MEMBRANE STRUCTURAL LIPID CHANGES IN FRESH-CUT CARROTS: REVISITING THE "WOUNDING AND AGING" PHENOMENON

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Abstract

Virtually no quantitative data exist for membrane lipid metabolism of fresh-cut (wound-stressed) vegetables and fruits, despite the inevitable consequences of wounding. One such result is the rupture of cell membranes and activation of membrane lipid catabolic processes. We have evaluated changes in membrane sterols and glycerolipids in shredded carrots, a popular fresh-cut commodity. 'Apache' and 'Caropak' carrots were shredded and stored under conditions similar to those used in retail markets (10°C and 95% RH). Between 0 and 10 days of storage, membrane lipid concentrations in the shredded tissue were determined using HPLC/evaporative light scattering detection. Total phospholipid and acylated sterol glycoside concentrations increase by as much as 31% and 135%, respectively, following 10 days of storage. These increases are indicative of membrane restructuring ("repair") processes, are accentuated by Ca^{2+} pretreatment, and appear to be cultivar-dependent. During storage, Ca^{2+} pretreatment also delays onset of increases in the ratios of free sterols to phospholipids and of free stigmasterol to free sitosterol (both senescence-driven processes); and as expected, Ca^{2+} pretreatment reduces cell permeability. However, Ca^{2+} pretreatment does not curb the rate of accumulation of phosphatidic acid, a membrane degradation product. Coexistence of membrane degradation and "repair" processes appears to be a characteristic feature of wounding and long-term storage of carrots. Further, Ca^{2+} pretreatment may prove to be a valuable approach for maintaining quality of fresh-cut carrots and possibly other fresh-cut products, owing to its stabilising influence on cell membrane systems.

1. Introduction

For years, studies have shown that over the short term (< 24 h), activation of membrane lipid catabolism is an immediate consequence of wounding in plant storage organs (see reviews of Kahl, 1974; Mazliak and Kade,r 1978; Theologis and Laties, 1980). The popularity of fresh-cut vegetables and fruits has increased greatly in the U.S. and Europe, but such products may often be stored for several days or longer prior to consumption. Despite the surging popularity, very little is known about membrane lipid metabolism of fresh-cut commodities. This ignorance should be addressed, because the severe injury to which fresh-cut tissues are subjected during preparation initiates membrane degradative reactions which shorten storage life. Controlling these catabolic processes could provide major benefits to the produce industry.

Complex lipids (membrane sterols and glycerolipids) are of fundamental importance in cell membrane integrity, cell viability, and the storage life of harvested plant tissues. In view of the importance of cell membrane deterioration in wounded plant tissues and the need to improve quality maintenance practices for fresh-cut products, we have evaluated membrane lipid changes during storage of shredded carrots, an important fresh-cut commodity.

2. Materials and Methods

'Apache' and 'Caropak' carrots were peeled and then shredded using a food processor to provide the test material. Freshly prepared shreds were immersed for 2 min in distilled water ('Apache' and 'Caropak') or in 1% w/v $CaCl_2$ ('Caropak' only). Controls (day-0 samples), 25 to 50 g fresh weight, were immediately frozen in liquid N_2, sealed under N_2 gas, and stored at -70°C to await lipid analyses. Additional samples were stored in 10-L plastic containers which were covered with polyethylene bags and aerated with humidified air at the rate of ca. 50 mL/min/g tissue. The containers were held in an environmentally controlled room at 10°C and 95% RH. Stored shreds were sampled at various intervals between 0 and 10 days of storage, frozen in liquid N_2, sealed under N_2 gas, held at -70°C with the 0-day samples, and saved for lipid analyses.

Frozen tissue was lyophilized, ground, and a total lipid extract was obtained. The total lipid extract was then divided into neutral lipid, glycolipid, and phospholipid fractions using preparative silica cartridges as in Picchioni et al. (1994). Free sterols in the neutral lipid fraction were isolated and quantified by capillary GLC-FID (Whitaker and Lusby, 1989). Component classes in the glycolipid and phospholipid fractions were separated and quantified by normal phase HPLC using authentic lipid class calibration standards with a Varex IIA evaporative light scattering detector (Picchioni et al., 1994). In our laboratories, this new HPLC method has reduced plant lipid analytical time and variability compared to the more time-consuming TLC method (Picchioni et al., 1996a). Typical chromatograms of phospholipid and glycolipid fractions from carrot tissue are shown in Figure 1.

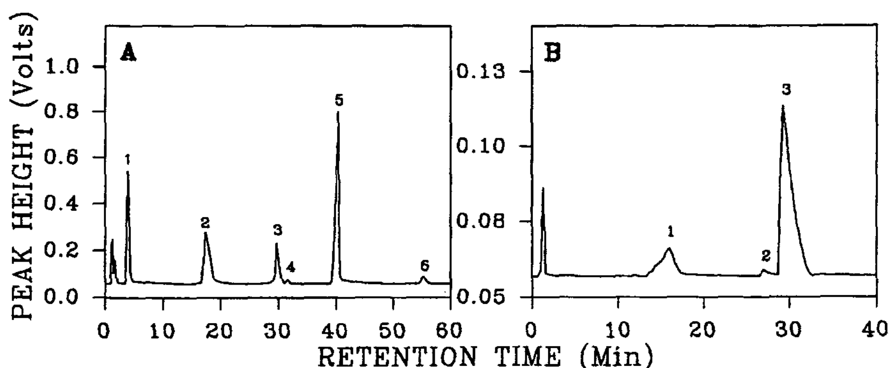

Figure 1. Representative HPLC chromatograms for phospholipids (A) and glycolipids (B) of shredded 'Apache' carrots. Phospholipid peaks (A): 2, phosphatidylethanolamine (PE); 3, phosphatidylinositol; 4, phosphatidic acid (PA); 5, phosphatidylcholine (PC); 6, lysophos-phatidylcholine [peak 1, digalactosyldiacylglycerol, is recovered in the phospholipid fraction during preparative chromatography and elutes on HPLC before phospholipids and near the void volume]. Glycolipid peaks (B): 1, acylated sterol glycoside; 2, sterol glycoside; 3, monogalactosyldiacylglycerol. Injections represent approximately 200 μg total phospholipid (A) and 100 μg total glycolipid (B). See Picchioni et al. (1994) for details on chromatographic conditions and hexane:*iso*PrOH:water gradient elution procedures.

3. Results and Discussion

At day-0, total phospholipid (TPL) concentration of 'Apache' and 'Caropak' tissue averaged 760 to 790 mg/100g dry wt (Figure 2A). Following 4 to 5 days of storage, TPL

concentration increased by 21% to 26% in both cultivars, and by the tenth day, 'Apache' shreds experienced a further increase in TPL concentration (31% greater than the day 0 value). In contrast, TPL concentration of 'Caropak' shreds ceased to increase following 4 days of storage. This may indicate that carrot genotypes differ in their capacity to synthesize membrane phospholipids following wounding and long-term storage.

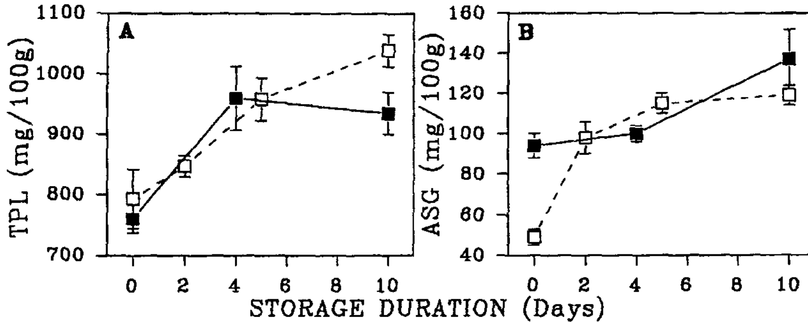

Figure 2. Total phospholipid (TPL) concentration (A) and acylated sterol glycoside (ASG) concentration (B) in 'Apache' (□) and 'Caropak' (■) shreds (pretreated with distilled water for 2 min) during storage at 10°C and 95% RH. Values for TPL calculated by summation of phospholipid classes as determined by integration of HPLC peaks. All concentrations are expressed on a dry tissue weight basis, and represent the mean ± SE of three replicates.

The pattern of increase in acylated sterol glycoside (ASG) concentration during storage also varied between cultivars (Figure 2B). In 'Apache' shreds, increases in ASG concentration occurred almost entirely during the first 5 days of storage (135% greater than the day 0 value), whereas a sizeable ASG increase in 'Caropak' shreds occurred toward the end of the 10-day storage period. The greatest increases in ASG concentration (0-2 days for 'Apache', 4-10 days for 'Caropak') were not associated with depletion of sterol glycoside and free sterol pools (Picchioni et al., 1994 and 1996b), indicating net synthesis of all membrane sterols during storage as well as the conversion of free sterols and sterol glycosides to ASG.

Recently, it has been postulated that accumulation of acylated sterol glycosides may serve an important metabolic function in stress-acclimation (Moreau and Preisig, 1993), and this has relevance to our findings here. Taken together, the time-dependent patterns (Figure 2A, 2B) strongly suggest that wound-stressed carrot tissues undergo an extended period of net membrane lipid synthesis essential for the biogenesis of new membranes. Ultrastructural change over a 10-day storage period (significant accumulation of rough endoplasmic reticulum) has been established (Picchioni et al., 1994), further supporting this likelihood.

Despite the apparent membrane "repair" processes in shredded carrots, variation in the relative distribution of phospholipid classes also occurs during storage, and the change is senescence-related. In 'Caropak' shreds, concentrations of the two most dominant phospholipids – C and PE – increased during 4 days of storage, but the increase in PE was greater than that of PC (42% and 18%, respectively, above the day 0 value). Thus, the ratio of PC:PE in 'Caropak' decreased sharply during this period, and between 4 and 10 days of storage (Figure 3A). A decrease in PC:PE ratio is known to impart rigidity to membrane bilayers (Leshem et al., 1992), which in turn has been associated with senescence and the loss of membrane protein function in plant cells (Duxbury et al., 1991). In marked contrast

to the 'Caropak' tissues, the PC:PE ratio in 'Apache' shreds was hardly affected during storage. This finding may be the result of genetic variation in the onset of senescence of fresh-cut carrots.

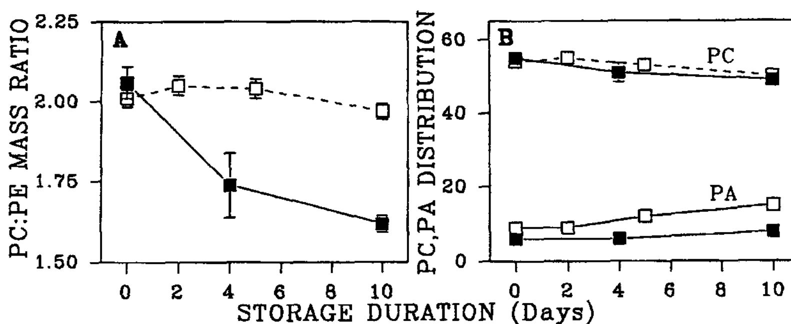

Figure 3. PC to PE mass ratio (A), and PC and PA distribution (B) in 'Apache' (□) and 'Caropak' (■) shreds (pretreated with distilled water for 2 min) during storage at 10°C and 95% RH. Distribution of PC and PA calculated as the weight percent of total phospholipid mass. All values represent the mean ± SE of three replicates. In most cases in (B), the SE is smaller than the symbol.

Another senescence-related trend in carrot shreds was a change in the relative proportion of PC and PA throughout storage (Figure 3B). In 'Apache' and 'Caropak', a reduction in the proportion of PC was closely matched by an increase in the proportion of PA (average PC reduction of 6% and average PA increase of 5% during 10 days of storage). PA is known to be a metabolic precursor in phospholipid synthesis (Moore, 1982) as well as a membrane phospholipid hydrolytic product (Larsson et al., 1990). The increase in the proportion of PA seems to arise, at least in part, from membrane hydrolytic activity (Picchioni et al., 1994).

Calcium pretreatment (1% $CaCl_2$) can increase the Ca^{2+} concentration of shredded carrots over 3-fold, which has been shown to improve storage quality of the tissue (Izumi and Watada, 1994). This may partly result from a deferral of senescence-related membrane physical and compositional changes, such as a reduction in cell permeability (Picchioni et al., 1994), and the absence of increase in the free sterol:phospholipid ratio (Table 1), which is characteristic of senescing plant organs (Thompson, 1988). In contrast to non-Ca^{2+}-treated 'Caropak' shreds (Table 1), the TFS:TPL ratio of non-Ca^{2+}-treated 'Apache' shreds did not increase during storage (Picchioni et al., 1994). This further demonstrates genotypic variation in the rate of senescence-related membrane lipid changes in wounded carrots. An increase in the proportion of stigmasterol relative to sitosterol during storage occurred in both 'Apache' and 'Caropak' (Picchioni et al., 1994, 1996b). Based on the findings of Schuler et al. (1991), this too may play a role in senescence of the tissue. In 'Caropak', however, this change was delayed by Ca^{2+} pretreatment (Picchioni et al., 1996b).

There is extensive documentation of the essential role of Ca^{2+} in delaying plant senescence at the membrane level (e.g., Ferguson, 1984). Irrespective of the role of Ca^{2+} in the *maintenance* of membrane integrity, the possibility that Ca^{2+} serves an essential biological function in active membrane *repair* processes (e.g., McNeil, 1991) has received little

attention in postharvest research. In 'Caropak' shreds, Ca^{2+} pretreatment accentuated increases in total phospholipid and monogalactosyldiacylglycerol concentrations throughout 10 days of storage (Table 1). However, the relative increase in PA content (e.g., Figure 3B) was not delayed by Ca^{2+} pretreatment (data not presented).

Calcium pretreatment also increased the concentrations of acylated sterol glycosides and sterol glycosides during the first 4 days of storage (*t*-tests significant at $p \leq 0.05$). These findings support the hypothesis that Ca^{2+} serves a role in regulating the repair of membrane systems (membrane lipid synthesis) in wounded carrot tissue.

Table 1. Influence of $CaCl_2$ (1% w/v) on changes in membrane lipid components in 'Caropak' shreds. Tissues were immersed in either $CaCl_2$ solution or distilled water (2 min), then stored for 10 days at 10°C and 95% RH. Values give the percentage change in concentration (dry matter basis) relative to values at Day-0. Abbreviations are: TPL, total phospholipid; TFS:TPL, total free sterol:total phospholipid molar ratio; ASG, acylated sterol glycoside; SG, sterol glycoside; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol.

Storage (*Days*) and Treatment	**Lipid concentration or ratio (% of average Day-0 value)**					
	TPL	TFS:TPL	ASG	SG	MGDG	DGDG
4 Days						
Water	126	99	107	51	104	110
$CaCl_2$	137	90	115	61	119	127
10 Days						
Water	123	113	146	66	109	123
$CaCl_2$	131	102	143	65	130	130
Significance[z]						
Days	NS	*	**	**	*	NS
Treatment	*	*	NS	NS	**	NS

[z]Completely randomized as a 2 x 2 factorial with three replicates. Nonsignificant (NS) or significant at $P \leq 0.05$ (*) or $P \leq 0.01$ (**); interactions were not significant.

Summary

The available knowledge on "wounding and aging" in plants is based heavily on the "tissue slice system" observed for 24 h or less. Historically, there has been little practical ground to study membrane lipid changes over longer periods, such as 10 days. In shredded carrots, both membrane degradation and "repair" processes appear to coexist during extended storage ("aging") and under environmental conditions that are relevant to the fresh-cut products industry. Accordingly, the growing interest in fresh-cut vegetables and fruits may provide an incentive to revisit the "wounding and aging" phenomenon in the laboratory.

Until further research can identify the enzyme systems and specific cellular membranes involved in the repair process, Ca^{2+} pretreatments may represent an environmentally-sound way to extend the storage life of fresh-cut carrots, and possibly other fresh-cut commodities. Our findings have also raised the possibility that carrot genotypes vary in their capacity for membrane restructuring following wounding. Supporting evidence includes variation in the

duration of increase in total phospholipid concentration, the presence or absence of an increase in the free sterol:phospholipid ratio, and the relative stability of the PC:PE ratio. Further study is needed to address and exploit such genotypic variation in the growing array of fresh-cut products.

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