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Elucidating the biochemical factors governing off-flavor perception in mandarins

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ABSTRACT

Mandarins tend to develop off-flavors during storage, and this is attributed to induction of ethanol fermentation metabolism and accumulation of ethanol and its by-products. In order to elucidate the biochemical factors contributing to off-flavor formation in mandarins, we conducted sensory and aroma-volatile analyses of 41 different mandarin varieties within the Israeli citrus breeding collection, at harvest and after 6 weeks of cold storage at 6 °C followed by 5 days at 20 °C. Descriptive sensory analysis with the aid of a trained panel revealed great diversity among mandarin varieties, in perceived off-flavors; and gas chromatography (GC) analysis revealed only a low correlation ($R = 0.14$) between ethanol levels and perception of off-flavors. Gas-chromatography mass-spectrometry (GC–MS) analysis of levels and compositions of total aroma volatiles during postharvest storage revealed general increases in levels of alcohols and ethyl esters, and consequent decreases in levels of monoterpenes, sesquiterpenes, and aldehydes. Detailed volatile analysis of diverse varieties with strong and weak off-flavors revealed that the aroma volatiles compositions and the ratios between chemical classes of volatiles have completely changed during storage in varieties with strong off-flavors, but were less altered in varieties with weak off-flavors. In addition, we observed a negative correlation ($R = -0.58$) between monoterpene levels and off-flavor perception, i.e., most varieties with weak off-flavors had relatively high terpene levels, which might mask the perception of off-flavors. Overall, it is suggested that perception of off-flavors in mandarins does not depend solely on accumulation of specific volatile compounds, such as ethanol, but rather evolves during storage because of general changes in juice aroma-volatiles profiles and compositions which create an atypical or spoiled flavor.

1. Introduction

During the last decade, there has been a continuous rise in consumption and global marketing of fresh, easy-to-peel mandarins, with a production forecast of 29 million tons per year (USDA, 2016). However, although they offer an attractive appearance and convenience for consumption, mandarins are much more perishable than other citrus varieties and, in particular, undergo deterioration in sensory acceptability and accumulation of off-flavors after harvest (Cohen, 1999; Tietel et al., 2011b).

Previous studies attributed development of off-flavors in mandarins mainly to induction of ethanol-fermentation metabolism and accumulation of high levels of ethanol; and this was demonstrated in several mandarin varieties (Cohen et al., 1990; Davis et al., 1967; Marcilla et al., 2009; Obenland et al., 2011; Shi et al., 2007; Tietel et al., 2010). Ethanol fermentation is a two-step process, catalyzed by pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH); it provides a major route for energy production under low-oxygen conditions (Geigenberger, 2003). Furthermore, accumulation of off-flavors is enhanced by application of wax coatings. These coatings restrict gas exchange through the peel surface, and result in increased CO_2 and reduced O_2 levels in the internal atmosphere of the fruit, leading, in turn, to stimulation of anaerobic respiration (Davis and Hofmann, 1973; Hagenmaier and Baker, 1993; Navarro-Tarazaga et al., 2007; Porat et al., 2005).

In addition to the direct effect of ethanol accumulation during storage on fruit-flavor perception, together with other acyl-CoAs

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ethanol also may serve as a substrate for subsequent esterification reactions catalyzed by alcohol acyl transferases (AATs), and thereby lead to accumulation of ethyl ester volatiles (Schwab et al., 2008). For example, it was reported that the levels of various ethyl esters, including ethyl acetate, ethyl propanoate, ethyl 2-methylpropanoate and ethyl 2-methylbutenoate, increased during postharvest storage of mandarins, and accumulation of such ethyl ester volatiles may also impact fruit-flavor perception (Obenland et al., 2011, 2013; Tietel et al., 2010, 2011a; Ummarat et al., 2015).

Despite the observed increases in ethanol levels during storage, it is unlikely that ethanol alone is the main cause of off-flavor perception, since: (1) ethanol has a relatively high odor threshold of 990,000 $\mu g L^{-1}$ (Czerny et al., 2008), and (2) off-flavor perception evolves from various sensations and not from alcoholic odor alone. Furthermore, in a recent study, Ummarat et al. (2015) evaluated aroma volatile levels of two different mandarin varieties, 'Pixie' and 'Gold Nugget', that differed in their tendencies to develop off-flavors after waxing and storage, and concluded that the observed differences in sensory quality between the two varieties could not be ascribed simply to accumulation of ethanol and ethyl esters.

In order to further elucidate the biochemical factors contributing to off-flavor formation in mandarins, we conducted sensory and aroma-volatile analyses of 41 mandarin varieties within the Israeli citrus breeding collection that vary in their susceptibility to development of off-flavors after storage. Comparison of results of sensory tests and aroma volatiles analyses, and use of heat maps and hierarchical clustering analysis enabled us to evaluate the interrelationships and correlations between the changes in specific volatile chemical classes, on the one hand, and perception of off-flavors after storage, on the other hand. The results obtained show that perceived off-flavors could not be attributed merely to accumulated ethanol and ethyl esters, but rather to creation of an atypical or spoiled flavor by general changes in profiles and compositions of juice-aroma volatiles during storage.

2. Materials and methods

2.1. Plant material and storage conditions

Fruits of 41 different mandarin varieties were obtained from the Israeli citrus breeding collection at the Agricultural Research Organization (ARO), the Volcani Center, Bet Dagan, Israel. The fruits of each variety were harvested at optimal maturity, as determined from a combination of maturity indices, including fruit size, color, and taste, and previous experience of the breeding teams as described previously (Goldenberg et al., 2014, 2015). After harvest, 50 fruits of each variety were washed and dipped for 20 s in a commercial 'Tag' polyethylene-based wax emulsion (Safe-Pack Ltd., Kfar Saba, Israel) containing Imazalil at 1000 $\mu L L^{-1}$. Then, the fruits were stored for 6 weeks at 6 °C and transferred to shelf-life conditions at 20 °C for five more days. The relative humidity (RH) was about 90–95% during cold storage and about 80–85% during shelf life.

2.2. Juice soluble solids contents and titratable acidity

Total soluble solids (TSS) content in the juice was determined with a PAL-1 digital refractometer (Atago, Tokyo, Japan), and acidity percentages were measured by titration to pH 8.3 with 0.1 M NaOH by means of a Model CH-9101 automatic titrator (Metrohm, Herisau, Switzerland). Each measurement comprised four replications, each using juice collected from three different fruits, i.e., a total of 12 fruits per measurement.

2.3. Sensory evaluations

Descriptive sensory analysis tests were conducted with the aid of a trained taste panel. The fruits were hand-peeled, and separated segments were cut into halves and placed in glass cups identified by randomly assigned three-digit codes. Each sample comprised a mixture of six to eight half-segments prepared from six different fruits. The trained sensory panel comprised 10 members – five males and five females, aged 25–62 – who routinely perform flavor tests of citrus fruits (Benjamin et al., 2013; Goldenberg et al., 2014, 2015; Tietel et al., 2010, 2011a). Each panelist assessed the various attributes of the samples according to an unstructured, 100-mm linear scale for each attribute. The scale ranged from "very weak" to "very strong", and sensory data were recorded as distances (mm) from the origin. The chosen sensory attributes were sweetness, sourness, bitterness, fruitiness, mandarin odor, off-flavor, gumminess, and juiciness.

Table 1
List of the 41 mandarin varieties tested.

Variety	Mandarin sub-group	Harvest date
Okitsu	Satsuma	3.10.13
Rishon	Common mandarin	3.10.13
Owari	Satsuma	13.10.13
GP-377	Clementine	15.10.13
Michal	Common mandarin	27.10.13
Oroval	Clementine	27.10.13
Lee	Common mandarin	4.11.13
Dubashi-Beni	Satsuma	13.11.13
Tami	Common mandarin	13.11.13
Caffin	Clementine	13.11.13
Fallglo	Common mandarin	13.11.13
Niva	Tangor	13.11.13
Nour	Clementine	25.11.13
Fairchild	Common mandarin	25.11.13
Orlando	Tangelo	26.11.13
Fina	Clementine	26.11.13
Idit	Common mandarin	1.12.13
Yusuf Efendi	Mediterranean	9.12.13
Merav	Common mandarin	9.12.13
Ponkan	Common mandarin	9.12.13
Tacle	Tangor	9.12.13
Dancy	Common mandarin	17.12.13
Minneola	Tangelo	17.12.13
Cami	Mediterranean	23.12.13
Or	Common mandarin	23.12.13
Yafit	Common mandarin	31.12.13
Temple	Tangor	31.12.13
Yifat	Common mandarin	6.01.14
Afourer	Common mandarin	20.01.14
Wilking	Common mandarin	6.01.14
Odem	Common mandarin	14.01.14
Sigal	Common mandarin	14.01.14
Pazit	Common mandarin	14.01.14
Shani	Common mandarin	20.01.14
Orit	Common mandarin	22.01.14
Kiyomi	Tangor	22.01.14
Winola	Common mandarin	9.02.14
Murcott	Tangor	18.02.14
Ortanique	Tangor	4.03.14
Hadas	Common mandarin	4.03.14
King	King	4.03.14

TSS, acidity and ripening ratio were determined on the day of harvest. Data are means ± SE of four replicates. The mandarin varieties tested belong to the following natural sub-groups: Common mandarin (*C. reticulata* Blanco), Satsuma mandarin (*C. unshiu* Marcovitch), King mandarin (*C. nobilis* Loureiro), Mediterranean mandarin (*C. deliciosa* Tenore), Clementine (*C. clementina* Hort. ex. Tan), and the mandarin hybrids Tangor (*C. reticulata* × *C. sinensis*, i.e., mandarin × orange hybrid) and Tangelo (*C. reticulata* × *C. paradise*, i.e., mandarin × grapefruit hybrid).