BARD

FINAL REPORT

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**Identification of Impact Aroma Compounds in Tomato:
Implications for New Hybrids with Improved
Acceptance through Sensory, Chemical, Breeding
and Agrotechnical Techniques**

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Investigators

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The Hebrew University

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DIRECTOR
AUTHORITY FOR RESEARCH
AND DEVELOPMENT

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Authorizing Official, Principal Institution

BARD Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	2			2
Submitted, in review, in preparation	4			4
Invited review papers	2			2
Book chapters	2			2
Books	0			0
Master theses			1 (in prep)	1
Ph.D. theses			2 (in prep)	2
Abstracts	3			
Not refereed (proceedings, reports)				

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

Dr. Florian Mayer SS No. 619-19-8061.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		3	3	6
Longer Visits (Sabbaticals)				

Description of Cooperation: see details of cooperation below.

Patent Summary: none

ABSTRACT

The tomato, a profitable vegetable crop in both the USA and Israel, has benefitted significantly from intensive breeding efforts in both countries, and elsewhere (esp. Holland). Modern hybrids are highly prolific and resistant to a variety of major pests. They produce attractive, firm fruit for both processing and fresh-marketing. In all cases, however, reduction in flavor and aroma have occurred concomitantly with the increase in yield. Sugars-acids ratio dominate fruit taste, whereas aroma volatiles (potent at minute ppb and ppt levels) contribute to the total characteristic tomato flavor. An increase in sugars (1-2%) contributes significantly to tomato fruit taste. However, because of energy reasons, an increase in fruit sugars is immediately compensated for by a decrease in yield. Our main objectives were to: (a) pinpoint and identify the major impact aroma components of fresh tomato; (b) study the genetic and environmental effects on fruit aroma; (c) determine precursors of appealing (flavors) and repelling (off-flavors) aroma compounds in tomato.

Addition of saturated salts blocked all enzymatic activities prior to isolation of volatiles by dynamic and static headspace, using solvent assisted flavor evaporation (SAFE) and solid phase micro-extraction (SPME) from highly favored (FA-612 and FA-624) and less preferred (R 144 and R 175) tomato genotypes. Impact aroma components were determined by gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and aroma extract dilution analysis (AEDA). The potent odorant (*Z*)-1,5-octadien-3-one, was identified for the first time in fresh tomato. From the ca. 400 volatile compounds in the headspace of fresh tomato, the following compounds are proposed to be impact aroma compounds: (*Z*)-3-hexenal, hexanal, 1-penten-3-one, 2-phenylethanol, (*E*)-2-hexenal, phenylacetaldehyde, β -ionone, β -damascenone, 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone (Furaneol[®]), (*Z*)-1,5-octadien-3-one, methional, 1-octen-3-one, guaiacol, (*E,E*)- and (*E,Z*)-2,4-decadienal and *trans*- and *cis*-4,5-EPOXY-(*E*)-2-decenal. This confirms the initial hypothesis that only a small number of volatiles actually contribute to the sensation of fruit aroma.

Tomato matrix significantly affected the volatility of certain impact aroma components and thus led to the conclusion that direct analysis of molecules in the headspace may best represent access of tomato volatiles to the olfactory receptors. Significant differences in certain odorants were found between preferred and less-preferred cultivars. Higher consumer preference was correlated with higher concentrations of the following odorants: 1-penten-3-one, (*Z*)-3-hexenal, (*E,E*)- and (*E,Z*)-2,4-decadienal and especially Furaneol, whereas lower consumer preference was associated with higher concentrations of methional, 3-methylbutyric acid, phenylacetaldehyde, 2-phenylethanol, and 2-isobutylthiazole.

Among environmental factors (salinity, N source, growth temperature), temperature had significant effects on the content of selected aroma compounds (e.g., 3-methylbutanal, 1-penten-3-one, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, 1-octen-3-one, methional, 2-phenylethanol, phenylacetaldehyde, and eugenol) in fresh tomatoes. Salt stress (20 mM NaCl) increased the content of odorants such as (*Z*)-3-hexenal, 2-phenylethanol and 3-methylbutanal in the R-144 cultivar whereas salinity had minor effects on 1-pentene-3-one, 2-isobutylthiazole and β -ionone.

This fundamental knowledge obtained by comprehensive investigation, using modern chemical, sensory and agrotechnical methodology will assist future attempts to genetically modify the concentrations of key odorants in fresh tomatoes, and thus keep the tomato production of Israel and the USA competitive on the world market.

ACHIEVEMENTS

The main objectives of this joint research were to pinpoint and identify impact aroma components of fresh tomato, study genetic, environmental, and agrotechnical effects on aroma composition, and determine possible precursors. Four commercial hybrids, comprised of two genotypes that are highly preferred (F₁s FA-612 and FA-624) and two genotypes that were less preferred (F₁s R 144 and R 175) by consumers were proposed to serve as the experimental model. These hybrids were grown in both Israel and the USA to provide fresh tomatoes for the entire project.

Saturated salts (either CaCl₂ or NaCl) were applied to block enzymatic activities prior to the isolation of volatiles and different isolation procedures were elaborated to ensure minimal effects of the isolation process on the volatile molecules. These were dynamic and static headspace, solvent-assisted flavor evaporation (SAFE) and solid phase micro-extraction (SPME). Identification of impact aroma components of fresh tomato was established by application of gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and aroma extract dilution analysis (AEDA). The potent odorant, (*Z*)-1,5-octadien-3-one, was identified for the first time in fresh tomato. The following constituents were determined in fresh tomatoes and found to be important contributors to fresh tomato aroma: (*Z*)-3-hexenal, hexanal, 1-penten-3-one, 2-phenylethanol, (*E*)-2-hexenal, phenylacetaldehyde, β -ionone, β -damascenone, 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone (Furaneol[®]), (*Z*)-1,5-octadien-3-one, methional, 1-octen-3-one, guaiacol, (*E,E*)- and (*E,Z*)-2,4-decadienal and *trans*- and *cis*-4,5-EPOXY-(*E*)-2-decenal. This is a relevant finding since although more than 400 volatiles have been identified in tomato only a small number actually contribute to its aroma. Significant tomato matrix effects on the volatility of certain impact aroma components were found suggesting that direct analysis of molecules in the headspace may best represent the access of tomato volatiles to the olfactory receptors.

A new sample preparation method was developed and applied for the quantification of Furaneol in fresh tomatoes. Furaneol, which possesses a sweet, pleasant odor, cannot be isolated by most of the commonly used sample preparation methods such as steam distillation or conventional dynamic headspace sampling, due to its high water solubility. Furaneol was found at the highest concentrations (660-1100 ppb) in a summer crop of home-grown tomatoes and at the lowest concentrations (38-180 ppb) in common ethylene-ripened, field-grown, supermarket tomatoes. The highly preferred FA-612 and FA-624 tomatoes (which

were perceived to have a sweeter odor) had higher concentrations of Furaneol than the less preferred R 144 and R 175 tomatoes.

The impact aroma components were quantified and odor units (odor unit = compound concentration/odor threshold) were calculated in the five tomato cultivars, R-144, R-175, FA-612, FA-624, and Josefina (also termed FA-139). Significant differences were found between the cultivars. Higher consumer preference was correlated with higher concentrations of the odorants, 1-penten-3-one, (Z)-3-hexenal, (E,E)- and (E,Z)-2,4-decadienal and especially Furaneol, whereas lower consumer preference was associated with higher concentrations of methional, 3-methylbutyric acid, phenylacetaldehyde, 2-phenylethanol, and 2-isobutylthiazole. Aroma simulation, a key step that indicates whether all important odorants and their concentrations have been correctly determined, was performed and we were able to simulate the aroma of the investigated cultivars and to distinguish between them by comparing the model mixtures with the corresponding authentic samples. Similarity cluster sensory analyses indicated that fortification of tomato juice derived from the less accepted 144 cultivar by (Z)-3-hexenal and Furaneol in an amount matched to that found in the preferred 624 cultivar, resulted in an aroma that was similar to that of the 624 cultivar, further suggesting the significance of these two odorants for the quality of fresh tomato.

The bioformation of the impact compounds, *cis*- and *trans*-4,5-epoxy-(E)-2-decenal and methional by enzymes in disrupted tomato tissue was established by using the ¹³C-labeled precursor compounds linoleic acid and methionine.

Salinity, N-source and temperature were explored in the study of environmental effects on fruit aroma and total soluble solids (TSS). In a number of growing seasons, clear interactions were noted between salinity and genotype. The concentration of Z-3-hexenal, 2-isobutylthiazole and β -ionone increased and hexanal decreased in salt-treated R-144 fruit and were not affected in cv. FA-612. High salt stress resulted in increased 2-phenylethanol in both tomato hybrids, but cv. R-144 was more affected. Phenylacetaldehyde increased significantly only in cv. R-144 and 3-methylbutanal level increased only in cv. FA612. Salt level had no measurable effect on other volatiles. The effect of growing temperature of a) 22/16; b) 28/22; and 34/24 {day/night temperature(°C) 14-h photoperiod} on the aroma volatile of vine ripe fruits indicated that the concentration of 3-methylbutanal, hexanal, (Z)-3-hexenal, (E)-2-hexenal, 2-phenylethanol and phenylacetaldehyde were markedly affected by temperature. At the lower growing temperature, the concentration of lipid-derived volatiles, (Z)-3-hexenal, (E)-2-hexenal and that of leucine-derived 3-methylbutanal increased while at 28/22°C the concentrations of hexanal, methional, and the phenylalanine-derived volatiles, 2-

phenylethanol and phenylacetaldehyde increased. Except of methional, higher temperatures (34/22°C) tended to decrease the concentration of most of the aroma volatiles. There were cultivar differences in the variation of aroma volatiles with growing temperature regime.

The effect of N-source and salinity on the yield performance and TSS were studied with two different genotypes (large fruit R-144 and small fruit FA-612). Our results suggest three possible ways of counteracting the adverse effects of salinity in hydroponically grown tomato: (1) the use of mainly NO_3^- with the addition of up to 1 mM NH_4^+ improves fruit size with minimal loss of fruit quality; (2) small fruit cultivars are less susceptible to salinity up to 45 mM NaCl, and (3) mild salinity of 20 mM NaCl in the nutrient solution hardly reduced yield but significantly improved fruit quality of R-144.

This fundamental knowledge, obtained by comprehensive investigation using modern chemical, sensory and agrotechnical methodology, will assist future attempts to genetically modify the concentrations of key odorants in fresh tomatoes, and thus keep the tomato production of Israel and the USA competitive on the world market.

Details of cooperation

The mode of cooperation in our project was based on expertise in flavor chemistry of tomatoes (Takeoka and Buttery), in sensory and chemical aspects of food flavors and off-flavors (Naim) and in physiology and breeding of tomatoes (Rabinowitch). Tomato seeds of five different hybrids developed in Rehovot by Rabinowitch's group, some with desirable and some with poor flavors, were forwarded to the Albany group and were successfully grown in California's central valley in the spring and summer of 2000, 2001 and 2002. The Albany group was able to supply authentic standards of some key tomato aroma compounds to Rehovot. Dr. Naim visited the Albany group twice, first in March 2000 and then in April 2001 (neither trip was funded by this BARD project). During these visits, we discussed the details of various phases of the research. A Ph.D. student, Yair Bezman from Rehovot, spent several days with the Albany group in August 2001 (supported by this project). He provided details about Rehovot's application of GC-O for the quantification of trace odorants. In turn, he was able to directly observe the sample preparation, chromatographic and spectrometric procedures used by the Albany group. During this visit Mr. Bezman also attended the ACS flavor symposium in Chicago where Dr. Mayer, a post-doc in Albany, presented an invited paper of our work. In August 2002, at the ACS meeting in Boston, Dr. Mayer, Dr. Takeoka and Dr. Naim were in attendance. Dr. Mayer presented an additional symposium paper and

Dr. Naim presented a poster.

Numerous e-mail messages have been routinely exchanged and the collaborative groups have developed close professional and personal working relationships.

Publications resulted from this Project

- 1) Buttery, R.G.; Takeoka, G.R.; Naim, M.; Rabinowitch, H.; Nam, Y. Analysis of furaneol using dynamic headspace sampling with sodium sulfate. *J. Agric. Food Chem.* **2001**, *49*, 4349-4351.
- 2) Mayer, F.; Takeoka, G.; Buttery, R.; Nam, Y.; Naim, M.; Bezman, Y.; Rabinowitch, H. Aroma of fresh field tomatoes. In *Freshness and Shelf Life of Foods*, Cadwallader, K.; Weenen, H. Eds., ACS Symposium Series No. 836, American Chemical Society: Washington, DC, Chapter 11, 2002.
- 3) Bezman, Y.; Mayer, F.; Takeoka, G.R.; Buttery, R.G.; Ben-Oliel, G.; Rabinowitch, H.D.; and Naim, M. Differential effects of aroma (*Lycopersicon esculentum*, Mill) matrix on the volatility of important aroma compounds. *J. Agric. Food Chem.* **2002** (in press).
- 4) Mayer, F.; Takeoka, G.; Buttery, R.; Whitehand, L.C.; Naim, M.; Bezman, Y.; Rabinowitch, H. Differences in the aroma of selected fresh tomato cultivars. In *Handbook of Flavor Characterization: Sensory, Chemical and Physiological Techniques*; Deibler, K.D.; Delwiche, Eds., ACS Symposium Series No., American Chemical Society, Washington, DC, (2002, in review).
- 5) Mayer, F.; Takeoka, G.; Buttery, R.; Whitehand, L.C.; Naim, M.; Bezman, Y.; Rabinowitch, H. New aspects of fresh tomato flavor – differences in the aroma of selected fresh tomato cultivars. *J. Agric. Food Chem.* (Will be submitted within a month).
- 6) Ben Oliel, G.; Kant, S.; Takeoka, G.R.; Buttery, G.; Naim, M.; Rabinowitch, H.D.; Kafkafi, U. Effect of ammonium nitrate ratio and salinity on yield and fruit quality of large and small tomato fruit hybrids. *J. Plant Nutr.* (2002, submitted).
- 7) Ben-Oliel, G.; Bezman Y.; Naim, M.; Takeoka, G.; Buttery, R.; Mayer, F.; Rabinowitch, H.D. The effect of temperature and salinity on aroma volatiles of vine-ripe tomato (*Lycopersicon esculentum* Mill.) (in preparation).
- 8) Ben-Oliel, G.; Naim, M.; Takeoka, G.; Buttery, R.; Rabinowitch, H.D. Effect of potassium and nitrogen source under salt stress on the composition of impact aroma volatiles of vine-ripe tomatoes. (in preparation).