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Physiological, biochemical and sensory characterization of the response to waxing and storage of two mandarin varieties differing in postharvest ethanol accumulation☆

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ABSTRACT

A loss of flavor quality often occurs with some varieties of mandarins (*Citrus reticulata* Blanco) following waxing and storage. This is thought to be due to the synthesis of ethyl esters which is stimulated by an accumulation of ethanol in the fruit as a result of anaerobic metabolism. The goal of this study was to determine the importance of postharvest ethyl ester accumulation to mandarin flavor in two important commercial mandarin varieties that differ in ethanol accumulation patterns. This study also aimed to enhance knowledge regarding the importance of ethanol accumulation to ethyl ester synthesis and overall flavor. In order to do this, comparisons were made between 'Pixie' (P) and 'Gold Nugget' (GN), mandarins that were previously identified as accumulating high and low amounts of ethanol, respectively, after waxing. In three of four harvests (H) at two different locations, P accumulated much higher concentrations of ethanol than GN after waxing and storage for 3 weeks at 5 °C and 1 week at 20 °C. Sensory panel analysis indicated that off-flavor development during storage was more pronounced in P than GN as were declines in overall acceptability. Flavor in fruit from Ojai, California (H4) was less negatively impacted by storage than fruit from the San Joaquin Valley of California (H1, H2, H3), for both varieties. Consistent with prior research, alcohols, esters and aldehydes were greatly altered in amount as a result of waxing and storage, with ethanol and the ethyl esters being the most prominent compounds, although ethyl ester concentration did not consistently relate to the amount of ethanol present. In H1 P had higher ethanol following storage than GN and correspondingly higher ethyl esters, while in H2 and H3 GN had significantly higher ethyl ester concentrations than P but did not have higher ethanol. Fruit from H4 had similar volatile concentrations between the varieties but ethanol was higher in P than GN. Internal oxygen concentrations in Pixie after waxing were lower than those in GN and likely were largely responsible for the greater ethanol accumulation observed in P. Following storage P tended to have higher pyruvate concentration and alcohol dehydrogenase (ADH) activity, while differences in pyruvate decarboxylase (PDC) activity were not consistent across harvests. Although the impact of waxing and storage on flavor was more negative for P than GN, the cause of this could not be simply ascribed to the greater tendency of P than GN to produce ethanol that in turn led to greater ethyl ester accumulation.

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1. Introduction

Worldwide the consumption of mandarins (*Citrus reticulata* Blanco) has dramatically increased over the last decade because of the rich, aromatic taste and ease of peeling. Their taste is mainly influenced by the levels of sugars and acids in the juice sacs and the relative ratios among them, while a mixture of different aroma volatiles, including alcohols, aldehydes, ketones, terpenes and esters affect aroma and overall flavor (Tietel et al., 2010a). Although consumer demand is increasing, mandarins are prone to developing off-flavors following commercial wax application and storage. The negative impact of the off-flavor on mandarin sensory quality has been well documented in a number of publications (Tietel et al., 2010b; Obenland et al., 2011, 2013).

Loss in mandarin sensory acceptability has been reported to be concomitant with a decline in acidity and a decreased concentration of terpenes and aldehydes, which influences typical citrus

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aroma, as well as increases of ethanol fermentation metabolism products and esters that may cause a sensation of over-ripeness and off-flavor (Tietel et al., 2011a). Increases in other volatiles derived from the catabolism of fatty acids and amino acids were also found to have occurred and may be involved in altering the flavor of the fruit through an enhancement in volatiles with fruity or musty aromas (Tietel et al., 2010a; Obenland et al., 2011). The importance of the ethyl esters, that rapidly increase following waxing of the fruit, has been increasingly recognized as a potential source of off-flavor. Formation of these esters is thought to be enhanced by increased concentration of the substrate, ethanol, in the fruit and the subsequent increase in ethyl ester concentrations along with the low odor thresholds of many of these compounds (Obenland et al., 2013).

Application of waxes to mandarins and other citrus imparts shine and reduces water loss and shrinkage following fruit washing. However, waxing can enhance the development of anaerobic conditions in the fruit by restricting gas diffusion through the peel (Porat et al., 2005). As with other types of citrus, in mandarins this metabolic shift toward anaerobiosis is associated with poor flavor quality (Hagenmaier, 2002). Shi et al. (2007) compared grapefruit and mandarins in an attempt to better understand the basis for the high susceptibility of mandarins to off-flavor formation and found lower peel permeability and higher ADH activity in mandarins compared to grapefruit, and suggested these as potential reasons for the difference between these two citrus types. The lower peel permeability restricted not only the exchange of CO_2 and O_2, which enhanced the likelihood of anaerobiosis within the fruit, but also the exit from the fruit of the off-flavor metabolites acetaldehyde and ethanol, potentially enhancing their accumulation.

Ethanol fermentation is a major route of ATP production that occurs under anaerobic conditions in two main steps. Firstly, pyruvate is decarboxylated to acetaldehyde by PDC, and then acetaldehyde is converted to ethanol by ADH (Tadege et al., 1999). Acetaldehyde can also be synthesized by enzymatic oxidation of ethanol, the reverse reaction of alcoholic fermentation catalyzed by ADH (Echeverría et al., 2004). Induction of these fermentative enzymes is linked with a decrease in oxygen, which is correlated with a decrease of energy status, ATP to ADP ratio (Zabalza et al., 2009). Exposure of mature citrus fruit to anaerobic conditions induced the expression of PDC and ADH messenger RNA, and increased the activity of PDC and ADH, potentially leading to alterations in the levels of acetaldehyde and ethanol in the fruit (Shi et al., 2007). Changes in fermentative metabolites of 'Clemenules' clementines and 'W. Murcott Afourer' mandarins were reported to be influenced by storage time, storage atmosphere and cultivar (Luengwilai et al., 2007).

Ethanol may not just have a direct impact on flavor, but may also serve as substrates for subsequent esterification reactions with acyl-CoA which is derived from fatty acid and amino acid catabolism, catalyzed by alcohol acyl transferase (AAT) (Tietel et al., 2010b; Tietel et al., 2011a). Esters are synthesized by AAT from the reactions of aliphatic and aromatic alcohols to their esters in the presence of acyl CoA in fruit and flowers (El-Sharkawy et al., 2005; Shan et al., 2012). In apple fruit, the levels of volatile production, AAT activity, and substrate were higher in peel than flesh. Alcohol acyl transferase enzyme activity was affected by ethylene, whereas ADH seems to be independent of ethylene modulation (Defilippi et al., 2005). Esterification in apple is also influenced by the concentration of alcohol (Berger and Drawert, 1984). In apple, the abundance of alcohol precursors was related to an increase in acetate ester production. However, the levels of ethanol and acetate ester production were not positively correlated with the activity of PDC, ADH and AAT, which suggested that substrate availability was a more important factor than enzyme activity for the development aroma during maturation of apple fruit (Echeverría et al., 2004). Low O_2 and/or high CO_2 concentrations have been reported to enhance activities of PDC and ADH but slightly reduce AAT activity, at least partially due to the decrease in cytoplasmic pH that occurs in avocado (Ke et al., 1994).

The authors have previously reported that variability exists among mandarin varieties in the amount of ethanol produced in response to either exposure to nitrogen gas or waxing and storage (Obenland and Arpaia, 2012). Subsequent research has expanded the number of mandarin varieties evaluated and verified that these varietal differences do exist (D. Obenland, unpublished data). Unknown was why these varieties differ in ethanol accumulation in response to waxing and also whether this dissimilarity in ethanol production relates to differences in ethyl ester production and subsequent sensory quality. In this study a comparison is made between 'Gold Nugget' (GN), a low ethanol producer, and 'Pixie' (P), a high ethanol producer, and physiological, biochemical and sensory characteristics of the two varieties before and after waxing and storage are quantified. In addition, multiple fruit harvests of both varieties have been conducted to determine the effect of harvest time and location on this phenomenon.

2. Materials and methods

2.1. Fruit and waxing or N_2 treatment

Multiple fruit harvests were employed to give an estimate of the impact of time of season on the postharvest response of the fruit. 'Gold Nugget' and P mandarins were harvested in 2013 on January 14 (H1), January 28 (H2), February 18 (H3), and May 1 (H4). Only fruit that were free of obvious surface disorders were used. Fruit for harvests 1–3 were obtained from sites near Exeter, CA located in the San Joaquin Valley. 'Gold Nugget' was harvested from the University of California Lindcove Research and Education Center (LREC) and P was obtained from a commercial orchard approximately 1.5 km from LREC. Fruit from H4 were obtained from growers in the Ojai Valley, CA, which is in southern California, approximately 250 km from LREC. These fruit were obtained from three separate growers and the grower lots were individually evaluated. All harvests occurred during the commercial harvest window for these two varieties in these two regions of California. Following harvest the fruit were transported to the LREC and stored overnight at 10–15 °C. The next day the fruit were cleaned and waxed on a research-scale packingline at LREC, utilizing a hot (43 °C) drench with JBT (Lakeland, FL, USA) Freshgard 75 WSG at 250 $\mu L\,L^{-1}$ and JBT Sta-Fresh 2109 pack wax as recommended by the manufacturer for use on mandarins. Wax was applied by air-assisted nozzles as the fruit moved over brushes, followed by passage through a 43 °C drying tunnel. After waxing the fruit were transported to the University of California Kearney Agricultural Center (UCKAC) in Parlier, CA and placed either at 15 °C for sensory analysis and sampling the next day (T1) or stored for 3 weeks at 7 °C and 1 week at 20 °C (T2) or 4 weeks at 7 °C (T3).

In experiments examining the timing of internal atmosphere and juice ethanol changes following waxing it was necessary to apply wax by hand instead of the packingline to enable shorter time periods to be examined. In these cases the same wax was used as for the packingline application but the wax was diluted (70 mL wax + 30 mL deionized water) prior to use. Application consisted of dipping or rolling the fruit in the diluted wax and wiping off the excess wax using a gloved hand. Drying of the hand-waxed fruit was performed on wire racks. Similar time course experiments were also performed with unwaxed fruit exposed to 100% N_2 to eliminate the diffusion of O_2 into the fruit and the potential influence of the peel on the production of ethanol and related off-flavor compounds. In this study, P and GN fruit were placed