Table 1) is atypical, because PA is usually rapidly hydrolyzed to diacylglycerol by phosphatidate phosphatase in the pathway of membrane lipid degradation associated with senescence (Paliyath and Droillard, 1992). Possibly, the extraction method used did not completely inactivate phospholipase-D, which could have contributed to the high PA levels. Total inactivation could have led to lower PA values on day 0 and, quantitatively, a more substantial increase during storage (C. Willemot, personal communication).

PA is a central precursor in PL synthesis (Joyard and Douce, 1979; Moore, 1982). Thus, whether the increase in PA was related to the overall increase in PL content, or whether it was a consequence of lipolytic activity, is unclear. Isotopic labelling studies are needed to elucidate this question. The indication that total PL began to increase before PA (Table 1) appears to support the lipolytic activity explanation.

Cell permeability (leakage) of all disks (pretreated with water or Ca-containing solutions) was similar following one day of storage (Table 3). However, a 45% increase in leakage from disks pretreated with water only occurred between 1 and 3 days storage, providing further evidence for membrane degradation. Between 3 and 5 days storage, average leakage from water-treated disks decreased, but was variable. Leakage of electrolytes from water-treated carrot slices increased to a similar degree during storage (data not shown).

In contrast to water-treated disks, leakage from Ca-treated disks remained constant between 1 and 3 days storage, then decreased 17–20% by the fifth day. This indicates that Ca pretreatments may increase membrane integrity and thus reduce the rate of senescence of cut and stored carrot tissues. Enoch and Glinka (1983) reported a similar finding using carrot disks during shorter experimental periods (4 hr in aqueous media).

Total steryl lipids (TSL = FS + ASG + SG) increased by 48% between 0 and 10 days storage (Table 4). This partly resulted from the marginal, 10% increase in total FS. It was mainly from the large increase in ASG, which doubled in only 2 days and showed further increases after 5 and 10 days. The increase in ASG was coincident with that of PL (predominantly PC and PE). This is consistent with the findings that, in higher plants, ASG synthesis is directly stimulated by PC (Forsee et al., 1974) or PE (Péaud-Lenoël and Axelos, 1972), which presumably serve as fatty acid donors.

The physiological basis of the ASG increase is not known, but sterol synthesis and conjugation could have a major influence on membrane properties (Benveniste, 1978). Reversible esterification/de-esterification and glycosylation/de-glycosylation have been suggested to exert modulatory effects on membrane organization and function (Wojciechowski, 1980). Moreau and Preisig (1993) demonstrated that ASG accumulates in plant cells during stress acclimation. Thus, the increase in ASG that we observed (following wounding stress) may be an indication of cell viability (R. Moreau, personal communication).

SG concentrations were comparatively low, which may reflect continued high levels of SG-6'-O-acyltransferase activity (Hartmann and Benveniste, 1987). Accumulation of ASG without depletion of FS and SG pools (Table 4) suggests continued FS synthesis and glycosylation during storage, although no substantial increase occurred in concentrations of FS and SG. However, the FS composition was altered following 10 days storage. The relative content of the two dominant sterols (stigmasterol and sitosterol) shifted, such that the ratio of stigmasterol:sitosterol increased from 0.30 to 0.45 during the 10-day period (Table 5). This specific change in FS composition has occurred during fruit ripening (Whitaker, 1988), and would be expected to result in decreased ordering and increased permeability of plant PL bilayers (Schuler et al., 1991).

The TSL:PL molar ratio decreased marginally during the 10-day storage (Fig. 1), but averages were not significantly different (statistical data not shown). The relative stability of this ratio may be adaptive. Coordinated regulation of sterol and PL pathways is believed to ensure optimal sterol:PL interaction in membranes (Burden et al., 1990). The free sterol: PL molar ratio decreased from 0.29 to 0.22 during 10 days storage (data not shown). This contrasts with earlier findings involving cabbage leaf disks aged at 15°C for up to 14 days

Table 5—Free sterol (FS) composition (weight percent of total FS) and ratio of stigmasterol to sitosterol in 'Apache' carrot shreds during 10 days storage at 10°C and 95% RH[z]

Storage (days)	Sterol moiety			Stigmasterol: sitosterol ratio
	Campesterol	Stigmasterol	Sitosterol	
0	12 ± 0.9	20 ± 5	68 ± 5	0.30 ± 0.09
2	11 ± 1.4	20 ± 3	69 ± 4	0.30 ± 0.06
5	10 ± 0.8	19 ± 2	70 ± 3	0.28 ± 0.04
10	10 ± 0.2	28 ± 3	62 ± 3	0.45 ± 0.07

[z] Each value is the mean ± standard deviation of three sample replicates.

Table 6—Galactolipid concentrations (mg/100g dry weight) in 'Apache' carrot shreds during 10 days storage at 10°C and 95% RH[z]

Storage (days)	Galactolipid[y]	
	MGDG	DGDG[x]
0	185 ± 8	366 ± 21
2	168 ± 1	323 ± 33
5	200 ± 42	337 ± 29
10	211 ± 17	361 ± 41

[z] Each value is the mean ± standard deviation of three sample replicates.
[y] Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol.
[x] DGDG was collected in the phospholipid fraction during preparative chromatography (thus quantitated with the phospholipid classes), but is included in this table for simplicity.

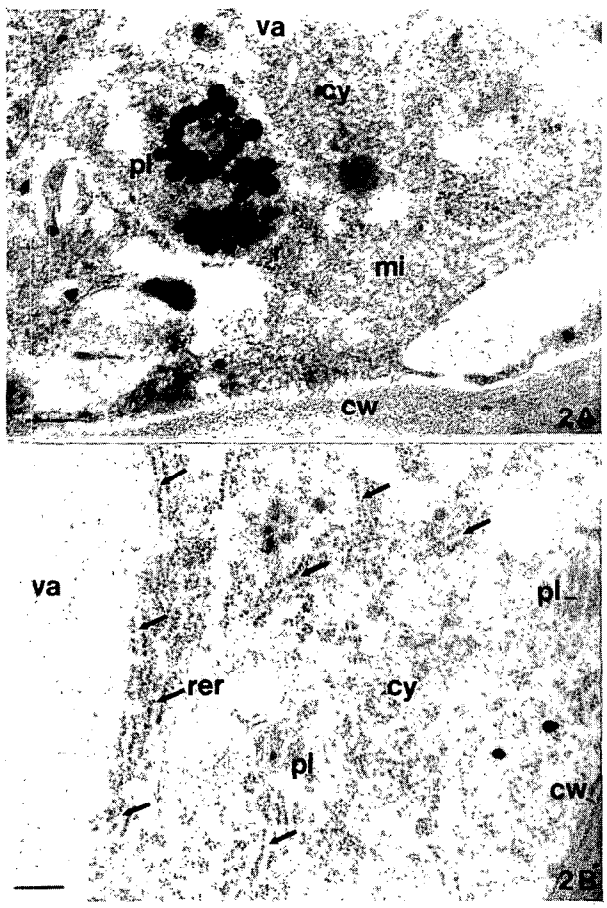

Fig. 2—TEM of thin sections showing typical vascular parenchyma cells in 'Apache' carrot shreds. Fig. 2A, portion of a parenchyma cell from a freshly shredded carrot. Fig. 2B, portion of a parenchyma cell from a carrot shred that had been stored for 10 days at 10°C and 95% RH. Cisternae of the ER (RER shown by arrows) are longer and more abundant in the stored tissue than in fresh tissue. Abbreviations used are as follows: cy, cytoplasm; cw, cell wall; mi, mitochondrion, pl, plastid; r, ribosome; rer, rough endoplasmic reticulum; va, vacuole. Bars represent 0.30 μm. (Magnification × 25000)

(Chéour et al., 1992), during which time a measurable increase in free sterol:PL was attributed to membrane degradative processes.

Slicing and cutting may advance the onset of senescence in plant organs (Huxsoll et al., 1988). In ripening fruit, measurable losses in membrane lipids and increases in the proportion of steryl lipids to PL are commonly reported (Thompson, 1988; Stanley, 1991). Thus, the coincident increases in PL and TSL and the lack of significant change in TSL:PL ratio in our study are contrary to known changes during genetically-programmed senescence. This suggests that, under the storage conditions we used, lightly processed carrots undergo a prolonged period of active lipid synthesis necessary for biogenesis of new membranes.

Previous studies involving plant storage organs (largely potato tuber) have demonstrated increases in lipid synthesis in wounded tissue, but typically only during the first several hours (e.g., < 24 hr) subsequent to wounding (Mazliak and Kader, 1978 and references cited therein). However, De Siervo (1990) reported a large reduction in PE concentration over an 8-day period in wounded tuber tissue of 2 potato cultivars. This indicates a major difference in membrane lipid metabolism of wounded potato tuber and wounded carrot storage root, 2 hr after wounding (Theologis and Laties, 1980) as well as following long-term incubation or storage (5–10 days).

On average, DGDG and MGDG were present in a mass ratio of almost 2:1. There was little or no indication that their concentrations changed over time (Table 6), demonstrating that storage did not result in a general increase in all lipids. Galactolipids are most abundant in plastidic membranes (Miernyk, 1985; Mudd, 1967), such as chromoplasts and amyloplasts of the carrot storage root (Grote and Fromme, 1984). Thus, the relatively constant MGDG and DGDG concentrations suggests the probability of site-dependent changes in membrane lipid metabolism.

Ultrastructural changes during storage

The mature carrot storage root primarily consists of secondary vascular tissue that contains the xylem and phloem parenchyma cells (Esau, 1940). Observations were limited to these cells, which appeared to be the most ultrastructurally and metabolically active. Parenchyma consisted of polygon-shaped cells, each with a large central vacuole and a thin parietal layer of cytoplasm that was bounded by the plasma membrane and pressed to the cell wall. In freshly shredded carrots, the cytoplasm contained a dense ribosome population, mitochondria, and plastids (primarily chromoplasts). Rough endoplasmic reticulum (RER) was only occasionally present (Fig. 2A). After 10 days storage, however, RER was the most conspicuous organelle. Several parallel layers of cisternae were found along the vacuole, with more randomly oriented membranes common in the cytoplasm (Fig. 2B). In order to establish with certainty that variations in RER lamellae occurred between fresh and stored tissues, large numbers or cells were examined. Observed changes in frequency or structure of cellular organelles (besides RFR) during storage were inconclusive.

The proliferation of RER supports the conclusion that membrane repair processes were active up to 10 days after wounding, since in plant cells, the ER is the primary site of PL and FS synthesis (Yamada et al., 1980; Moore, 1982; Browse and Somerville, 1991; Hartmann and Benveniste, 1987). In addition, the constant TSL:PL ratio suggests the synthesis of complete membranes with all their components, as indicated by the increase in RER. Such repair processes may be intensified following Ca pretreatments (Table 3), which would likely result in improved shelf life of lightly processed carrots. However, further study is necessary to verify this.

The greater abundance of RER in stored compared to freshly-cut tissue was somewhat expected, since only hours

after excision, the proliferation of RER is characteristic of wound tissue (Asahi, 1978). Also, ER strands are seen infrequently as part of cells in the resting state (Kahl, 1982). However, ER biogenesis in wounded plant storage organs has received very little evaluation during long-term periods (e.g., 10 days). Jackman and Van Steveninck (1967) reported a similar finding in beetroot disks aged up to 8 days at 24°C. In their study, ER lamellae were reduced to vesicles 2 hr after excision, but formed a near continuous layer within the cytoplasm 2 days later, which persisted up to 8 days.

Presumably, a buildup of RER would be of major importance in the wound repair mechanism. In addition to its role in membrane lipid synthesis, ER (RER) would be essential in the enzyme induction processes known to occur within 12 hr of wounding in storage organs (Kahl, 1983). Also, chemical modification of cell walls during wound healing, such as transport and secretion of phenolics and other precursors in lignin and suberin synthesis (Kahl, 1983; Kolattukudy, 1978), probably depends on ER vesiculation (Benveniste, 1978). For example, Babic et al. (1993) showed that, among four cultivars, storage stability of carrot shreds correlated with the rate of chlorogenic acid accumulation in the tissue 24 hr after wounding.

Cell walls of injured carrot root tissue (disks) are also known to accumulate large amounts of hydroxyproline-containing polypeptides/glycoproteins (e.g., extensin), even when aged as long as 6 days at 30°C (Chrispeels, 1969). In carrot disks, extensin biosynthesis and cell secretory capacity are specifically enhanced in response to excision and incubation (Chrispeels et al., 1974). Furthermore, ER lamellae or bound ribosomes may be involved in the extensin glycosylation process (Sadava and Chrispeels, 1978), in translation of extensin mRNA (Barckhausen, 1978), and in the secretion of extensin (Brodl and Ho, 1992).

Further study is needed to determine specific cellular membranes affected by these metabolic changes and to identify key enzymes involved in the repair process. Possibly, such enzymes can be genetically manipulated in the development of new cultivars with extended shelf life.

CONCLUSION

MEMBRANE LIPID METABOLISM of lightly processed (wound-stressed) carrots undergoes major modifications during 10-days storage. The most pronounced changes appear to reflect net synthesis of PL and ASG, development of RER, and membrane biogenesis. The simultaneous accumulation of PA and an increase in cell permeability, however, suggest coordinated involvement of catabolic and anabolic reactions. Also, an increase in proportion of stigmasterol relative to sitosterol during storage may indicate a senescence relationship.

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