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Elucidating the Roles of Ethanol Fermentation Metabolism in Causing Off-Flavors in Mandarins

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ABSTRACT: To elucidate the roles of ethanol fermentation metabolism in causing off-flavors, 'Mor' mandarins were exposed to anaerobic atmospheres for 0, 2, 4, 7, and 10 days to gradually increase juice ethanol and acetaldehyde levels through enhanced fermentation. Exposure to anaerobic atmosphere caused progressive decline in fruit sensory quality, from nearly "good" to "very bad", because of decreased typical mandarin flavor and increased sensation of 'musty' and 'ethanol' off-flavors. GC-MS analysis revealed significant ($p \leq 0.05$) increases in the contents of 12 aroma volatiles, including the ethanol fermentation metabolites ethanol and acetaldehyde, and several fatty acid and amino acid catabolism derivates, 7 of which were ethyl esters, which suggests that they were esterification products of ethanol and acyl-CoA's derived from fatty acid and amino acid catabolism. These de novo synthesized anaerobiosis-regulated ethyl esters impart 'pungent', 'ethereal', 'waxy', 'musty', and 'fruity' notes. Overall, these results suggest that besides the direct effects of ethanol and acetaldehyde, downstream ethanol esterification products may also be involved in causing off-flavor sensation in mandarins.

KEYWORDS: ethanol, mandarin, off-flavor, postharvest

INTRODUCTION

During the past decade, there has been a continuous rise in the consumption and global marketing of fresh, easy-to-peel mandarins, with production forecast to exceed 20 million metric tons in 2010.[1] However, despite their attractive appearance and convenience for consumption, mandarins are much more perishable than other citrus varieties and suffer from much shorter storage lives of just a few weeks after harvest.[2,3] One of the major problems in maintaining mandarin fruit quality is rapid deterioration in flavor and sensory acceptability, mainly because of the accumulation of off-flavor volatiles, which shortens fruit flavor life.[4]

Ethanol fermentation is a two-step process in which pyruvate is first decarboxylated to acetaldehyde by pyruvate decarboxylase (PDC) and acetaldehyde is subsequently converted to ethanol by alcohol dehydrogenase (ADH).[5] Activation of ethanol fermentation metabolism provides a major route for ATP synthesis and energy production via the glycolysis pathway under low-oxygen conditions.[6,7]

In previous studies, it was suggested that induction of ethanol fermentation metabolism, which results in accumulation of ethanol and acetaldehyde, is the main cause for formation of off-flavors during postharvest storage of citrus fruit.[8−10] This is especially true in commercially wax-coated fruit, because the application of waxes imparts shine and reduces water loss but also restricts gas exchange through the peel, which results in enhanced CO_2 and reduced O_2 levels in the internal atmosphere of the fruit, leading, in turn, to stimulation of ethanolic fermentation.[11−14] Furthermore, continuous increases in ethanol and acetaldehyde levels, concurrent with observed decreases in fruit flavor acceptability, were reported also during postharvest storage of various mandarin varieties.[8,15−17]

To better understand and elucidate the roles of ethanol fermentation metabolism in causing off-flavors in mandarins, we exposed 'Mor' mandarins to anaerobic atmospheres for various periods of 0, 2, 4, 7, and 10 days, thus inducing gradual increases in juice ethanol and acetaldehyde levels because of enhanced ethanolic fermentation, and evaluated the changes in fruit sensory quality and in content and composition of aroma volatiles. This enabled us to determine whether accumulation of high levels of ethanol indeed affected fruit flavor and whether this accumulated ethanol might serve as a substrate together with various acyl-CoA's, for subsequent esterification reactions that lead to accumulation of ethyl esters, which also may affect fruit flavor, as previously observed during low-oxygen storage of apples.[18−20] We chose the 'Mor' mandarin as a model system because, as a seedless progeny of 'Murcott',[21] it has a rich and aromatic flavor but suffers from the development of off-flavors after harvest.[8,14] Furthermore, in previous studies, we observed increases in *PDC* gene expression levels as well as in ethanol and acetaldehyde contents during postharvest storage of this variety, which indicated the potential importance of ethanol fermentation metabolism in governing perceptions of off-flavor in these fruits.[16,22] In the present study, we describe that ethanol and acetaldehyde not only directly affect the perceived flavor of mandarins but also that ethanol further acts as a key metabolic precursor in the formation of other volatiles that impart off-flavors to stored mandarins.

MATERIALS AND METHODS

Plant Material and Exposure to Anaerobic Atmospheres. 'Mor' mandarins (*Citrus reticulata* Blanco) were harvested from a

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commercial citrus plantation at Nir Zvi, Israel, and were used on the day of harvest. Fruits were exposed to anaerobic atmospheres as described previously:[23] they were placed in 30 L airtight plastic containers that were flushed with pure N_2 for about 10 min until the O_2 concentrations were below 0.5%. The containers were then sealed and kept at 20 °C for 2, 4, 7, or 10 days. Control fruits were kept in cardboard boxes at the same temperature for similar periods. The containers were flushed with N_2 twice daily, in the morning and afternoon, to ensure that O_2 levels remained below 0.5% and to remove excess CO_2. After the containers were opened, fruits were kept for 1 h in air at 20 °C and then taken for chemical and sensory analysis. Each treatment included 40 fruits, and the experiments were repeated twice with similar results.

Chemicals. Authentic chemical standards of the volatiles acetaldehyde, acetic acid, ethyl acetate, ethyl propanoate, ethyl 2-butenoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 3-methylbutanol, 2-methylbutanol, and ethyl 2-methylbutanoate were purchased from the Sigma Flavor & Fragrances catalogue (Sigma-Aldrich, St. Louis, MO). Ethanol (GC grade, 99.8%) was purchased from Sigma Chemical catalog (Sigma-Aldrich).

Sensory Evaluation. Sensory quality was tested on the day of harvest and after the various periods of anaerobic exposure. Fruits were peeled, and separated segments were cut into halves and placed in covered glass cups. Each treatment included a mixture of six to eight cut segments per panelist, prepared from five different fruits. Fruit taste was evaluated by a sensory panel of 10 members (five males and five females) aged from 25 to 62 years. Each panelist assessed the various attributes of the samples according to an unstructured 100 mm scale, with the anchor points 'very weak' and 'very strong' for each attribute, and sensory data were recorded as distances (mm) from the origin. The samples were identified by means of randomly assigned three-digit codes. In addition, panelists were requested to rate overall fruit taste on a scale of 1−5 in which 1 = very bad, 2 = bad, 3 = fair, 4 = good, and 5 = excellent. Panelists were asked to rinse their mouths with cold water between samples. The sensory panel gathered prior to the experiment to define the provided sensory attributes and practiced by performing open discussion panels to normalize flavor attribute intensities among all members. All sensory panel members were technicians or students working at the Department of Postharvest Science at the ARO, the Volcani Center, already familiar with sensory analysis of mandarins. The sensory attributes included taste (sweet, sour, bitter), odor (mandarin flavor and musty and alcohol off-flavors), and mouthfeel sensations (juicy and gummy).

Juice Sugar and Acid Contents. Total soluble solids (TSS) content in the juice was determined with a PAL-1 digital refractometer (Atago, Tokyo, Japan), and acid content was assessed by titration to pH 8.3 with 0.1 N NaOH by means of a CH-9101 automatic titrator (Metrohm Herisau, Switzerland). TSS and acidity measurements included five replications, each of juice collected from three different fruits.

Analysis of Aroma Volatiles. Aroma volatiles were extracted from homogenized segments as described previously.[16] Fruits were hand-peeled, weighed, and blended for 30 s with an equal weight of 30% NaCl to inhibit enzymatic degradation. Aliquots (2 mL) were placed in 10 mL glass vials, and 5 μL of 1-pentanol (Sigma-Aldrich) diluted 1:1000 (v/v) in water was added as an internal standard. The vials were stored at −20 °C pending analysis. At each time point, aroma volatiles were determined in three replicate measurements, each involving three different fruits, that is, a total of nine fruits per time point.

Aroma volatiles were identified by gas chromatography (GC) coupled with mass spectrometry (MS). Prior to analysis, samples were thawed at room temperature and were allowed to equilibrate for 5 min at 40 °C. Afterward, volatiles were extracted from the vials' headspaces by solid-phase microextraction (SPME) using 1-cm-length stable flexible fibers coated with a 50/30 μm layer of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellefonte, PA). Volatiles were extracted from the vials' headspaces during incubation at the same temperature of 40 °C for an additional 25 min. The extracted volatiles were injected to a model 7890A gas chromatograph (Agilent, Palo Alto, CA), equipped with an HP-5 column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA), using an autosampler (CTC PAL, Zwingen, Switzerland), by desorption for 2 min at 250 °C into the splitless inlet. The oven was programmed to run at 50 °C for 1 min, then to ramp up to 160 °C at 5 °C min^{-1} and to 260 °C at 20 °C min^{-1}, and finally to remain at that temperature for 4 min. The helium carrier gas flow was set at 0.8 mL/min. The effluent was transferred to a model 5975C mass spectrometer detector (Agilent) that was set to scan the m/z range from 40 to 206 at 7.72 scans s^{-1} in positive ion mode, and mass spectra in electron impact mode were generated at 70 eV. Chromatographic peaks were identified by comparing the mass spectrum of each component with the U.S. National Institute of Standards and Technology (NIST) 2006 Mass Spectral Library. Identification of aroma volatiles was further confirmed by calculating their linear retention indices (RI) by using a series of n-alkanes (C5−C20) and comparing their values with various published databases, namely, that of Adams,[24] the University of Florida Citrus Flavor Database,[25] and the LRI Database.[26] The detection limit of the aroma volatiles was approximately 10−15 $\mu g\ L^{-1}$.

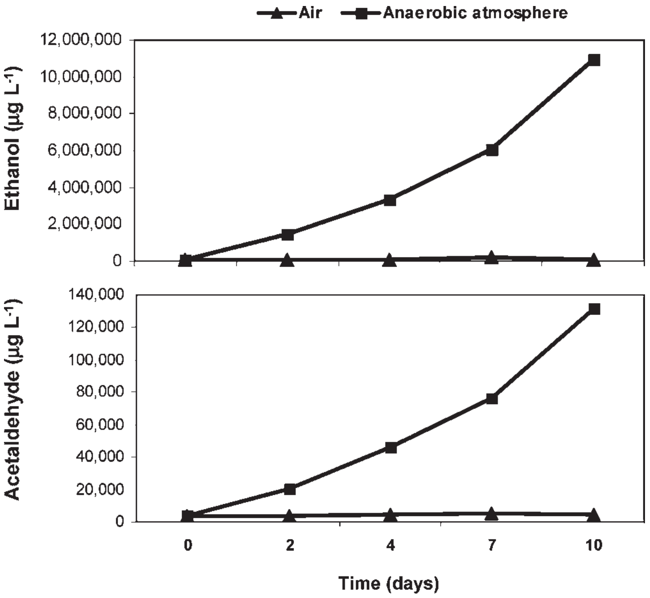

Figure 1. Effects of different times of anaerobic exposure on ethanol and acetaldehyde levels in 'Mor' mandarin juice. Fruits were kept for 0, 2, 4, 7, or 10 days in air or in anaerobic atmosphere ($O_2 < 0.5\%$) at 20 °C. Data are the mean of three measurements, each of juice collected from three different fruits.

The identities of volatiles of which contents increased following exposure to anaerobic conditions, namely, acetaldehyde, ethanol, acetic acid, ethyl acetate, ethyl propanoate, ethyl 2-butenoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, 3-methylbutanol, 2-methylbutanol, and ethyl 2-methylbutanoate, were confirmed by comparison with authentic chemical standards (Sigma-Aldrich). Quantification of all volatiles was performed according to calibration curves determined with pure chemical standards (Sigma-Aldrich); each calibration curve included five points in the range of 0.01X to 10X in water (X represents volatile concentration in mandarin segments after 10 days of anaerobic exposure). Quantification of ethanol and acetaldehyde was further verified by GC analysis according to the method of Davis and Chace.[27]

Statistical Analysis. One-way analysis of variance (ANOVA) and Tukey's HSD pairwise comparison tests were applied by means of the SigmaStat statistical software (Jandel Scientific Software, San Rafael, CA), and Microsoft Office Excel programs.

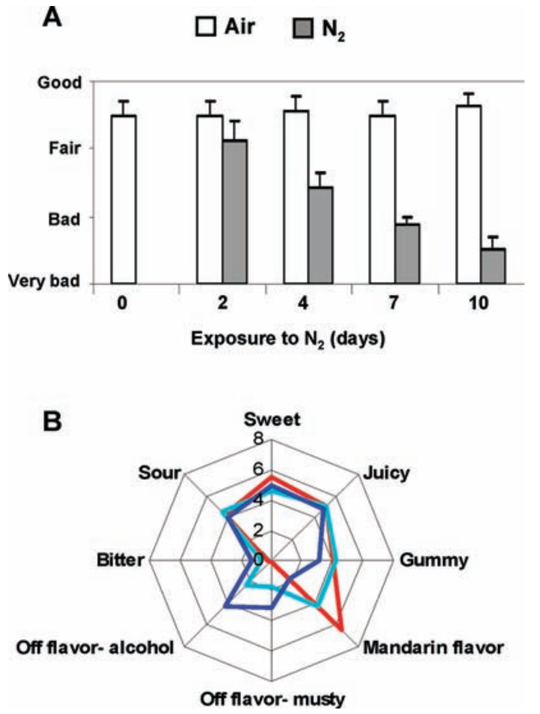

Figure 2. Effects of various times of exposure to anaerobic atmosphere on sensory quality of "Mor' mandarins. Sensory evaluations were conducted by a trained panel at harvest (0) and after 2, 4, 7, or 10 days of exposure to air or anaerobic atmosphere ($O_2 < 0.5\%$) at 20 °C: (A) overall taste score evaluations; (B) detailed flavor profile analyses. Data are the mean ± SE of 10 replications.

RESULTS

Effects of Anaerobic Atmospheres on Ethanol Fermentation. Exposure of 'Mor' mandarins to anaerobic atmospheres ($O_2 \leq 0.5\%$) for 2, 4, 7, and 10 days resulted in gradual increases in the ethanol fermentation metabolism products, ethanol and acetaldehyde (Figure 1). For example, at these time points ethanol levels increased from ~50,000 μg L^{-1} at time 0 to 1,457,000, 3,360,000, 6,040,000, and 11,000,000 μg L^{-1}, respectively, and acetaldehyde levels increased from 3,580 μg L^{-1} at time 0 to 20,300, 46,400, 76,800, and 131,400 μg L^{-1}, respectively (Figure 1). In control fruits kept for similar periods in air, we did not detect any notable changes in ethanol and acetaldehyde levels (Figure 1). Overall, exposure of 'Mor' mandarins to anaerobic atmospheres for various periods allowed modification of internal juice ethanol and acetaldehyde levels in a controlled and systematic manner.

Effects of Anaerobic Atmospheres on Fruit Flavor. Exposure of 'Mor' mandarins to anaerobic atmospheres resulted in a dramatic decrease in fruit flavor acceptability (Figure 2A). Exposure of fruits to anaerobic atmospheres for 2, 4, 7, and 10 days resulted in a gradual decrease in flavor acceptability from between 'fair' and 'good' at time 0 to nearly 'very bad' (Figure 2A). Detailed analysis of sensory quality at time 0 and after exposure to anaerobic atmospheres for 4 and 10 days revealed that the observed decrease in sensory quality was due to a gradual decrease in the perception of typical mandarin flavor and gradual increases in the perception of 'musty' and 'alcohol' off-flavors (Figure 2B). The flavor of control fruits held in air did not change during the course of the experiment (Figure 2).

Table 1. Effects of Various Times of Exposure to Anaerobic Atmosphere on TSS and Acid Contents in Juice of 'Mor' Mandarins[a]

treatment	TSS (%)	acid (%)	TSS/acid ratio
air			
at harvest	13.1 a	2.1 a	6.2 a
after 2 days	13.5 a	2.0 a	6.7 a
after 4 days	13.8 a	2.0 a	6.9 a
after 7 days	13.7 a	2.1 a	6.5 a
after 10 days	13.6 a	2.1 a	6.5 a
anaerobic atmosphere			
at harvest	13.1 a	2.1 a	6.2 a
after 2 days	13.0 a	2.0 a	6.5 ab
after 4 days	13.5 a	1.8 ab	7.5 ab
after 7 days	13.0 a	1.8 ab	7.2 ab
after 10 days	12.6 a	1.7 b	7.4 ab

[a] Fruits were kept for 0, 2, 4, 7, or 10 days in air or in anaerobic atmosphere ($O_2 < 0.5\%$) at 20 °C. Data are the mean of five measurements, each of juice collected from three different fruits. Different letters within each column indicate significant differences at $p \leq 0.05$.

Effects of Anaerobic Atmospheres on juice TSS and Acid Contents. The taste and aroma of citrus fruits are determined by the levels of sugars and acids and of aroma volatiles. We found that exposure of 'Mor' mandarins to an anaerobic atmosphere did not have any significant effect on juice TSS levels, which were in the range of 13.1−13.8% (Table 1). In contrast, the same exposure resulted in a small but significant decrease in juice acid levels from 2.1% at time 0 to 1.7% after 10 days (Table 1), whereas in control fruits stored in air juice acid contents barely changed (Table 1). The small decrease in acid levels resulted in a gradual increase in the juice TSS/acid ratio from 6.2 at time 0 to 7.4 after 10 days of anaerobic exposure (Table 1). Overall, the small changes observed in juice TSS and acid levels during anaerobic exposure likely did not affect the sensation of 'sweet' and 'sour' sensory attributes and, thus, probably had negligible effects on fruit flavor (Figure 2).

Effects of Anaerobic Atmospheres on Aroma Volatiles Contents. To identify aroma volatiles of which contents increased or decreased in homogenized segments of 'Mor' mandarins following anaerobic exposure, we conducted detailed GC-MS analysis at time 0 and after 4 and 10 days of anaerobic exposure. Pairwise comparisons of volatile contents after exposure to the extreme treatment of 10 days in an anaerobic atmosphere revealed that the contents of 12 volatiles significantly ($p \leq 0.05$) increased as compared with their initial levels observed at time 0 (Table 2). These anaerobiosis-regulated mandarin volatiles included the ethanol fermentation metabolites ethanol and acetaldehyde, as well as seven volatiles derived from fatty acid catabolism (acetic acid, ethyl acetate, ethyl propanoate, ethyl 2-butenoate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate) and three derived from amino acid catabolism (3-methylbutanol, 2-methylbutanol, and ethyl 2-methylbutanoate).[28] It is noteworthy that 7 of the 12 identified anaerobiosis-regulated volatiles were ethyl esters (Table 2). Further pairwise comparisons conducted after anaerobic exposure of the fruits for the shorter period of just 4 days revealed that the only volatiles for which contents significantly increased during this early stage were ethanol and acetaldehyde (data not shown). Detailed analysis of the contents of these 12 volatiles at time 0 and after

Table 2. Volatiles for Which Contents Significantly Increased ($p \leq 0.05$) in Homogenized Segments of 'Mor' Mandarin after 10 Days of Exposure to Anaerobic Atmosphere at 20 °C, As Compared with Their Initial Levels at Harvest[a]

compound	RI	concn at harvest (μg L^{-1})	concn after 10 days in anaerobic atmosphere (μg L^{-1})	odor threshold (μg L^{-1})	aroma description
ethanol fermentation					
acetaldehyde	513	29	131,000	63[35]	pungent, solventy[25]
ethanol	526	85	11,072,000	2,000,000[35]	ethanol[29]
fatty acid catabolism					
acetic acid	599	57	3000	180,000[35]	pungent, stinging[25]
ethyl acetate	612	44	3562	3000[36]	ethereal, fruity[25]
ethyl propanoate	710	nd	168	20[36]	sweet, fruity, ethereal[25]
ethyl 2-butenoate	842	nd	132		pungent, rum-like[29]
ethyl octanoate	1197	nd	6	0.1[36]	waxy, musty, fruity[29]
ethyl decanoate	1395	nd	1.2	0.032[37]	sweet, oily[29]
ethyl dodecanoate	1596	0.1	2	0.49[37]	sweet, waxy, soapy[29]
amino acid catabolism					
3-methylbutanol	729	nd	819	980[35]	malty[25]
2-methylbutanol	732	nd	750	3700[35]	green, ethereal[25]
ethyl 2-methylbutanoate	847	nd	12	0.063[35]	fruity, apple-like[25]

[a] Data are the mean of three measurements, each of juice collected from three different fruits. nd, not detected. All compounds were identified and quantified using calibration curves of authentic reference standards. The cited references indicate the sources for odor thresholds and aroma descriptions.

4 and 10 days of exposure to air or anaerobic atmospheres is displayed in Figure 3. It can be seen that the contents of all of these volatiles increased specifically after anaerobic exposure but were either not affected or not detected at all in control fruits held in air (Figure 3).

Comparison of the observed concentrations of the identified mandarin anaerobiosis-regulated volatiles with their published odor thresholds showed that the observed concentrations of acetic acid, 3-methylbutanol, and 2-methylbutanol were below their odor thresholds and, therefore, they probably did not have any effects on fruit flavor perception (Table 2). In contrast, the concentrations of ethanol and acetaldehyde, and also of all other detected anaerobiosis-regulated volatiles, were near or above their reported thresholds, so that they probably contributed to the observed changes in fruit flavor (Table 2). To show the putative effects of the identified anaerobiosis-regulated mandarin volatiles on fruit flavor, we included in Table 2 their aroma descriptions as provided by the University of Florida Citrus Flavor database[25] and the Good Scents Co.[29] As seen in Table 2, these volatiles imparted mainly 'pungent', 'ethanol', 'fruity', 'oily', 'waxy', and 'malty' notes, which most likely contributed to the observed decrease in fruit flavor quality and, especially, to the observed increase in 'musty' and 'alcohol' off-flavor sensations (Figure 2).

In contrast to the significant increases in the contents of these 12 volatiles, we have not detected any significant decreases at $p \leq 0.05$ in contents of aroma volatiles. Nevertheless, even though not significant, we yet observed at least 2-fold decreases in the contents of nine volatiles; that is, their final concentrations after 10 days of anaerobic exposure were less than half of their initial levels at harvest. The volatiles for which concentrations decreased after exposure to anaerobic atmospheres included four terpenes (sabinene, α-terpinene, carvone, and copaene), four aldehydes [octanal, decanal, (*E*)-2-decanal, and undecanal], and 1 alcohol (1-nonanol), all of which are known to impart typical citrus notes and, therefore, the decrease in their concentrations may result in the observed decrease in the sensation of typical mandarin flavor (Figure 2B).

DISCUSSION

It was postulated that induction of ethanol fermentation metabolism and accumulation of high levels of ethanol and acetaldehyde are responsible for the formation of off-flavors in commercially wax-coated citrus fruits.[8−11,30,31] In the present study, we found that anaerobic exposure of 'Mor' mandarins resulted in significant increases in the levels of ethanol and acetaldehyde, as well as of 10 other volatiles derived from catabolism of fatty acids and amino acids; 7 of the latter were ethyl esters (Table 2). In light of these findings, we hypothesize that in addition to the direct effects of ethanol and acetaldehyde on fruit flavor perception, high levels of ethanol serve also as substrates for subsequent esterification reactions with acyl-CoA's derived from fatty acid and amino acid catabolism; a reaction catalyzed by alcohol acyl transferases (AAT's)[28] and resulting in accumulation of various ethyl esters, which also may affect fruit flavor sensation (Figure 4). Indeed, in previous genome-wide transcriptome analysis studies of 'Mor' mandarin juice sacs during storage, we observed significant up-regulation of transcripts involved in fatty acid and amino acid catabolism, thus possibly resulting in the formation of fatty acid- and amino acid-derived acyl-CoA's required as substrates for AAT activities.[22] Besides ethyl esters, we also noted the accumulation of other compounds, such as 3-methylbutanol and 2-methylbutanol, also derived from catabolism of branched-chain amino acids.[28,32]

It is important to mention that volatile odor thresholds may be heavily influenced by the matrix composition and, therefore, the volatile odor thresholds in mandarin juice matrices might somewhat differ from those reported in pure water.[33,34] Nevertheless, because the observed concentrations of most of the anaerobiosis-regulated mandarin volatiles were way above their reported thresholds, we assume they most likely affect fruit odor (Table 2; Figure 3). For example, ethyl acetate and ethyl propanoate may contribute 'ethereal' and 'fruity' notes; ethyl octanoate, ethyl decanoate, and ethyl dodecanoate may provide unpleasant 'waxy', 'oily', and 'soapy' notes; and ethyl 2-methylbutanoate probably imparts a strong 'fruity' (over-ripe) note (Table 2).

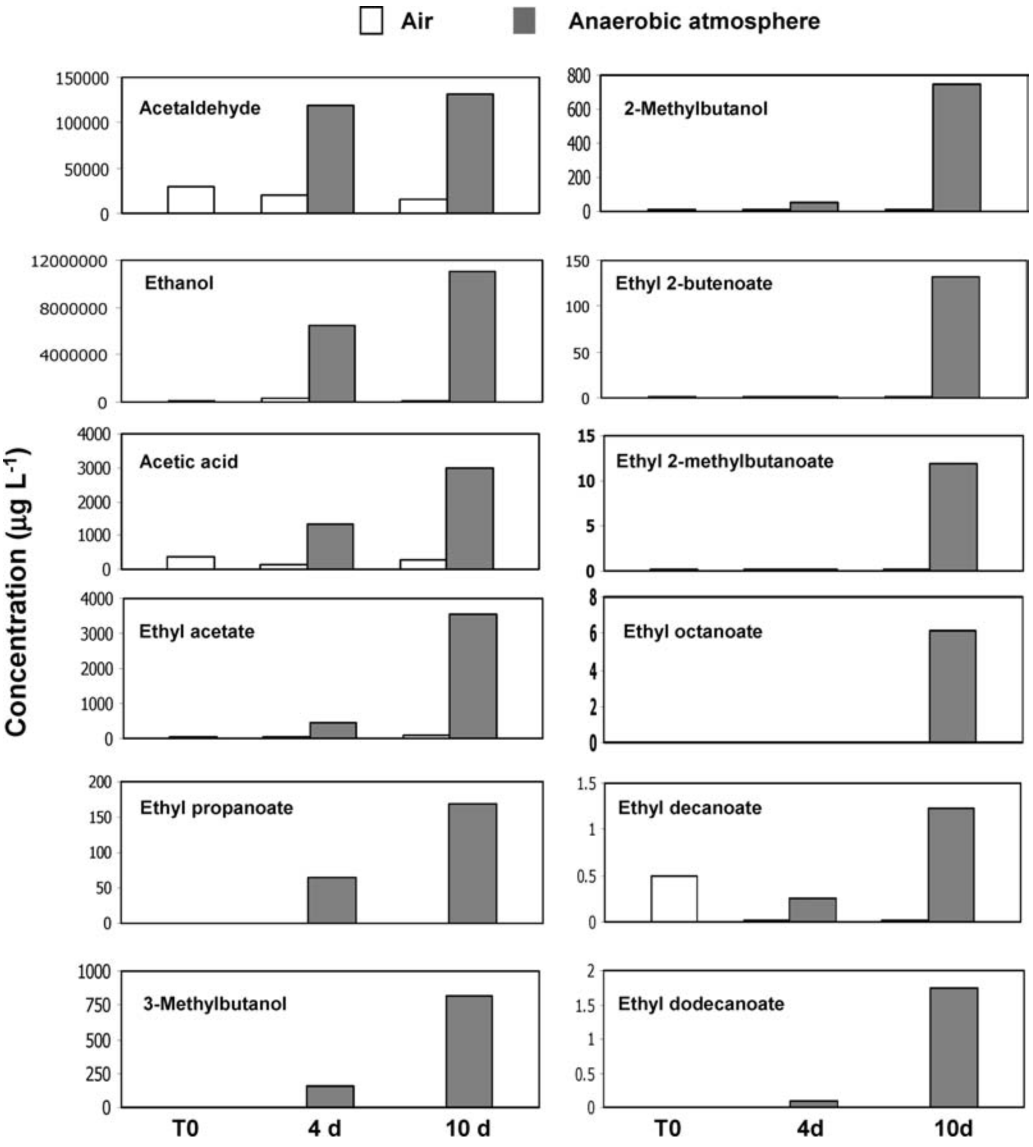

Figure 3. Effects of various times of exposure on the levels of 12 volatiles for which contents significantly increased ($p \leq 0.05$) in 'Mor' mandarin segments following exposure to anaerobic atmosphere ($O_2 < 0.5\%$) at 20 °C. Data are the mean of three measurements, each of samples collected from three different fruits.

Finally, we do not know the odor threshold of ethyl 2-butenoate, but it may account for the reported undesired 'pungent' and 'rum-like' notes (Table 2). Overall, it can be seen that accumulation of these de novo synthesized anaerobiosis-regulated ethyl esters imparts 'pungent', 'ethereal', 'waxy', 'musty', 'oily', and 'fruity' notes that probably contribute to the observed decrease in fruit sensory quality following exposure to anaerobic atmospheres (Figure 2B). Similar increases in contents of these and other ethyl ester volatiles were observed previously after storage of apples in low-oxygen atmospheres.[18,19] In that case, it was hypothesized that conversion of ethanol to ethyl esters allowed its decapitation, because esters are more volatile and can easily evaporate out of apple fruits.[18]

Our present hypothesis that high levels of ethanol serve as substrates for downstream esterification reactions in mandarin segments is further supported by studying the accumulation kinetics of these volatiles. At the early time point of just 4 days after exposure to anaerobic atmosphere, ethanol levels increased from very low levels at time 0 ($\sim$50,000 μg L^{-1}) to moderate levels of approximately 3,360,000 μg L^{-1}, but at that time point we did not observe any significant increases in contents of ethyl esters. In contrast, after 10 days of anaerobic exposure, ethanol content increased to a very high level of 11,000,000 μg L^{-1}, with consequently significant increases in the contents of its various esterification products (Table 2; Figures 1 and 3).

Furthermore, we found that the contents of many of the ethanol esterification products that increased after anaerobic exposure of 'Mor' mandarins, such as ethyl acetate, ethyl propanoate, ethyl 2-butenoate, ethyl octanoate, ethyl dodecanoate, and ethyl 2-methylbutanoate, also increased during postharvest storage of these fruits.[16] This great similarity in the compositions of aroma volatiles of which contents increased both after prolonged storage and after anaerobic exposure suggests that induction of ethanol fermentation metabolism is indeed a key process in causing off-flavor formation in mandarins and that accumulation of ethyl esters is most likely involved in driving flavor

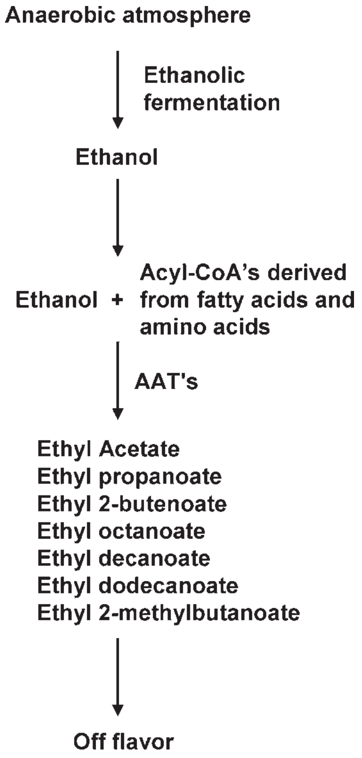

Figure 4. Model describing the proposed mechanism of metabolic changes in 'Mor' mandarin flesh during storage, which is responsible for the formation of off-flavor volatiles and impaired sensory acceptability.

deterioration. In accordance with our present findings, significant increases in contents of various ethyl esters, including ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate, ethyl 2-butenoate, and ethyl 2-methylbutenoate, were also reported during postharvest storage of California 'W. Murcott' and 'Owari' mandarins.[17]

Overall, in light of the present findings, we propose that not only ethanol and acetaldehyde, the primary metabolites of the ethanol fermentation pathway, but also downstream ethanol esterification products are involved in causing the off-flavor sensation in mandarins. In the future, further biochemical analyses of putative AAT enzyme activities, including definition of substrate preferences, will shed light on the metabolic processes that lead to ethyl ester biosynthesis in mandarin juice sacs.

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