Cover Page

BARD Project Number: IS-4368-10

Date of Submission of the report: June 18, 2014

Project Title: Identification of key aroma compounds and optimization of postharvest handling procedures in order to improve sensory quality of mandarins

Investigators

Principal Investigator (PI):

Ron Porat

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Anne Plotto

Collaborating Investigators:

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Institutions

ARO, the Volcani Center

USDA, ARS, Florida

University of California

USDA-ARS, Parlier, CA

USDA, ARS, Florida

USDA, ARS, Florida

ARO, The Volcani Center

Keywords: *not* appearing in the title and in order of importance. Avoid abbreviations.
Citrus, off-flavors

Abbreviations commonly used in the report, in alphabetical order:

GC-MS, gas chromatography mass spectrophotometry; GC-O, gas chromatography olfactometry.

Budget: IS: \$120,000

US: \$180,000

Total: \$300,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	1	1	4	6
Submitted, in review, in preparation		3		3
Invited review papers				
Book chapters	1			1
Books				
Master theses		1		1
Ph.D. theses			1	1
Abstracts		6		6
Not refereed (proceedings, reports, etc.)		4		4

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Sophie Deterre, PhD from AgroParisTech, France. Dr. Deterre came with the status of Visiting Scientist, not an employee of the USDA. She worked from July 2012 through September 2013.

Faraj Hijaz, University of Florida, funded through a Specific Cooperative Agreement between USDA and University of Florida, from March to August 2012.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	3			3
Longer Visits (Sabbaticals)				

Description Cooperation:

The US and Israeli partners were in continuous cooperation by e-mails to discuss the research progress, share data and prepare the research reports. At March 2012, the US Co-PI, Anne Plotto, as well as the Californian collaborators, Mary Lu Arpaia and David Obenland, visited Ron Porat in Israel, and participated in the *Fruit Flavor and Aroma* workshop of the Monselise and Bar - Akiva Memorial Foundation that took place in the ARO, Israel.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				-
Issued (allowed)				-
Licensed				-

Abstract

Flavor acceptability is one of the most important quality parameters of fresh mandarin fruit. The general goals of this research were to explore the sensory attributes and aroma volatile profiles of various mandarin varieties, and evaluate how postharvest storage conditions affect fruit sensory quality and accumulation of off-flavors. The specific research objectives were: 1) Assess sensory quality and aroma profile compositions of various commercial and new mandarin varieties in CA, FL and Israel; 2) Characterize the effects of postharvest storage conditions on sensory quality and aroma profile composition of mandarins; 3) Identify key aroma compounds in mandarins; 4) Study the inheritance of flavor traits in a progeny of *Poncirus* and mandarin hybrids; and 5) Propose possible biochemical and molecular pathways that may be involved in governing off-flavor development during postharvest storage of mandarins. Sensory evaluations revealed notable differences between mandarin varieties and among seasons and harvest dates. Furthermore, it was found that mandarin flavor acceptability decreased during postharvest storage and following application of wax coatings, and this was attributed mainly to decreases in sourness and fruitiness and accumulation of off-flavors. The main aroma volatiles whose contents increased during storage and that are probably involved in imparting off-flavor sensations were the anaerobic metabolites ethanol and acetaldehyde, as well as various ethyl esters, such as ethyl acetate, ethyl propanoate, ethyl 2-methyl butanoate, ethyl octanoate, etc. Gas chromatography-olfactometry (GC-O) studies revealed the identification of ~25 mandarin aroma-active compounds, which impart various fatty, rubbery, fruity, citrus, floral, green and mushroom notes, suggesting that the unique flavor of mandarins evolves from a mixture of several aroma-active compounds. *Citrus* x *Poncirus* hybrids had complex chemical composition, with large number and quantity of esters and sesquiterpenes derived from the *Poncirus* ancestor. At last, transcript profiling analysis of mandarin flesh during storage using the Affymetrix® Citrus Genome Array revealed significant up-regulation of transcripts involved in ethanol fermentation metabolism, and in amino acid and fatty acid catabolism pathways, both processes are required for energy production by the fruit. Overall, we propose that increased levels of ethanol together with accumulation of acyl-CoA's derived from fatty acid and amino acid catabolism during storage, provide substrates for further down-stream esterification reactions catalyzed by alcohol acyltransferases (AAT's) that lead to the formation of ethyl esters, which greatly contribute to perception of over-ripe and off-flavor sensations.

Achievements

Significance of main scientific achievements and innovations

In this research, we studied and characterized the biochemical constituents that determine the unique flavor of mandarins, including the identification of key aroma-active compounds in fresh and stored fruit. In this respect, we found that the main aroma attributes of mandarin fruit are fruity, floral, citrus, green/grassy, fatty, metallic/rubber, herbal and mushroom notes. Like in oranges, we observed that there is not just one specific character impact compound imparting typical mandarin flavor, but that mandarin flavor is rather generated from a combination of several different aroma-active compounds.

Furthermore, we noticed that one of the major problems in postharvest handling of mandarins is the decrease in sensory acceptability after harvest, which results from a combination of decreased acidity and fruity flavors and increase in off-flavors. Moreover, we found that the decrease in mandarin sensory quality after harvest was enhanced by later harvests, application of wax coatings and extended storage duration. This storage effect on flavor was influenced by storage temperatures, especially by transfer of the fruit to shelf life conditions.

By studying the biochemical and molecular mechanisms presumably involved in regulating mandarin flavor deterioration after harvest, we concluded that off-flavors are formed by stimulation of ethanol fermentative metabolism during delayed harvest and postharvest storage, which is enhanced following application of wax coatings and exposure to anaerobic conditions. Accordingly, the presence of high levels of ethanol, together with accumulation of acyl-CoA's derived from fatty acid and amino acid catabolism during storage; provide substrates for further esterification reactions catalyzed by alcohol acyltransferases (AAT's). This leads to the accumulation of high levels of ethyl ester volatiles that contribute to perception of over-ripeness and off-flavors.

Based on the above findings, we propose a model that describes the biochemical mechanisms leading to increased synthesis of ethanol and ethyl ester volatiles during postharvest storage, which purportedly leads to the development of off-flavors (Fig. 1). According to this model, during storage there is a simultaneous induction of ethanol fermentation metabolism and of fatty acid and amino acid catabolism. Accordingly, the increased levels of ethanol together with accumulation of acyl-CoA's derived from fatty acid and amino acid catabolism provide substrates for further esterification reactions catalyzed by alcohol acyltransferases (AAT's). This then leads to the formation and accumulation of ethyl esters which results in off-flavors and also a sensation of over-ripeness or fermentation (Fig. 1).

Fig. 1. Model describing the biochemical pathways involved in stimulation of ethanol fermentation and ethyl ester synthesis, responsible for the formation of over-ripe and off-flavor sensations during postharvest storage of citrus fruit. AAT, alcohol acyltransferase.

Agricultural impacts of the research findings

Conduction of this study greatly improved our current knowledge regarding the sensory characteristics of mandarins, and the biochemical constituents that determine its unique flavor. Moreover, conduction of this study greatly improved our current understanding regarding the postharvest factors, such as storage temperatures and durations, that influence off-flavor development, and regarding the biochemical and molecular mechanisms involved in off-flavors accumulation.

Details of cooperation

Conduction of this BARD funded research included collaboration between three main research groups from Israel, Florida and California. The Israeli and Californian groups focused mainly on conduction of sensory and aroma volatile analysis studies during postharvest storage, whereas the Floridian group focused mainly on evaluation of mandarin sensory quality and aroma volatile composition during ripening. In addition,

The PI's group in Israel further conducted genome-wide microarray gene expression evaluations, and the Co-PI's group in Florida further identified aroma-active compounds from mandarin fruit.

List of publications

1. Reviewed papers:

- [1] Tietel, Z., **Plotto, A.**, Fallik, E., Lewinsohn, E., **Porat, R.** (2011). Taste and aroma of fresh and stored mandarins. *J. Sci. Food Agric.* 91: 14-23.
- [2] Tietel, Z., **Porat, R.**, Weiss, K., Ulrich, D. (2011). Identification of aroma-active compounds in fresh and stored 'Mor' mandarins. *Int. J. Food Sci Technol.* 46: 2225-2231.
- [3] Tietel, Z., Feldmesser, E., Lewinsohn, E., Fallik, E., **Porat, R.** (2011). Changes in the transcriptome of 'Mor' mandarin flesh during storage: emphasis on molecular regulation of fruit flavor deterioration. *J. Agric. Food Chem.* 59: 3819-3827.
- [4] Tietel, Z., Lewinsohn, E., Fallik, E., **Porat, R.** (2011). Elucidating the roles of ethanol fermentation metabolism in causing off-flavors in mandarins. *J. Agric. Food Chem.* 59: 11779–11785.
- [5] Tietel, Z., Lewinsohn, E., Fallik, E., **Porat, R.** (2012). Importance of storage temperatures in maintaining flavor and quality of mandarins. *Postharvest Biol. Technol.* 64: 175-182.
- [6] **Obenland, D.**, Collin, S., Sievert, J., **Arpaia, M.L.** (2013). Mandarin flavor and aroma volatile composition are strongly influenced by holding temperature. *Postharvest Biol. Technol.* 82: 6-14.

2. Book chapters:

- [1] **Porat, R.**, Deterre, S., Giampaoli, P., **Plotto, A.** (2014). The flavor of citrus fruit. In: *Biotechnology in Flavor Production*, second edition, (Havkin-Frenkel, D, Dudai, N.; Eds.), Wiley-Blackwell, Oxford (in press).

3. Not refereed papers

- [1] **Plotto, A.**, **Baldwin, E.**, **McCollum, G.T.**, Gmitter, F.G., Jr. (2011). Sensory evaluation of tangerine hybrids at multiple harvests. *Proc. Fla. State. Hort. Soc.* 124:260-263.
- [2] Biotteau, A., Leclair, C., **Plotto, A.**, **McCollum, G.T.** (2012). A comparative study of 'Fallglo' and its seedless mutation 'US Early Pride' tangerines by sensory evaluation. *Proc. Fla. State Hort. Soc.* 125:272-275.

- [3] Leclair, C., **Plotto, A., McCollum, G.**, Bai, J., Biotteau, A., **Baldwin, E.**, Reyes-De-Corcuera, J., Chervin, C. (2012). Gas chromatography-olfactometry analyses of volatiles produced by 'Fallglo' and 'US Early Pride' mandarins. Proc. Fla. State Hort. Soc. 125:264-271.
- [4] Deterre, S., **McCollum, G.**, Leclair, C., Bai, J., Manthey, J., Salvatore, J., Raithore, S., **Baldwin, E., Plotto, A.** (2013). Secondary metabolite composition in *Citrus × Poncirus trifoliata* hybrids. Proc. Fla. State. Hort. Soc. 126 : *in press*.