Variability in Waxing-Induced Ethanol and Aroma Volatile Production among Mandarin Genotypes

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Abstract

Mandarins often develop off-flavors during storage that impact consumer acceptance and it would be useful to develop mandarin cultivars that are less susceptible to postharvest flavor loss. Ethanol has long been identified as being a compound identified with flavor loss in citrus. A range of diverse mandarin genotypes were screened for their ability to produce ethanol in response to both waxing and storage or a 2-h exposure to nitrogen gas and were found to differ greatly in ethanol content both at harvest and after treatment. Headspace measurements of intact fruit were not predictive of internal juice ethanol concentrations and exposure to nitrogen did not accurately mimic the effect of waxing and storage on ethanol accumulation. High ethanol production was associated with enhanced accumulation of other alcohols and esters that likely influence flavor. Screening for ethanol production could potentially identify mandarin genotypes that differ in the propensity to produce off-flavor volatiles and aid in better understanding the relationship between ethanol production and aroma volatile accumulation.

INTRODUCTION

Mandarins are increasing in consumer popularity but are prone to the development of off-flavors during storage. This loss in flavor is one of the most important factors that limit mandarin postharvest quality. Researchers have identified parameters that influence flavor loss, including storage time and temperature (Obenland et al., 2011), and composition of the wax coating (Hagenmaier, 2002; Porat et al., 2005). During storage extensive alterations in the aroma volatile profile occur, including large increases in alcohols and esters and decreases in aldehydes and terpenes, that are believed to be major reasons for the poor flavor quality (Tietel et al., 2010; Obenland et al., 2011). The origin of these compounds is not entirely known but many of these changes are believed to occur as a result of the wax applied during packing that leads to anaerobic conditions within the fruit and enhanced alcohol and ester production. Although it has major disadvantages, the use of some type of coating is needed to prevent water loss and to provide a more-pleasing exterior appearance to the consumer.

Potential means to prevent or minimize the occurrence of off-flavor in mandarins includes both changes in postharvest handling techniques and, potentially, in the development of mandarin genotypes that are less susceptible to flavor loss during storage. Genotypic variation in aroma volatile production has been reported in a range of different mandarin hybrids (Miyazaki et al., 2011), but the authors of this study focused on the volatile profiles at harvest and did not investigate postharvest effects. It would be useful to determine the degree of genetic variability that exists in current and upcoming populations of mandarins for postharvest flavor loss, but this is complicated by the time-consuming nature of flavor volatile analysis which makes it difficult to evaluate large numbers of genotypes. Fermentation products, such as ethanol, have been often implicated as potential causes for postharvest flavor loss in citrus (Baldwin et al., 1995; Hagenmaier, 2002), are easy to measure, and could be a suitable marker indicating poor

flavor development. In this study we evaluated the usefulness of this type of screening by evaluating a number of mandarin genotypes for the ability to produce and accumulate ethanol in response to either a nitrogen atmosphere or waxing and storage and then determined the relationship of ethanol production to the synthesis of other aroma volatiles.

MATERIAL AND METHODS

'Kuno Wase', 'Kawano Wase' and 'Dobashi Beni' Satsuma mandarins (*Citrus unshui* Marcovitch), averaging 75 g in weight, were harvested from research plots at the University of California, Lindcove Research and Education Center (LCREC) in Lindcove, CA during the time for commercial maturity of these cultivars. The fruit were transported to the United States Department of Agriculture in Parlier, CA (Parlier USDA) where they were left at ambient laboratory temperature (~20°C) overnight. The following day the fruit were placed individually into 3.79 L jars, the jars flushed with nitrogen gas for 10 min and then sealed. After 2 h in nitrogen a 0.5 ml gas sample was withdrawn from the headspace of the closed jar using a syringe and injected into a Shimadzu GC 14A gas chromatograph (Columbia, MD, USA) equipped with a FID detector and a 2 m × 5 mm × 2.6 mm column packed with 5% Carbowax 20M on 60/80 Carbopack B (Supelco, St. Louis, MO, USA). Numbers of fruit individually measured for each cultivar were 10, 18 and 12, for 'Kuno Wase', 'Kawano Wase' and 'Dobashi Beni', respectively. A period of 2 h in nitrogen had been previously found to induce the accumulation of headspace ethanol in the headspace that was readily measureable while no ethanol was detectable when the nitrogen was replaced with air (data not shown). This methodology of using nitrogen to induce ethanol accumulation was tested in this study as a potential means to rapidly screen large numbers of mandarin germplasm for the rate of anaerobic ethanol production. Since it was necessary that this method accurately predict the concentration of internal ethanol, each fruit was juiced and an aliquot of the juice quantified for ethanol using a kit (Genzyme Diagnostics P.E.I. Inc., Charlottetown, PE, Canada) based upon the conversion of ethanol to acetaldehyde by alcohol dehydrogenase. This quantification method was previously demonstrated to give the same results as would be obtained by headspace analysis of the juice (data not shown).

In order to make comparison to the postharvest processing that would occur commercially, eight additional mandarin cultivars ('W. Murcott', 'Gold Nugget', 'Temple', 'Pixie', 'Ellendale', 'Minneola', 'Orlando' and 'Sue Linda') were obtained from LREC, transported to the Parlier USDA and evaluated for the ability to produce ethanol when the fruit were exposed to nitrogen gas for a period of 2 h as described above or when waxed and stored to simulate commercial packing and marketing. The first four cultivars listed are classified as *Citrus reticulata* Blanco and the final four as *Citrus* × *tangelo*. Waxing was conducted by hand by dipping the fruit into undiluted Fruit-A-Peel C carnauba wax (Fruit Growers Supply, Sherman Oaks, CA, USA) and wiping off by gloved hand the excess wax. These fruits were then stored for either 1 week at 20°C or 2 weeks 5°C followed by 1 week at 20°C. Fruit sizes ranged from a low of 73 g for 'Pixie' to a high of 157 g for 'Ellendale'. Following nitrogen gas exposure or waxing and storage the fruit were peeled, individually juiced and assayed for ethanol using the Genzyme assay kit. Juice from two "high" ethanol producers ('Pixie' and 'Minneola') and two "low" producers ('W. Murcott' and 'Gold Nugget') was saved for aroma volatile analysis with a 5-ml portion from each fruit being placed into a sealed glass vial in addition to 5 ml of saturated sodium chloride. An internal standard of 1-pentanol was then added to the vial and the sample frozen at -20°C until the analysis. Ten fruit were utilized per treatment for both the nitrogen gas and waxing tests, with fruit being individually measured for ethanol concentration.

Volatile samples were thawed and analyzed for aroma volatiles by solid phase microextraction (SPME) and gas chromatography/mass spectrometry as previously detailed (Obenland et al., 2011). This analysis was performed to determine the relationship between ethanol production and aroma volatile concentration for each of the

mandarin cultivars tested. Each storage treatment within a cultvar was measured with six replicates, with each replicate being the pooled juice from two different fruit. Volatiles were identified by use of Wiley/NBS library spectra, retention indices and the retention time of standards when available. Peaks of interest were semi-quantified by comparison of peak areas to a standard curve made in deodorized mandarin juice using the internal standard (1-pentanol).

Statistical significance between storage treatments for ethanol or other aroma volatiles was determined using a one-way analysis of variance and Tukey's test to perform the multiple mean comparisons (SPSS, Chicago, Ill., USA). Principal component analysis on the aroma volatile data was performed using XLStat (New York, New York, USA).

RESULTS AND DISCUSSION

Exposing mandarin genotypes to nitrogen gas for 2 h, followed by headspace measurement, potentially offered a rapid and easy means of screening large numbers of mandarin genotypes for the ability to produce ethanol and other off-flavor components. Headspace ethanol measurements were, however, found to relate very poorly with the actual ethanol content of the fruit as determined by direct measurements of the juice (Fig. 1). The reasons for this discrepancy are unclear but could be due to fruit-to-fruit differences in the ability of ethanol to diffuse through the peel. Such differences have been noted between grapefruit and 'Murcott' mandarins (Shi et al., 2007) but not previously between individual mandarin fruit. This result indicated that ethanol measurements for comparisons between citrus genotypes, or even between individual fruit, needed to be made using juice rather than intact fruit.

As an additional test of the ethanol screening methodology, eight diverse cultivars of mandarins were waxed to simulate commercial conditions and compared to fruit that had received a 2 h treatment in nitrogen. Although this treatment had been found in prior testing to be effective in inducing the accumulation of ethanol in the headspace above intact mandarin fruit in other cultivars, only in 'W. Murcott' and 'Orlando' was the ethanol present in the juice of the treated fruit significantly different ($P \leq 0.05$) than that in the control fruit (Fig. 2). In contrast, waxing and storage greatly enhanced ethanol in every mandarin cultivar, with the additional time in cold storage not having any impact in all but 'W. Murcott' (Fig. 3). Clearly, a 2 h nitrogen treatment did not mimic waxing in these cases and is not suitable as part of a quick screen for ethanol production capability. It is possible that a longer duration of nitrogen exposure as was previously used in mandarins to initiate anaerobiosis (Shi et al., 2005) would be useable, but this was not evaluated. What was noteworthy in this portion of the study, however, was that there were large and significant differences ($P \leq 0.05$) observed among these mandarin types in both the amount of ethanol at harvest and following storage.

Assays of aroma volatiles were performed on the two cultivars with the lowest ethanol concentrations ('W. Murcott' and 'Gold Nugget') after waxing and storage and compared to two cultivars with much higher concentrations ('Minneola' and 'Pixie') to evaluate the relationship between differential amounts of ethanol production and aroma volatile content (Table 1). Given their more consistent changes due to storage, only alcohols, esters, ketones and aldehydes are shown in this table, although other hydrocarbons were identified and quantified. Most of the evaluated compounds increased in amount during storage, with the exception of a few of the alcohols and aldehydes. Principal component analysis conducted on the same data, but including the hydrocarbon compounds, indicated an association of high ethanol production with higher levels of esters and alcohols (Fig. 4). Previous research has shown that high levels of ethanol can enhance ester production in apples (Mattheis et al., 1991; Rudell et al., 2002).

It is worth noting that a number of the esters that greatly increased due to storage, and especially so in the high ethanol producers, such as ethyl isobutyrate, ethyl butanoate and ethyl 2-methyl butanoate, have relatively low odor thresholds (1 μg/L or less) and require only low concentrations to have an impact on flavor. These compounds have

fruity aromas that are not directly objectionable but that could together act to greatly alter flavor. Certain alcohols, such as 3-methylbutanol and 2-methylbutanol, were only present in high ethanol producers that had been stored. These alcohols both have a malty aroma, but have higher odor thresholds (1000 and 320 µg/L, respectively) than the esters. 'W. Murcott' mandarins were unique from the other mandarin cultivars examined in that they accumulated far greater amounts of ketones and aldehydes as a result of storage (Table 1, Fig. 4).

CONCLUSIONS

The mandarin genotypes examined displayed substantial differences in both the amount of ethanol present in freshly-harvested fruit and in the amount produced in response to waxing and storage. These differences in ethanol concentrations were associated with enhanced accumulation of other alcohols and esters which likely influence flavor. Even though this study would need to be repeated in different locations and different years to fully substantiate these differences, the study does illustrate the potential utility of screening for ethanol production to estimate the degree of postharvest accumulation of other esters and alcohols that could contribute to off-flavor. It also opens the possibility to use this screening approach to more fully understand the importance of the role of fermentative metabolism and ethanol accumulation to flavor loss during mandarin storage.

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Tables

Table 1. Concentrations (μg/L)[a] of aroma volatiles present in mandarins immediately after harvest and following waxing and 3 weeks (2 weeks 5°C + 1 week 20°C) of storage, tr=trace amount.

Compound		Storage time (weeks)							
		W. Murcott		Gold Nugget		Mineola		Pixie	
		0	3	0	3	0	3	0	3
Alcohols	Ethanol (mg/L)	58	595*	322	365	267	2364*	239	1938*
	3-Methylbutanol	tr	tr	tr	tr	tr	72*	tr	75*
	2-Methyl-1-butanol	tr	tr	tr	tr	tr	42*	tr	44*
	Linalool	49	31	254	34*	131	42*	122	60*
	4-Terpineol	26	29	104	22*	52	25*	41	24*
Esters	Ethyl acetate	0	720*	1753	2922*	55	1397*	487	5812*
	Ethyl propanoate	tr	36*	92	201*	6	43*	36	320*
	Ethyl isobutyrate	tr	tr	tr	8*	tr	11*	tr	73*
	Ethyl butanoate	tr	tr	18	37*	tr	21*	tr	466*
	Ethyl 2-methylbutanoate	tr	tr	tr	6*	1	30*	tr	209*
	3-Methylbutyl acetate	tr	tr	tr	tr	tr	10*	tr	9*
	2-Methylbutyl acetate	tr	tr	tr	tr	tr	7*	tr	8*
	Octyl acetate	tr	17*	256	60*	118	101	185	106
	Decyl acetate	37	30	102	19*	52	53	64	34
Ketones	1-Penten-3-one	8	20*	7	7	6	8	6	8*
	Carvone	15	52*	tr	12*	8	8	15	8
Aldehydes	Pentanal	20	121*	9	11*	8	19*	9	15*
	Hexanal	157	1675*	20	24	36	62	28	19
	E-2-Hexenal	8	10	27	21	17	13	18	47*
	Heptanal	24	187*	17	16	13	26*	15	19
	Octanal	55	103*	179	48*	171	65*	386	66*
	E-2-Octenal	13	137*	tr	tr	9	9	8	6
	Nonanal	37	80*	83	44*	61	46	106	47
	E-2-Nonenal	10	61	tr	tr	4	6	tr	tr
	Decanal	0	40*	529	38*	137	45*	223	54*

[a] Star after value indicates statistical significance between initial (0 week) and 3 weeks storage within a cultivar.

Figures

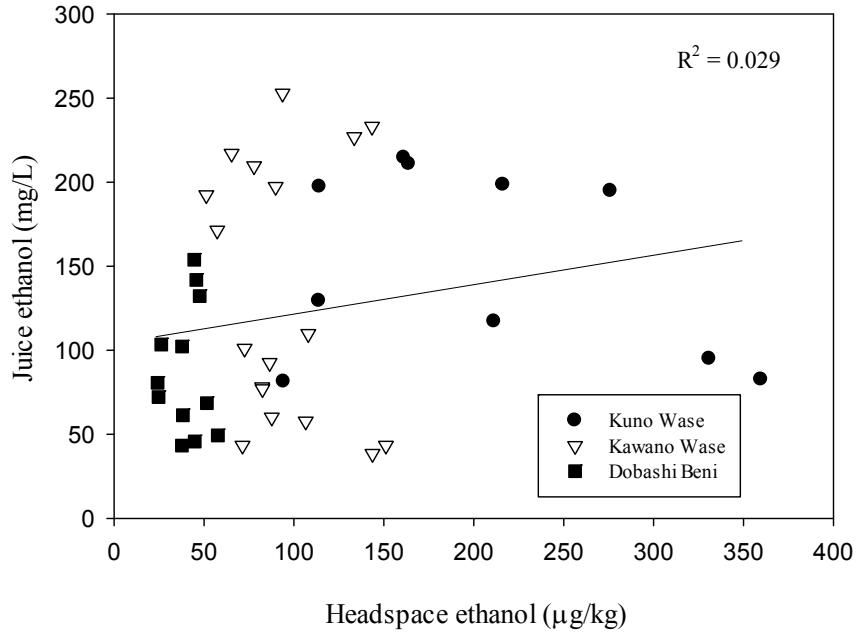

Fig. 1. Correlation between headspace ethanol measured above the intact fruit in a sealed jar and juice ethanol from the same fruit of three mandarin cultivars exposed to 2 h of nitrogen. Each point indicates an individual fruit.

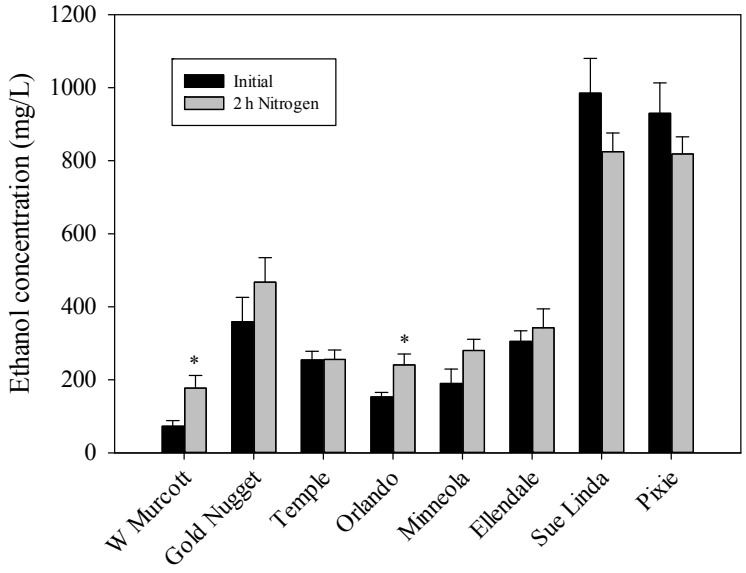

Fig. 2. Comparison of ethanol concentration measured from the juice of eight cultivars of mandarins at harvest (initial) or subjected to a 2-h exposure in nitrogen. Each bar represents the mean of 10 fruit which were individually measured with the bar indicating standard error. Star indicates a significant difference ($P \leq 0.05$) from initial within cultivar.

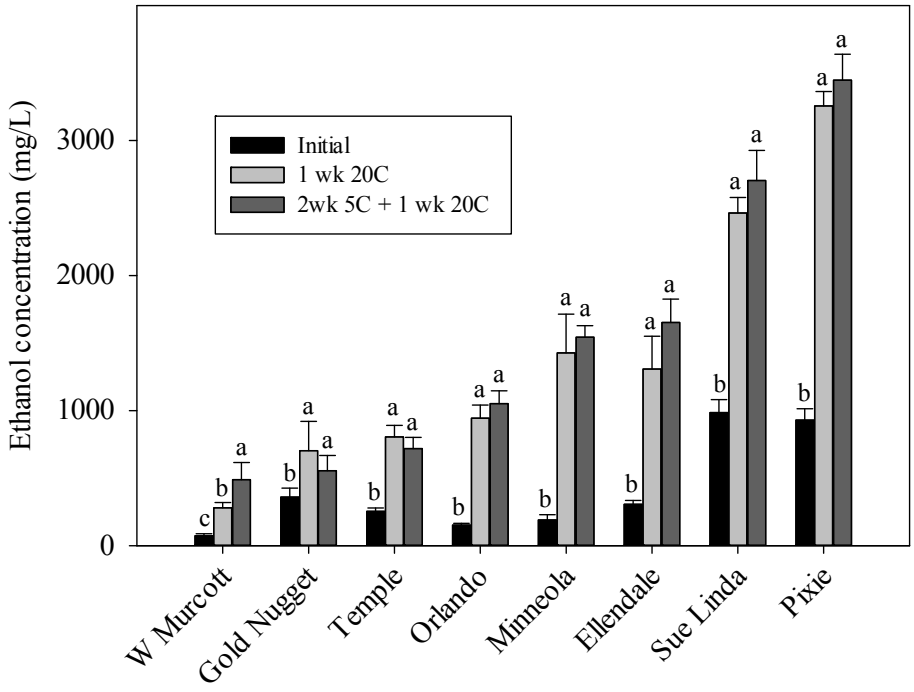

Fig. 3. Comparison of ethanol concentrations measured from the juice of eight cultivars of mandarins at harvest (initial) or waxed and stored at either 1 week 20°C or 2 weeks 5°C+1 week 20°C. Each bar represents the mean of 10 fruit which were individually measured with the bar indicating standard error. Bars within a cultivar with different letters are significantly different (P≤0.05).

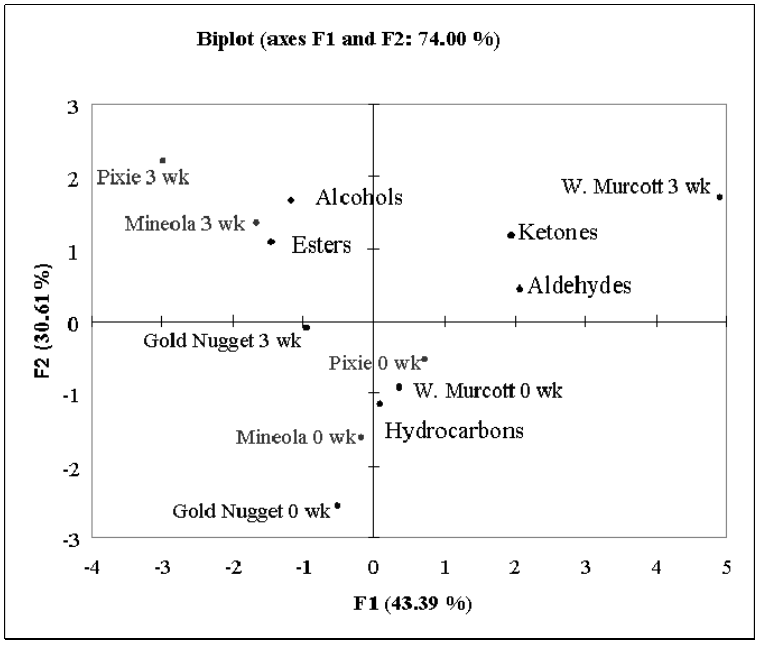

Fig. 4. Principle component analysis biplot of aroma volatiles present in mandarins with high (in red) and low (in blue) ethanol content after waxing and storage at 5°C for 2 weeks followed by 1 week at 20°C