Grapefruit Gland Oil Composition Is Affected by Wax Application, Storage Temperature, and Storage Time

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The effect of wax application, storage temperature (4 or 21 °C), and storage time (14 or 28 days after wax application) on grapefruit gland oil composition was examined by capillary gas chromatography. Wax application decreases nonanal and nootkatone levels. β-Pinene, α-phellandrene, 3-carene, ocimene, octanol, *trans*-linalool oxide, and *cis*-*p*-mentha-2,8-dien-1-ol levels increase, but limonene levels decrease, with temperature. Levels of α-pinene, limonene, linalool, citronellal, α-terpineol, neral, dodecanal, and α-humulene decrease with time. Levels of α-phellandrene, 3-carene, ocimene, and *trans*-linalool oxide increase with time. No compound level was affected by the interactive action of temperature and wax application, suggesting that these two factors cause grapefruit oil gland collapse (postharvest pitting) through means other than changing gland oil composition. Compounds that are toxic to the Caribbean fruit fly (α-pinene, limonene, α-terpineol, and some aldehydes) decrease with time, thus suggesting grapefruit becomes increasingly susceptible to the fly during storage.

Keywords: *Gland oil; wax application; temperature; time; postharvest pitting*

INTRODUCTION

Postharvest pitting of white grapefruit is characterized by clusters of collapsed oil glands that develop over the surface of the fruit (Petracek et al., 1995). The collapse is stimulated by wax application and high-temperature storage (≥10 °C) common in handling of grapefruit. Disorders with similar morphologies and etiologies have also been observed in Fallglo tangerines (Petracek et al., 1998) and Temple oranges (Petracek et al., 1997). Gland oil constituents are largely terpenes and sesquiterpenes and are highly phytotoxic (Soule and Grierson, 1986). Oil gland collapse may be due to changes inside the gland oil or changes of the surrounding cell walls directly contacting gland oil, either of which may result in increased susceptibility of the surrounding cell walls to gland oil. Because postharvest pitting causes substantial losses during storage of citrus fruit, we have been interested in improving our understanding of peel physiology to help predict or control this disorder.

It has been necessary to subject grapefruit to adverse physical conditions to kill Caribbean fruit fly eggs or larvae present before the grapefruit are exported from Florida to locations such as Japan. Cold storage was recommended as one of such adverse conditions (Ismail et al., 1986). Naturally occurring oxygenated compounds, some of which are present in grapefruit peel oil, have been found to be toxic to Caribbean fruit fly larvae (Davis et al., 1976; Styer and Greany, 1983). Therefore, any change in peel oil might affect the survival of the larvae in grapefruit (Greany et al., 1983).

This paper reports the effects of storage temperature, wax application, and storage time on grapefruit gland oil composition because of concerns about (1) whether changed gland oil composition is the direct cause of postharvest pitting and (2) whether treatments (waxing, temperature, and time) may affect fruit susceptibility to fruit flies by altering certain compound contents. To serve these purposes, oil taken directly from the oil gland by syringe was analyzed.

MATERIALS AND METHODS

Plant Material. Mature Marsh white grapefruit were harvested from groves in Fort Pierce, FL, on February 8, 1998. Fruit were packed and stored at the Citrus Research and Education Center in Lake Alfred, FL, on February 11. Fruit were washed on roller brushes with FMC Fruit Cleaner 395 (FMC, Lakeland, FL), waxed, and stored at 21 °C. Wax application consisted of coating the fruit with a commercially available shellac-based wax (FMC) on roller brushes and drying the fruit at 60 ± 5 °C for 2 min. Fruit were not degreened with ethylene or treated with fungicide.

Internal Gas Level. Grapefruit internal gas level was analyzed as previously described (Petracek et al., 1998). Briefly, septa were created by applying dabs (~1 cm in diameter) of Dow 3140 RTV silicone (Midland, MI) to the stylar end of the fruit. Gas samples (20 mL) were taken from the internal air space of the fruit by syringe. Samples were analyzed by a flow-through system consisting of O_2 **(**model 26112, Orbisphere Laboratories, Geneva, Switzerland) and CO_2 (model LI-6251, Li-Cor, Lincoln, NE) gas analyzers connected in series with N_2 used as the carrier gas.

Chromatography. Samples were analyzed on a Hewlett-Packard 5890 high-resolution capillary gas chromatography with a 0.32 mm i.d. × 30 m DB-5 column (Restek, Bellefonte, PA). The initial oven temperature was 32 °C, held for 5 min, then ramped at 7 °C min^{-1} to 260 °C, and held there for 5 min. The injector temperature was 260 °C, the detector temperature was 260 °C, and the hydrogen carrier gas flow was 50 cm/s. Retention index values were established using standards. Peaks were identified by using retention indices and by mass spectral information obtained from standards and the literature.

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Table 1. Effect of Waxing and Temperature on Internal O_2 and CO_2 Levels of Marsh Grapefruit[a]

	21 °C		4.5 °C	
	no wax	wax	no wax	wax
O_2 (%)	18.0 c	2.5 a	19.2 c	12.1 b
CO_2 (%)	2.1 a	6.2 c	2.1 a	3.2 b

[a] Internal O_2 and CO_2 levels were measured 24 h after waxing ($n = 10$ fruit). Mean separation within columns was by Duncan's new multiple-range tested at $\alpha = 0.05$.

Sample Preparation. The fluid from three to five intact oil glands was extricated from the middle of the stylar end and equator of the fruit with a syringe (Rouseff et al., 1996) and dissolved in 0.1 mL of methylene chloride. Two microliters of the solution was injected immediately for GC analysis. The punctured part of the fruit was sealed by Dow 3140 RTV silicone. Six fruit were tested for each treatment. The experimental design was three factor with two levels. The parameters are as follows: waxed and not waxed, 4 and 21 °C storage temperature, and 14 and 28 days storage time. Factorial analysis was performed by PlotIT (SPE, Haslett, MI).

RESULTS AND DISCUSSION

Twenty-seven volatile components in grapefruit oil extracted from the peel were determined as listed in Table 2. Wax application decreased nonanal and nootkatone at 95% level and 99% level, respectively. Nonanal was specifically decreased by waxing after 28 days and at 21 °C. Nootkatone was decreased by waxing at 21 °C but not at 4 °C. Limonene level decreased with temperature. Levels of *β*-pinene, α-phellandrene, 3-carene, ocimene, octanol, *trans*-linalool oxide, and *cis*-*p*-mentha-2,8-dien1-ol increased with temperature. Levels of α-pinene, limonene, linalool, citronellal, α-terpineol, neral, dodecanal, and α-humulene decreased with time. Levels of α-phellandrene, 3-carene, ocimene, and *trans*-linalool oxide increased with time.

Waxing significantly decreased grapefruit internal O_2 level and increased internal CO_2 level. (Table 1). Shaw et al. (1990) found that a low-oxygen (<1%) controlled atmosphere treatment had little effect on Valencia orange essence oil composition, whereas changes in terpene, alcohol, and aldehyde levels occurred in Pineapple orange essence oil. Because both nonanal and nootkatone are formed through oxidation (Erduran and Hotchkiss, 1995; Wilson and Shaw, 1978; Drawert et al., 1984), lowered internal O_2 by wax application might be responsible for the decrease in their levels.

Linalool was found to be the most phytotoxic compound in citrus oil gland (Wild, 1992). Neither wax application nor high-temperature storage affected its level. Limonene was found to be second in phytotoxicity (Wild, 1992). Wax application did not affect its level, whereas high temperature decreased its level. Neither nonanal or nootkatone, two compounds affected by wax application, was known to possess remarkably high or low phytotoxicity. Moreover, not a single compound level was affected by waxing and temperature interactive effect. Because wax application combined with high-temperature storage stimulates postharvest pitting (Petracek et al., 1995), it seems that pitting was caused by means other than changing gland oil composition. Changes in oil gland wall composition by wax application and high-temperature storage that impact susceptibility to gland oil may be the cause of postharvest pitting.

Aldehydes are more toxic to Caribbean fruit fly than alcohols and ketones (Davis et al., 1976), which may be

Table 2. Percentage Composition of Marsh Grapefruit Peel Oil Affected by Waxing, Storage Temperature, and Storage Time

		day 14				day 28										
		21.0 °C		4.5 °C		21.0 °C		4.5 °C		significance[a,b]						
compound	index	no wax	wax	no wax	wax	no wax	wax	no wax	wax	w	T	t	wT	wt	Tt	wTt
α-pinene	938	0.62	0.627	0.63	0.62	0.60	0.614	0.61	0.612			*				
sabinene	976	0.77	0.723	0.688	0.693	0.850	0.777	0.540	0.677							
β-pinene	980	0.08	0.079	0.067	0.063	0.093	0.084	0.061	0.067		*					
myrcene	991	1.94	1.912	1.939	1.948	1.932	1.931	1.893	1.911							
octanal	1004	0.67	0.765	0.649	0.734	0.710	0.670	0.660	0.509							
α-phellandrene	1009	0.07	0.057	0.076	0.075	0.190	0.173	0.134	0.096		**	***			***	
3-carene	1023	0.04	0.050	0.053	0.050	0.256	0.272	0.194	0.151		**	***			***	
limonene	1039	92.63	92.25	92.943	92.949	89.083	90.853	92.668	92.467		***	***			**	
ocimene	1051	0.27	0.305	0.223	0.187	0.384	0.238	0.273	0.281		**	***				
γ-terpinene	1067	0.06	0.064	0.056	0.037	0.170	0.133	0.108	0.103							
octanol	1074	0.03	0.048	0.019	0.020	0.042	0.020	0.010	0.020		*					
cis-linalool oxide	1082	0.03	0.043	0.030	0.036	0.028	0.018	0.010	0.054							
trans-linalool oxide	1099	0.07	0.071	0.075	0.070	0.328	0.307	0.213	0.180		***	***			***	
linalool	1107	0.10	0.084	0.076	0.083	0.050	0.054	0.042	0.089			**				
nonanal	1110	0.11	0.096	0.099	0.109	0.148	0.101	0.125	0.092	*				*		
cis-*p*-mentha-2,8-dien-1-ol	1149	0.07	0.078	0.089	0.095	0.239	0.174	0.051	0.023		*				**	
citronellal	1161	0.05	0.049	0.063	0.056	0.038	0.034	0.040	0.054			*				
α-terpineol	1210	0.05	0.046	0.031	0.036	0.011	0.018	0.009	0.023			***				
decanal	1216	0.50	0.514	0.480	0.501	0.495	0.514	0.499	0.469							
neral	1258	0.05	0.051	0.046	0.043	0.032	0.032	0.041	0.032			*				
α-copaene	1418	0.084	0.095	0.084	0.0908	0.0901	0.0900	0.09	0.0900							
dodecanal	1419	0.11	0.097	0.098	0.092	0.069	0.071	0.078	0.084			**				
trans-caryophyllene	1456	0.17	0.172	0.168	0.171	0.178	0.165	0.158	0.172							
α-humulene	1491	0.03	0.033	0.030	0.027	0.023	0.020	0.024	0.026			**				
γ-cadinene	1502	0.11	0.106	0.112	0.111	0.111	0.114	0.117	0.123							
nootkatone	1847	0.43	0.282	0.297	0.282	0.636	0.309	0.315	0.322	**	*	**				
total		99.12	99.025	99.204	99.264	96.883	97.87787	99.052	98.816							

[a] Factors w, T, t, wT, wt, and Tt represents waxing, temperature, time, waxing interacting with temperature, waxing interacting with time, and temperature interacting with temperature, respectively. [b] *, **, and *** represent confidence levels of 95%, 99%, and 99.9%, respectively.

due to the role of aldehydes as potent uncouplers of oxidative phosphorylation (De Greef and Van Sumere, 1966). Three of five aldehydes listed in Table 2 decreased with time. In addition, α-pinene, limonene, and α-terpineol are the three compounds that showed toxicity to Caribbean fruit fly in Styer and Greany's (1983) work, and all of the three compounds decrease with time (Table 2). Therefore, grapefruit might become increasingly susceptible to Caribbean fruit fly with time in storage.

Nootkatone levels increased with storage time but were reduced by wax application and cold storage (Table 2). Wilson et al. (1990) found that increases in nootkatone content in grapefruit oil with maturity paralleled those reported in navel orange for valencene, the level fo which changed concomitantly with biochemical and physiological changes associated with senescence (Coggins et al., 1969). Nootkatone is probably formed in vivo from valencene through 2-hydroxyvalencene (nootkatol), because several cell suspension cultures from citrus were able to convert valencene to nootkatone (Drawert et al., 1984). Because the level of valencene in grapefruit is low and thus difficult to quantify, nootkatone may serve as a senescence indicator or predictor in grapefruit. It is interesting that for all compounds levels affected by both temperature and time, including α-phellandrene, 3-carene, limonene, ocimene, *trans*-linalol oxide, and nootkatone, higher temperature and longer time have the same effect (increase or decrease). Each of these compounds may be a candidate for senescence indicator or predictor because longer storage time and/or higher temperature promote senescence. On the other hand, because wax application and cold storage can decrease nootkatone level, both might be used to slow grapefruit senescence.

Waxed and stored grapefruit may be processed into juice. The peel oil in grapefruit contributes to the characteristic flavor of grapefruit juice because some oil is introduced into the juice during extraction. Among compounds that show treatment effects (Table 2), nootkatone and limonene are most important to grapefruit juice flavor, whereas α-terpineol contributes negatively to grapefruit flavor (Pino et al., 1986). Nootkatone has a typical grapefruit aroma and exhibits a low odor threshold (Berry et al., 1967). It is believed to be a major flavor impact compound (MacLeod and Buigues, 1964).

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