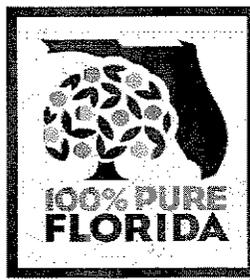


**SIXTIETH
CITRUS PROCESSORS'
and
SUBTROPICAL TECHNOLOGY
CONFERENCE**

OCTOBER 22, 2009



**CITRUS RESEARCH AND EDUCATION CENTER
COOPERATIVE EXTENSION SERVICE, IFAS
UNIVERSITY OF FLORIDA**

**STATE OF FLORIDA
DEPARTMENT OF CITRUS**

**U.S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
CITRUS AND SUBTROPICAL PRODUCTS LABORATORY**

**Processors' Meeting Session
Chairpersons**

1950-68	Fred Wenzel
1969	Warren Savant, Robert Rutledge
1970	David Hamrick, Ralph Brincklow
1971	Arlen Jumper, Henry Cragg
1972	Art Mathias, Marvin Walker
1973	Howard Trumm, Omer McDuff
1974	Dave Hamrick, Arlen Jumper
1975	Bob Vilece, Ray Dennison
1976	Charlie Hendrix, Ken Hastings
1977	Bob Williard, Dick Matthews
1978	Morris Ratcliff, Lou Taylor
1979	James Kirk, Omer McDuff
1980	Ken Fox, Sam Ahmed
1981	Allen Clark, Bob Cook
1982	Dave Mixon, Richard Bogey
1983	Kurt Vahle, Marshall Dougherty
1984	Charlie Varsel, Bob Ferguson
1985	Lew Fain, Chip Bettle
1986	John Neiswanger, Dick Westmoreland
1987	Fred Fulks, Lindsey Richards
1988	Laura Sublett, Willis Wheeler
1989	Kevin Gaffney, George Truitt
1990	Al Assar, Paul Ballentine
1991	Victor Clark, Joe Johnson
1992	Roger Waters
1993	Richard Bogey, Louise Mang
1994	Jose Matos
1995	Dave Johnson
1996	Phil Herndon
1997	Harold Pollack
1998	Clifford Beasley, Jr.
1999	Sean Frielich
2000	Howard Nivens
2001	Sandy Barros, Renee Goodrich
2002	Bill Dubose
2003	Robert Kryger
2004	Garry Merritt, Jose Rodriquez
2005	Jamie Goodner
2006	Dan King, Robert Kryger
2007	Renee Goodrich, José Reyes-De-Corcuera
2008	Elizabeth Baldwin, José Reyes-De-Corcuera
2009	Kevin Gaffney, Kristen Gunter

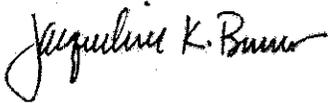
**Subtropical Technology
Conference Chairpersons**

1992	Gordon Hartman
1993	Ruxton Villet
1994	Ruxton Villet
1995	Ruxton Villet
1996	Charlie Hendrix
1997	Kevin Gaffney
1998	Gary Merritt
1999	John Neiswanger
2000	Lisa Rath
2001	Howard Nivens

FOREWARD

Welcome to the 60th meeting of the Citrus Processors and Subtropical Conference Meeting at UF/IFAS Citrus Research and Education Center. We are proud to co-host this conference with the USDA Agricultural Research Service and the Florida Department of Citrus. Cooperation among these research agencies, together with guidance from citrus growers, harvesters and processors, enables us to address challenges and provide solutions for sustainability and profitability. Producing tomorrow's citrus products requires multidisciplinary research within and across all agencies. This spirit of discovery and cooperation is the cornerstone of our citrus industry.

Today you will hear our latest collective research addressing important citrus industry topics. It is our hope that this shared information will be useful to your business. Feedback from our partners will assist us in meeting your needs today and in the future. Best wishes for a productive 2009-10 citrus processing season.



Jacqueline Burns
Interim Director and Professor
UF, IFAS Citrus Research and Education Center
Lake Alfred

On behalf of the Scientific Research Department (SRD) staff at CREC, the FDOC and the Florida Citrus Commission, I am pleased to welcome you to the 60th Annual Citrus Processing and Technology Conference. As the issues of disease in our citrus industry demand our focus, our team has directed significant effort toward disease-related issues, and has also entered into cooperative efforts with both the USDA and UF/IFAS research teams in seeking appropriate responses to disease issues.

The FDOC SRD team has as a primary goal the provision of support to our marketing and public relations programs focused on the nutritional content, and health and wellness benefits, of citrus products. Another primary FDOC responsibility is associated with monitoring both supply side and finished products with respect to regulatory, label, and other compliance factors. Our programs of monitoring citrus juices have been expanded to include both greater numbers of samples and new monitoring programs for certain byproducts – citrus oils and citrus pulp pellets. Our monitoring programs also are tracking any changes that may be associated with the onset of diseases such as HLB, and the changes in production and/or harvesting procedures that may influence the quality of both orange and grapefruit products.

We are pleased to report, that at this point of our industry's battle with diseases such as HLB, we have not identified major changes in the components associated with quality, nutritional, or health and wellness benefits of commercial products. The changes we are recognizing appear to be similar to those the industry recognizes and successfully manages with immature, or stressed, trees and fruit. You may hear reports that specific fruit affected by disease may indeed demonstrate characteristics that differ from non-affected fruit, but our monitoring of commercial citrus products does not detect a significant impact of that fruit if it manages to enter commercial channels.

Our activities associated with supporting the health and wellness message continue and our staff maintains scientific currency and awareness of progress in their specialty fields through cooperative efforts with other research agencies, and participation in domestic and international professional meetings. Staff is responsible for providing our marketing team with consumer-friendly translations of the extensive world-wide research associated with health, wellness and nutrition studies associated with both citrus and competing products. The FDOC sponsorship

of external clinical studies continues to provide a positive return on investment as these studies establish the human health benefits of citrus products and the mechanisms underlying the beneficial events. Clinical research continues to demonstrate the association of beneficial effects in such areas as cardiovascular protection, bone health, cognition and brain neural function, weight management, nutrient density, blood pressure, and lipid metabolism. Recent studies suggest that citrus juices and specific citrus components may not only work at the physiological/biochemical levels in tissues and organs, but may indeed have direct influence on genetic expression at the gene level.

Major research efforts and funding sources will continue to be needed for crop diseases such as HLB. As major funding needs were identified for HLB and canker research, the Florida Citrus Commission directed major re-allocations of the FDOC budget toward support of citrus disease research. In FY 2007-2008, disease research expenditures allocated from the advertising trust fund reached \$2MM, rose to \$7.3MM for FY 2008-09, and have been budgeted at \$10MM for FY 2009-10. The FDOC, by contracting with the National Academy of Sciences to obtain independent evaluation of research needs and proposed research projects, provided a framework for an effective and accountable evaluation system for citrus research funding.

Today, we offer reports on both disease issues and other research issues within the industry. Our critical focus is the severe threat HLB poses for our industry; however, we must continue our much-needed research efforts on other significant problems, and not risk losing the momentum and advances established over many years.

Please feel free to contact any of our Staff if you have any questions or comments on our research programs. Welcome to the 60th Annual Citrus Processing and Technology Conference.

Dan King, Ph.D.
Director, Scientific Research
Florida Department of Citrus, Lake Alfred

On behalf of the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Citrus & Subtropical Products Laboratory (USCSPL), I would like to welcome you to the Subtropical Technology portion of this conference. We are pleased to join the University of Florida, Citrus Research and Education Center (CREC), and Florida Department of Citrus (FDOC) in the presentation of the Processor's Day and Subtropical Technology Program.

We are at the end of our five-year project plan cycle for all four of our research projects. Currently, we have two quality and two by-product projects that conduct research on citrus and subtropical/tropical products. By commodity, our research is about 70 percent on citrus, which is proposed to be maintained in the next five-year cycle. We appreciate the input we have received from industry through our Liaison Committee and are in the process of writing new five-year plans that will be reviewed by panels put together by ARS. We are proposing to combine the "Flavor" and "Fresh Fruit" projects into one larger project, which will expand into the area of nutritional quality, while maintaining the two citrus by-product projects. We are incorporating more research on citrus canker and greening into our proposed plans, and have received outside funding for work in these areas, some of which will be presented in this program.

Work on canker and greening as well as flavor work with mandarin breeding lines has been a joint effort with the ARS lab in Ft. Pierce, FDOC, CREC and the UF Indian River Research and Education Center. Collaborative efforts by university, state and federal labs are what is needed to solve the serious issues facing Florida agriculture and the citrus industry, in particular. As always we welcome any discussion or input concerning our research plans, and extend an open invitation to peruse our publications, visit our facility and talk with our scientific staff.

Elizabeth A. Baldwin
Research/Location Leader
USDA/ARS Citrus & Subtropical Products Laboratory, Winter Haven

October 22, 2009

**60th ANNUAL CITRUS PROCESSORS' AND
SUBTROPICAL TECHNOLOGY CONFERENCE MEETING**

**Citrus Research and Education Center
Cooperative Extension Service, IFAS
University of Florida**

**State of Florida
Department of Citrus**

**U.S. Department of Agriculture
Agricultural Research Service
Citrus and Subtropical Products Laboratory**

Program Committee: Michelle Danyluk, D. King & E. Baldwin

8:15 a.m. Registration and Coffee

8:45 a.m. Announcements and Welcome

**Jacqueline Burns, Interim Director
Citrus Research and Education Center
Lake Alfred**

**Dan King
Director of Scientific Research
Florida Department of Citrus
Lake Alfred**

**Elizabeth Baldwin, Research Leader
Citrus & Subtropical Products Laboratory
Winter Haven**

- 9:30 am FAST CENTRIFUGAL PARTITION CHROMATOGRAPHY AS A PREPARATIVE CHROMATOGRAPHIC TECHNIQUE FOR CITRUS NATURAL PRODUCTS
J. Manthey¹
- 9:45 am ISOTOPIC MARKERS FOR ORIGIN DETERMINATION OF ORANGE JUICES AND BLENDS
M. Azik² and D. McLean²
- 10:00 am LACTONE RING OPENING OF 6', 7'-DIHYDROXYBERGAMOTTIN BY CITRUS-PATHOGENIC FUNGI DIMINISHES ITS CYTOCHROME P450 3A4 INHIBITORY ACTIVITY
K. Myung¹, J. Manthey¹ and J. Narciso¹
- 10:15 am IMPROVED ANALYSIS OF SIMPLE SUGARS AND GALACTURONIC ACID IN HYDROLYZED CITRUS WASTE
W. Widmer¹

10:30 - 11:00 ORANGE JUICE BREAK

- 11:00 am NANOSTRUCTURAL CHARACTERIZATION OF ENZYMATICALLY MODIFIED PECTIN
R. Cameron¹, G. Luzio¹, B. Savery³, P. Vasu⁴ and M. Williams⁴
- 11:15 am FUNCTIONAL CHARACTERIZATION OF CITRUS PECTIN MODIFIED WITH A THERMALLY TOLERANT PECTIN METHYLESTERASE
G. Luzio¹
- 11:30 am COMPARISON BETWEEN GRAPEFRUIT JUICES FROM HLB INFECTED AND CONTROL TREES
F. Valim², F. Jabalpurwala⁷, P. Cancalon² and R. Rouseff⁷
- 11:45 am EFFECT OF SEASONAL VARIATION ON QUALITY OF 'VALENCIA' ORANGE
J. Bai¹, E. Baldwin¹, A. Plotto¹, J. Manthey¹, T. Mccollum⁵, M. Ireys⁶
And G. Luzio¹

12:00 pm - 1:30 pm LUNCH

- 1:30 pm CHARACTERIZATION OF AROMA VOLATILES IN SELECT TANGERINE HYBRIDS BY GAS CHROMATOGRAPHY-OLFACTOMETRY
T. Miyazaki⁷, A. Plotto¹, E. Baldwin¹, J. Reyes De Corcuera⁷ and F. Gmitter⁷
- 1:45 pm NATURAL FLAVOR SYNTHESIS ENHANCED BY UTILIZING HIGH PRESSURE AND "GREEN" SOLVENT TECHNOLOGIES
M. Eisenmenger⁷ and J. Reyes De Corcuera⁷

2:00 pm **GRAPEFRUIT JUICE SULFUR VOLATILE PROCESSING PATTERNS AND PRECURSORS**

F. Jabalpurwala⁷, F. Valim² and R. Rouseff⁷

2:15 pm **DEOXYGENATION OF FRUIT JUICES AND MODEL SOLUTIONS USING IMMOBILIZED ENZYME REACTOR**

N. Ponagandla⁷, and J. Reyes De Corcuera⁷

2:30 - 2:45 BREAK

2:45 pm **MICROBIOLOGICAL EVALUATION OF MECHANICALLY HARVESTED CITRUS FRUIT**

L. Friedrich⁷, T. Spann⁷, R. Ebel⁸ and M. Danyluk⁷

3:00 pm **MECHANICAL HARVESTING OF FLORIDA CITRUS – PROGRAM OVERVIEW**

S. Barros² and D. King²

3:15 pm **FLAVOR ANALYSIS OF ‘HAMLIN’ AND ‘VALENCIA’ JUICE FROM GREENING (HUANGLONGBING) TREES IN 2009**

S. Dea¹, A. Plotto¹, F. Valim², E. Baldwin¹, T. Mccollum⁵ and M. Irely⁶

3:30 pm **USE OF ELECTRONIC TONGUE AND NOSE SENSORS TO DIFFERENTIATE BETWEEN JUICE FROM GREENING (HUANGLONGBING) AND HEALTHY FRUIT**

A. Plotto¹, X. Bredzinski⁹, E. Baldiwn¹, T. Mccollum⁵ and M. Irely⁶

¹ USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

² Florida Department of Citrus, Scientific Research, Lake Alfred, FL

³ Arkansas Biosciences Institute and College of Agriculture, Arkansas State University, Jonesboro, AR

⁴ Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

⁵ USDA-ARS US Horticultural Research Laboratory, Ft. Pierce, FL

⁶ US Sugar Corp, Clewiston, FL

⁷ University of Florida, Citrus Research and Education Center, Lake Alfred, FL

⁸ University of Florida, Southwest Florida Research and Education Center, Immokalee, FL

⁹ Alpha-Mos, Toulouse, France

9:30 am

Fast Centrifugal Partition Chromatography as a Preparative Chromatographic Technique for Citrus Natural Products

J. Manthey¹

Fast centrifugal partition chromatography (FCPC) is a preparative-scale separations methodology based on the principles of counter current chromatography. Separations by FCPC are typically achieved with higher recoveries and with lower solvent use compared to conventional column chromatography. HSCPC, run with a Kromaton A200, was used to separate groups of compounds in mixtures of polymethoxylated flavones obtained from orange oil. Biphasic separations with *n*-hexane/ethyl acetate/methanol/water (1/0.8/1/1, v/v/v/v) solvent mixtures, using either the organic phase as the stationary phase in the descending mode, or with the aqueous phase as the stationary phase in the ascending mode allowed for efficient separations of the polymethoxylated flavones within 2 h chromatographic runs. No loss in resolution occurred for scale-up from 20 mg to 400 mg sample injection. Quantitative recoveries were achieved. Purification of the main PMFs is achieved by FCPC, as well as significant simplifications of the isolations of minor PMF constituents by subsequent column chromatography.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

9:45 am

Isotopic Markers for Origin Determination of Orange Juices and Blends

M. Azik² and D. McLean²

Citrus fruits and juices contain organic compounds and inorganic minerals. The major inorganic elements in orange juice are potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg). There are also other elements in orange juice at trace levels such as rubidium (Rb), strontium (Sr), palladium (Pd), barium (Ba), lanthanum (La) and cerium (Ce). The mineral constituents of citrus fruit are mainly dependent upon the soil (geographic origin) where the citrus plant grows (1). It is expected that single-strength and frozen concentrate citrus juices should also have the same mineral constituents of the source citrus fruit.

The geographical origin of orange juices has been studied previously using ICP-AES (inductively coupled plasma atomic emission spectrometer) and Py-MS (pyrolysis mass spectrometer) techniques measuring the concentrations of major elements and the patterns of pyrolysis products of some organic compounds in the juice (2-4). The goal of this study was to determine and measure the concentrations of interference free isotopes of specific trace elements as markers in single-strength and frozen concentrate orange juices from Florida, Brazil, and blends consisting of Florida juices blended Brazil juices. ICP-MS (inductively coupled plasma mass spectrometer) data was used to create a trace element database from various juice sources.

Samples were collected from Florida and Brazil origin during a harvesting season for each growing region, with some samples provided by USDA import sampling programs. Frozen concentrate orange juices were reconstituted with deionized water (DI) to 11.8° Brix and single-strength juices were used as acquired. All juice samples were microwave digested with 70% nitric acid in closed Teflon vessels with programmed temperature increases up to 220°C. After cooling and addition of internal standards, samples were diluted with DI water prior to analysis by ICP-MS. A fully quantitative analytical method was developed, including the generation of standard calibration curves for chosen isotopes using standards and blank solutions. This method was then utilized for

analysis of specific trace elements found in original source and blended juice samples.

The ICP-MS spectra showed that the mass peaks of ^{85}Rb , ^{88}Sr , ^{105}Pd , ^{138}Ba , ^{139}La and ^{140}Ce isotopes were at comparable levels, which facilitated the distinction of the orange juice from Florida and Brazil. These specific isotopes were chosen as they did not require any isobaric corrections. It was found that Florida orange juices had significantly higher concentrations of ^{88}Sr , and ^{105}Pd than Brazil juices. Conversely, the concentrations of ^{85}Rb , ^{138}Ba , ^{139}La and ^{140}Ce were higher in juices from Brazil than Florida. The differences in the concentrations of these isotopes were also utilized to distinguish blends of Florida and Brazilian juice containing as little as a 5% blend of juices. In addition to differences in absolute concentration values of specific trace elements, the ratios of concentrations of isotopes such as $^{88}\text{Sr}/^{85}\text{Rb}$, $^{88}\text{Sr}/^{138}\text{Ba}$, $^{85}\text{Rb}/^{138}\text{Ba}$, $^{138}\text{Ba}/^{105}\text{Pd}$ and $^{85}\text{Rb}/^{105}\text{Pd}$ were utilized to further distinguish Florida, Brazilian, and various levels of blended juices.

This current research improves upon previous efforts with this technology to build trace element databases from analysis of both pure and blended juices, both single strength and concentrates, from various worldwide citrus producing areas. It is hypothesized that such databases may be used to 'train' artificial neural network software in the identification of the country of origin both pure and blended citrus juices. Further research will continue to extend the range of juices and blends included in the databases and improve the accuracy of identification of country of origin. Immediate targets are to extend the capability to blends of more than two juice origins, and for identifying additional countries of origin for imported juices.

Reference:

1. Nagy, S. 1977. Inorganic elements in "**Citrus Science and Technology**". Eds. Nagy, S., Shaw, P.E., Veldhuis, M.K. The Avi Publishing Company, Inc., Westport CT. 479-495
2. Nidkel, S., Nagy, S. and Attaway, J.A. 1988. Trace metals: Defining geographical origin and detecting adulteration of orange juice in "**Adulteration of Fruit and Beverages**". Eds. Nagy, S., Attaway, J.A. Rhodes, M. Marcel Dekker Inc., New York. 81-105.
3. Bayer, S., McHard, J.A. and Winefordner, J.D. 1980. Determination of the geographical origins of frozen concentrated orange juice via pattern recognition. **J. Agri. Food Chem.** 28:1306-1307.
4. Gracia-Wass, F., Hammond, D., Mottram, D.S. and Gutteridge, C.S. 2000. Detection of fruit juice authenticity using pyrolysis mass spectroscopy. **Food Chemistry.** 69(2):215-220.

10:00 am

Lactone ring opening of 6', 7'-Dihydroxybergamottin by Citrus-Pathogenic Fungi Diminishes its Cytochrome P450 3A4 Inhibitory Activity

K. Myung¹, J. Manthey¹ and J. Narciso¹

Furanocoumarins (FCs) are a class of aromatic compounds in grapefruit that inhibit human intestinal cytochrome P450 3A4 (CYP3A4). Since fungi metabolize polycyclic aromatic hydrocarbons, we hypothesized that certain fungi might also metabolize FCs into forms that may be inactive as CYP3A4 inhibitors in the "grapefruit-drug interactions". Six citrus-pathogenic fungi were observed to metabolize 6', 7'-epoxybergamottin (1) into three major metabolites, including 6', 7'-dihydroxybergamottin (2), bergaptol (3), and an unknown compound, 4-(6', 7'-dihydroxygeranyloxy)-5-(2"-carboxyethyl)-6-hydroxybenzofuran (4). The compound 4 was also found to be a major metabolite of compound 2, where the lactone ring of compound 1 was reduced and cleaved. In Table 1, the compound 4 showed poor inhibitory activity against CYP3A4 ($IC_{50} > 172.0 \mu M$), compared to the known inhibitor 2 ($IC_{50} = 0.81 \mu M$). These results demonstrate a potential use of the fungal enzymes to make the grapefruit FCs inactive.

Table 1. Effects of lactone ring opening of DHB on CYP3A4 inhibition

Compounds	IC_{50} (μM)
6',7'-dihydroxybergamottin (2)	0.81 ± 0.50
4-(6', 7'-dihydroxygeranyloxy)-5-(2"-carboxyethyl)-6-hydroxybenzofuran (4)	$> 172.0^a$

^a indicates highest concentration used for the inhibition assay of CYP3A4 by 4. Data represent means \pm standard deviations of triplicates.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

10:15 am

Improved Analysis of Simple Sugars and Galacturonic Acid in Hydrolyzed Citrus Waste

W. Widmer¹

While the most accurate method for analysis of sugars in biomass is based on gas chromatography of trimethylsilane or alditol acetate derivatives of sugars, the derivation method is time consuming and laborious. In comparison, sample preparation for sugar analysis using liquid chromatography is a simple water extraction procedure, however analysis of hydrolyzed biomass materials are either poorly resolved for some sugars or analysis times are long. High performance ion moderated partition (HPIMP) chromatography using a lead column does not give good resolution for some sugars present in enzyme or chemical hydrolysates of biomass. In addition, galacturonic acid (an indicator of pectin hydrolysis) is retained and not eluted by this column. Since pectin is a major polysaccharide component in citrus waste, galacturonic acid analysis is of interest and requires a separate analytical technique if a lead based HPMIP column is used for sugar analysis. High performance anion-exchange chromatography using a Dionex PA-1 column with pulsed amperometric detection (PAD) provides a much better separation of mono and disaccharide sugars, and a gradient method was developed by Clarke et al. (1991) using a post column addition that also allows analysis of galacturonic and other acidic sugars along with neutral simple sugars in hydrolyzed materials. However, the time required for analysis using this method is lengthy at 70 min. The method of Clarke et al. (1991) was modified to reduce analysis time to 40 min, equivalent to the analysis time required using HPIMP. The method gives good resolution for arabinose, rhamnose, galactose, xylose, glucose, fructose, sucrose, cellobiose, galacturonic acid, and glucuronic acid and its application to hydrolyzed citrus waste materials will be discussed.

Reference:

1. Clarke, A.J., Sarabia, V., Keenleyside, W., MacLachlan, P.R. and Whitfield, C. 1991. The compositional analysis of bacterial extracellular polysaccharides by high-performance anion-exchange chromatography. **Anal. Biochem.** 199:68-74.

11:00 am

Nanostructural Characterization of Enzymatically Modified Pectin

R. Cameron¹, G. Luzio¹, B. Savery³, P. Vasu⁴ and M. Williams⁴

Pectin methylesterase (PME) hydrolyzes C6 methyl esters of galacturonic acid (GA) residues in pectin homogalacturonan regions. Both the degree of esterification (DE) and ester spatial distribution affect pectin functionality. We have created a demethylated pectin series with a unique, thermally tolerant PME (TT-PME) and characterized the modified nanostructure. A model pectin (initial DE of 94%) was demethylated to 90, 80, 70, 60 and 50% DE at pH 7.5 and 4.5. A minimum estimate of demethylated block size was obtained by limited digestion with endo-polygalacturonase. With demethylation to 80% and 70% DE at pH 7.5 maximum demethylated block size increased from 6 to 27 respectively and from 27 to 41 when the DE was lowered to 60%. Similar changes also occurred with demethylation at pH 4.5. Average demethylated block size increased more with decreasing DE in the pH 4.5 series. Demethylation patterns obtained with the TT-PME will be compared to those previously reported for the salt independent PME from citrus. These results indicate pectin nanostructure can be manipulated and tailored by enzymatic treatment under controlled conditions.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

³Arkansas Biosciences Institute & College of Agriculture, Arkansas State University, Jonesboro, AR

⁴Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand

11:15 am

Functional Characterization of Citrus Pectin Modified with a Thermally Tolerant Pectin Methyltransferase

G. Luzio¹

Degree of esterification (DE) is a primary determinant for applications involving pectin. It has been demonstrated that the yield stress behavior of pectin in the presence of calcium ions is dependent on the type of deesterification (ordered vs. random) as well as the overall DE, but these pectin structures were not characterized. This work will be important for new high-volume applications involving suspension such as using citrus peel for drilling muds or for paper additive products.

More recently a non-calcium sensitive pectin with a 94 % degree of methylation was demethylated at lower degrees of methylation (DM) using a thermally tolerant pectin methyltransferase to examine the effects on functional properties, such as rheology, relative to DM. Pectin's functional properties and reactivity toward calcium and other cations is dependent on the amount of methylated galacturonic acid units and their distribution pattern within the homogalacturonan stretches formed from demethylation.

Storage modulus (G') and calcium sensitivity increased with decreasing DM with maximum G' occurring at 50 % DM. Onset of storage modulus was observed at DM values near 70%. Storage modulus maximized when the pectin to calcium ratio reached a value of approximately 5 to 1. Recovery of storage and loss modulus after exposure shear, which is important for suspension, was determined as a function of pH at which the demethylation occurred and will be discussed. Mixing demethylated pectin with narrow-range size-classes of oligogalacturonic acids showed that small galacturonic acid oligomers with a degree of polymerization less than 25 has a significant effect on modulus and modulus recovery.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

11:30am

Comparison Between Grapefruit Juices from HLB Infected and Control Trees

F. Valim², F. Jabalpurwala⁷, P. Cancalon² and R. Rouseff⁷

Citrus greening also known as Huanglonghing (HLB) or yellow dragon disease, has being described as one of the most important and destructive citrus diseases. It has significantly reduced citrus production in Asia, Africa and now is a major treat for Brazil and US.

The main symptoms of the disease in the tree are leaf yellowing, with reduced productivity and small and misshapen fruit. Fruit from HLB infected trees is reported to be bitter, but there is little to no information on juice quality parameters. All previous reports have dealt with oranges. This will be one of the first studies on HLB effects on grapefruit juice flavor compounds and other juice quality parameters.

Grapefruit from control and HLB-infected trees were harvested from a commercial grove in the East coast of Florida throughout the harvesting season. When compared to control grapefruit harvested at the same time, fruit from HLB-infected trees had higher acids and lower sugars, resulting in lower Brix/acid ratio which is characteristic of immature fruit. Juice from HLB-infected trees had higher vitamin C and Naringin content in average. Those differences were higher at the beginning of the season. Aroma profile of juices from control versus HLB-infected trees presented few differences. Juice from fruit from HLB-infected trees exhibited a less balanced aroma profile when compared to control juice.

²Florida Department of Citrus, Scientific Research, Lake Alfred, FL

⁷University of Florida, Citrus Research and Education Center, Lake Alfred, FL

11:45 am

Effect of Seasonal Variation on Quality of 'Valencia' Orange

J. Bai¹, E. Baldwin¹, A. Plotto¹, J. Manthey¹, T. Mccollum⁵, M. Irey⁶ and G. Luzio¹

'Valencia' oranges were harvested from February to June, 2007, and the effect of harvest time on fruit and juice quality was investigated. After reaching a peak in peel color in March, peel regreening occurred and juice content decreased. Soluble solids content (SSC) remained constant at 10.3-11.0% regardless of harvest time. However, juice from later harvested fruit had much lower titratable acidity (TA). Thus, the SSC/TA ratio steadily increased from 10.4 in February to 25.5 in June harvested fruit. When individual sugars and acids were analyzed, it was found that the ratio increased due to a decrease in citric acid and an increase of sucrose over the season. Pectin content in juice increased with delayed harvest time, possibly due to a softening of albedo and membrane tissues that resulted in small amounts of these materials entering the juice during processing. Ascorbic acid content decreased throughout the harvest season. Phenolic hydroxycinnamic acids (HCAs), an unidentified alkaloid, flavonoids including narirutin (NR), 6,8-di-C-glucosyl apigenin (DCGA) and narirutin-4'-glucoside (NRG), as well as sesquiterpenoid limonoids including limonin glucoside (LG), nomilin glucoside (NG), nomilinic acid glucoside (NAG), limonin (L) and nomilin (N) aglycones were measured over the season. Contents of HCAs decreased continually until May then increased in June, and alkaloid continually increased during the entire harvest season. Contents of flavonoids decreased or remained constant. Limonoids, including L and N, the major bitterness contributors, decreased over the season except LG and NG, which peaked in May. Volatile production for most compounds increased with delayed harvest time at least until May (some dropped off in June), including acetaldehyde, octanal, hexanal, decanal, ethanol, hexanol, E-2-hexenol, linalool, octanol, α -pinene, mycene, limonene, ethyl butanoate, ethyl hexanoate and methyl butanoate. Some compounds, such as methanol valencene and ethyl acetate decreased with delayed harvest. Z-3-Hexenol and α -terpineol showed

similar patterns, decreasing in April and May and increasing thereafter. The results indicate that fruit harvested in earlier in the season had better quality in terms of higher juice content, better SSC/TA ratio (April to May, 15.1-18.6 ratio), higher levels of ascorbic acid, flavonoids and other secondary metabolites. However, fruit harvested later in the season likely had more aroma and lower levels of bitter components.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

⁵USDA-ARS US Horticultural Research Laboratory, Ft. Pierce, FL

⁶US Sugar Corp., Clewiston, FL

1:30 pm

Characterization of Aroma Volatiles in Select Tangerine Hybrids by Gas Chromatography-Olfactometry

T. Miyazaki⁷, A. Plotto¹, E. Baldwin¹, J. Reyes De Corcuera⁷ and F. Gmitter⁷

Aroma volatiles in orange juice have been well studied¹ but little information is available on those found in fresh tangerine. Five of 25 tangerine hybrids studied in the 2007-2008 season were analyzed by gas chromatography-olfactometry (GC-O) using the time intensity (Osme) method. The choice of samples to analyze by GC-O was determined from their differences by sensory evaluation in a trained panel² and from their volatile profile. Fruit were harvested and gently squeezed to avoid peel oil volatiles in the juice. Aliquot samples (2.5 mL) were put into 20 mL GC vials with an equal volume of saturated sodium chloride to stop any potential enzymatic activity. Aroma volatiles were sampled using headspace solid phase microextraction (SPME) method, and analyzed by GC-mass spectrometry (GC-MS) and GC-O. Three panelists evaluated the samples by GC-O.

Forty nine odorants were found by all three panelists in a consensus and comprised of monoterpenes, aldehydes, esters, alcohols, ketones, a phenol (thymol) and an ether (1,8-cineole). Hexanal, ethyl 2-methylbutanoate, unknown peak (No. 9), 1-octen-3-one, β -myrcene, 1,8-cineole, linalool, and (*E,E*)-2,4-nonadienal with descriptors of green/grassy, fruity, metallic, mushroom, metallic, green, floral and fatty, respectively, were intense aroma compounds in all five samples. Differences between sample aroma profiles were found for compounds having descriptors of fruity, green/metallic/fatty, terpeney and green/grassy as the main notes, and five other aroma categories. Perceived aroma intensity determined the specific aroma character of each sample. GC-O results partially confirmed the sensory characteristics of some samples (8-9 \times Val4x with orange aroma and a sample with undetermined parentage having characteristic pumpkin and fatty aroma and flavor); however, sugars, acids and other non-volatile compounds also contribute to flavor.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

⁷University of Florida, Citrus Research and Education Center, Lake Alfred, FL

1:45 pm

Natural Flavor Synthesis Enhanced by Utilizing High Pressure and “Green” Solvent Technologies

M. Eisenmenger⁷ and J. Reyes De Corcuera⁷

Isoamyl acetate has a banana flavor and is used as an ingredient. When synthesized from a lipase-catalyzed reaction it can be considered a “natural” flavor ingredient. This study examines the application of high hydrostatic pressure (HHP) on the apparent kinetics of immobilized and free lipase (*Candida antarctica* lipase B) in a biphasic ionic liquid (IL)-alcohol system. ILs have become an attractive alternative media because they can enhance stability, enantioselectivity, product yield, and reaction rate of enzyme reactions [1-4] while being nontoxic and nonvolatile [5]. Also, ILs utilized in a bi-phasic system can improve downstream separation, thus improving production efficiency [5-7]. The application of IL and HHP technologies has been explored separately for enzyme catalysis [8-10]. This study is the first to explore the combination of these technologies. Conversion of isoamyl acetate was up to 10-fold greater with free lipase compared to immobilized lipase after 3 h at 300 MPa and 80 °C (Fig. 1 and 2). Rate of catalysis by free lipase was also up to 15 and 25-fold greater at 500 MPa than at 0.1 MPa at 40 or 80 °C respectively (Fig 3). Pressure had no effect on activation energy (E_a) of free lipase which was 55.6 ± 4.2 and 56.2 ± 4.6 kJ mol⁻¹ at 0.1 and 300 MPa respectively (Fig 4). Likewise, temperature had no effect on activation volume (ΔV^\ddagger) of free lipase which was -16.1 ± 1.5 and -16.7 ± 1.4 cm³ mol⁻¹ at 40 and 80 °C respectively. It was also observed that after treatment at pressures greater than 400 MPa, the free lipase is temporarily suspended in a semi-solid IL phase (Fig 5). This “temporary immobilization” has not been previously described, and may be useful in aiding the separation of phases between reaction cycles.

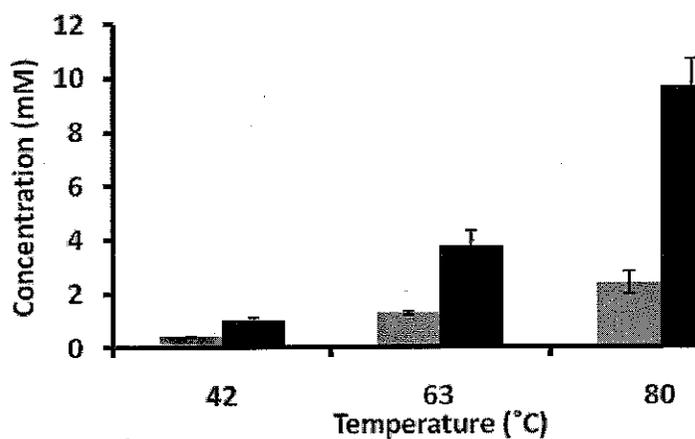


Figure 1. Concentration (mM) of isoamyl acetate produced after 3 h incubation per unit enzyme of immobilized CALB at (■) 0.1 or (■) 300 MPa. Error bars represent standard deviation.

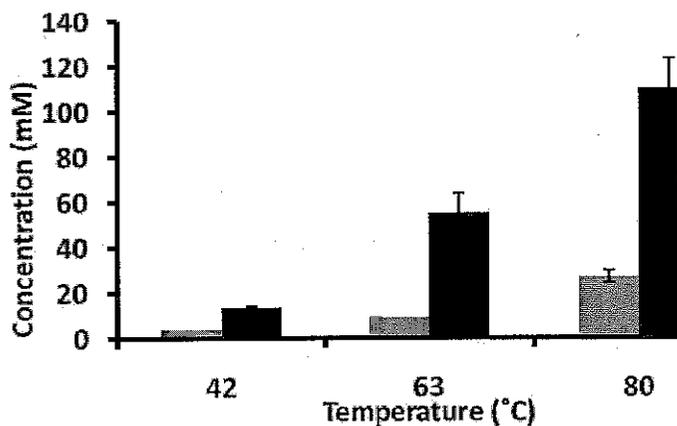


Figure 2. Concentration (mM) of isoamyl acetate produced after 3 h incubation per unit enzyme of free CALB at (■) 0.1 or (■) 300 MPa. Error bars represent standard deviation.

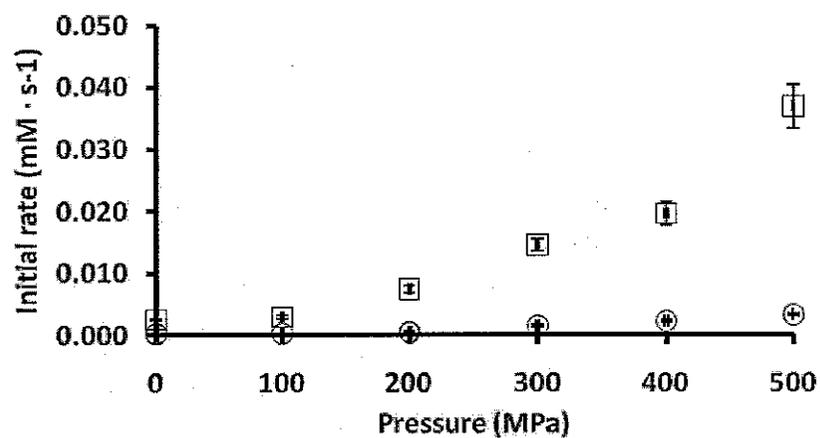


Figure 3. Apparent initial rate per unit enzyme of free CALB at (○) 40 or (■) 80 °C. Error bars represent standard error of linear regression.

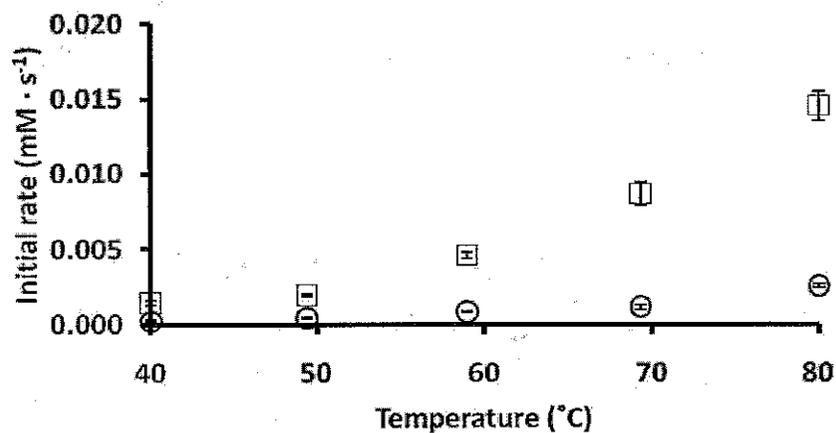


Figure 4. Apparent initial rate per unit enzyme of free CALB at (○) 0.1 or (■) 300 MPa. Error bars represent standard error of linear regression.

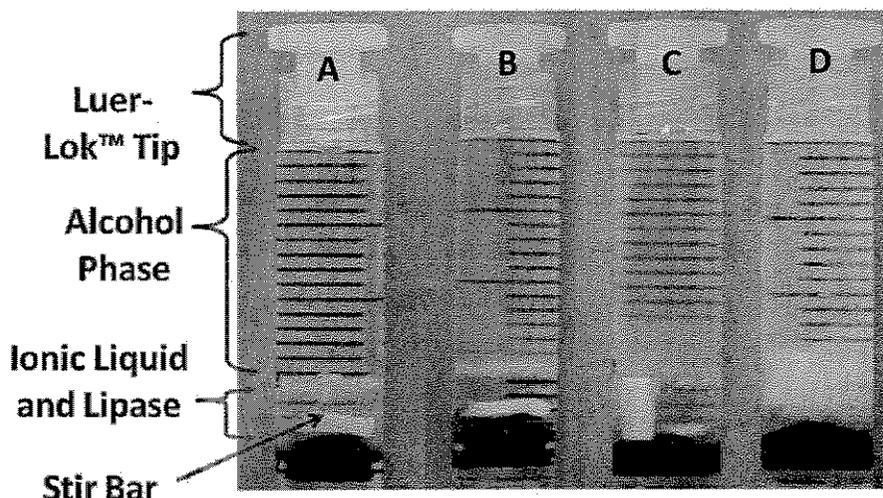


Figure 5. Reaction vessel with ionic liquid biphasic mixture and free lipase. Fig. 5A is the native enzyme-IL, Fig. 5B is after incubation at 0.1 MPa, Fig. 5C is following incubation between 100 and 300 MPa, Fig 5D is after incubation ≥ 400 MPa.

Reference:

1. Feher, E., Major, B., Belafi-Bako, K. and Gubicza, L. 2007. On the background of enhanced stability and reusability of enzymes in ionic liquids. **Biochem Soc. Trans.** 35:1624-1627.
2. Contesini, F.J. and Carvalho, P.D. 2006. Esterification of (RS)-ibuprofen by native and commercial lipases in a two-phase system containing ionic liquids. **Tetrahedron-Asymmetry.** 17:2069-2073.
3. Xin, J.Y., Zha, Y.J., Zhao, G.L., Zheng, Y., Ma, X.S., Xia, C.G. and Li, S.B. 2005. Enzymatic resolution of (R, S)-Naproxen in water-saturated ionic liquid. **Biocatal Biotransform.** 23:353-361.
4. Lozano, P., DeDiego, T., Carrie, D., Vaultier, M. and Iborra, J.L. 2001. Over-stabilization of *Candida antarctica* lipase B by ionic liquids in ester synthesis. **Biotechnol Lett.** 1(23):1529-1533.
5. Van Rantwijk, F. and Sheldon R.A. 2007. Biocatalysis in ionic liquids. **Chem. Rev.** 107:2757-2785.
6. Feher, E., Major, B., Belafi-Bako, K. and Gubicza, L. 2009. Semi-continuous enzymatic production and membrane assisted separation of isoamyl acetate in alcohol-ionic liquid biphasic system. **Desalination.** 241:8-13.
7. Hernández, F.J., de los Ríos, A.P., Gómez, D., Rubio, M. and Villora, G. 2006. A new recirculating enzymatic membrane reactor for ester synthesis in ionic liquid/supercritical carbon dioxide biphasic systems. **Applied Catalysis B: Environmental.** 67:121-126.

8. Eisenmenger, M.J. and Reyes-De-Corcuera, J.I. 2009. High hydrostatic pressure increased stability and activity of immobilized lipase in hexane. **Enzyme Microb. Tech.** 45:118-125.
9. Feher, E., Illeova, V., Kelemen-Horvath, I., Belafi-Bako, K., Polakovic, M. and Gubicza, L. 2008. Enzymatic production of isoamyl acetate in an ionic liquid-alcohol biphasic system. **J. Mol. Catal B: Enzym.** 50:28-32.
10. Husum, T.L., Jorgensen, C.T., Christensen, M.W. and Kirk, O. 2001. Enzyme catalysed synthesis in ambient temperature ionic liquids. **Biocatal Biotransform.** 19:331-338.

2:00 PM

Grapefruit Juice Sulfur Volatile Processing Patterns and Precursors

F. Jabalpurwala⁷, F. Valim² and R. Rouseff⁷

Grapefruit (*Citrus paradisi* McFayden) has a unique flavor due to the combination of sweet-tart and slight bitter taste with a distinctive aroma. This aroma differentiates grapefruit from other members in the citrus family and has been attributed primarily to volatile sulfur compounds, VSC's. VSC's exist at ng/Kg levels in grapefruit juices, GFJ (1-4). Therefore, their identification and quantification using conventional aroma isolation and detection procedures is extremely difficult so little quantitative information on these compounds exist.

A highly sensitive, sulfur specific analysis employing SPME and GC-PFPD with three internal standards has been developed to quantify grapefruit VSC's. This is the first comprehensive method for GFJ sulfur volatiles. SPME parameters (fiber type, headspace atmosphere, extraction time and temperature) were optimized to increase sensitivity. Over 30 VSC's were detected in GFJ's, of which 18 were identified and represented over 90% total sulfur peak area. VSC's were quantified in 10 fresh hand-squeezed (FHS), 11 not from concentrate (NFC) and 9 reconstituted from concentrate (RFC) juice samples. Twenty VSC's were present in significantly higher concentrations ($p < 0.05$) in heated juices than in fresh juices. Principal component analysis, PCA and discriminant analysis revealed unique volatile sulfur patterns which differentiated juices as fresh unpasteurized, pasteurized not from concentrate and reconstituted juice from concentrate. 1-*p*-menthene-8-thiol, a grapefruit character impact compound, also increased dramatically with thermally processing and subsequent storage.

GC-Olfactometry studies revealed 51 aroma active compounds in fresh and canned reconstituted from concentrate juice. Six aroma peaks identified as VSC's, including 1-*p*-menthene-8-thiol, were perceived in higher intensities in canned juice. Four 'fresh grapefruit or spicy pungent' smelling peaks were detected only in fresh juice and tentatively identified as nootkatone, 1,10-dihydronootkatone, beta- sinensal or isoeugenol and eugenol. Model juice studies with sulfur amino acids revealed thermal generation of specific VSC's

observed in commercially processed juices. Methionine produced primarily methional. S-methyl methionine produced primarily dimethyl sulfide. Aromas of both were perceived more intensely in canned juice compared to fresh juice.

Reference:

1. Jella, P., Rouseff, R., Goodner, K. and Widmer, W. 1998. Determination of key flavor components in methylene chloride extracts from processed grapefruit juice. **J. Agric. Food Chem.** 46(1):242-247
2. Buettner, A. and Schieberle, P. 1999. Characterization of the most odor-active volatiles in fresh, hand-squeezed juice of grapefruit (*Citrus paradisi* Macfayden). **J. Agric. Food Chem.** 47(12):5189-5193
3. Lin, J., Jella, P. and Rouseff, R. 2002. Gas chromatography-olfactometry and chemiluminescence characterization of grapefruit juice volatile sulfur compounds. In. **Heteroatomic Aroma Compounds.** 826:102-112
4. Buettner, A. and Schieberle, P. 2001. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi* Macfayden) by quantitation and flavor reconstitution experiments. **J. Agric. Food Chem.** 49(3):1358-1363

²Florida Department of Citrus, Scientific Research, Lake Alfred, FL

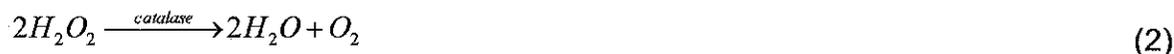
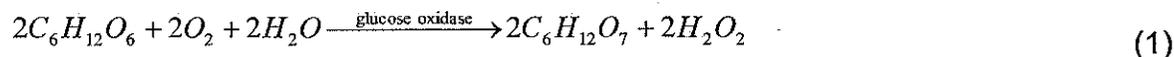
⁷University of Florida, Citrus Research and Education Center, Lake Alfred, FL

2:15 PM

Deoxygenation of Fruit Juices and Model Solutions using Immobilized Enzyme Reactor

N. Ponagandla⁷, and J. Reyes De Corcuera⁷

Dissolved oxygen (DO) affects the quality of fruit juices by reacting with ascorbic acid and flavor components. Degradation of ascorbic acid is one of the major causes of color and quality changes during storage (Rassis and Saguy, 1995; Trammell and others 1986). To prevent these deleterious effects, fruit juices are often deaerated prior to pasteurization. Industrial deaeration using vacuum deaeration resulted in significant reduction of volatiles (Jordan and others 2003). Enzymatic deaeration using glucose oxidase and catalase (GOx-Cat) in solution has been reported (Sagi and Mannheim, 1990; Pickering and others 1998; Parpinello and others 2002). Glucose oxidase oxidizes D-glucose to D-gluconic acid and hydrogen peroxide according to equation 1. Catalase decomposes hydrogen peroxide to water and oxygen as per equation 2.



Thus for each mole of oxidized glucose the enzymatic method removes half a mole of oxygen (Sagi and Mannheim, 1990). The objectives of this study were to develop and test a laboratory prototype deaeration reactor immobilizing GOx-Cat in electrochemically generated poly-o-phenylenediamine on the interior wall of platinized platinum reactor.

Platinum tubes were immobilized with 10, 25 and 50 g L⁻¹ GOx to maximize reactor performance. Operational stability was greater for 10 and 25 g L⁻¹ compared to 50 g L⁻¹ where film detachment was observed (data not shown). Performance for 10 and 25 g L⁻¹ was similar. Both, flow rate and reactor length affected reactor performance. Table 1 shows that decreasing the flow rate from 0.075 to 0.025 mL min⁻¹ increased oxygen removal from 22.6 % to 46.3%. Increasing the length from 3 cm to 12 cm helped in removing more than 90% DO at a flow rate of 0.025 mL min⁻¹.

Table 1. Effect of flow rate and length on the performance of reactor

Length (cm)	Flow rate (mL min ⁻¹)	Initial [DO] (µM)	Final [DO] (µM)	%DO removed	Retention time (sec)
3	0.075	279±11.3	216.5±27.6	22.55±6.7	31.8
3	0.05	279.5±2.1	187±15.6	33.1±6.1	63.6
3	0.025	284.3±3.8	153±31.2	46.3±10.3	95.5
12	0.075	250	91	63.6	127.4
12	0.05	242	58	76	254.7
12	0.025	274±0.7	23±1.41	91.6±0.6	382.4

Table 2. Performance of 12 cm enzyme reactor with model OJ solution, glucose solution and pineapple juice

Solution	Flow rate (mL min ⁻¹)	Initial [DO] (µM)	Final [DO] (µM)	% DO removed	°Brix	% acidity	pH
150 mM glucose solution	0.025	265	21.5	91.9		0.0	7.0
Model OJ solution	0.025	274.7±14.2	29.6±16.6	89.2±6.0		0.8	3.5
Pineapple juice	0.025	281.3±7.8	23.5±2.5	91.7±0.6	15.75±0.17	0.59±0.03	3.01

In both model solutions and pineapple juice approximately 90% DO was removed using a 12 cm reactor at a flow rate of 0.025 mL min⁻¹ with a retention time of 6.4 min.

Reference:

- Jordan, M.J., Goodner, K.L. and Laencina, J. 2003. Deaeration and pasteurization effects on the orange juice aromatic fraction. **Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology** 36(4):391-396.
- Parpinello, G.P., Chinnici, F., Versari, A. and Riponi, C. 2002. Preliminary study on glucose oxidase-catalase enzyme system to control the browning of apple and pear purees. **Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology** 35(3):239-241.
- Pickering, G.J., Heatherbell, D.A. and Barnes, M.F. 1998. Optimising glucose conversion in the production of reduced alcohol wine using glucose oxidase. **Food Research International** 31(10):685-692.
- Rassis, D. and Saguy, I.S. 1995. Oxygen effect on nonenzymatic browning and vitamin C in commercial fruit juices and concentrate. **Food Science and Technology-Lebensmittel-Wissenschaft & Technologie** 28(3):285-290.

5. Sagi, I. and Mannheim, C.H. 1990. The effect of enzymatic oxygen removal on quality of unpasteurized and pasteurized orange juice. **Journal of Food Processing and Preservation** 19(4):253-266.
6. Trammell, D.J., Dalsis, D.E. and Malone, C.T. 1986. Effect of oxygen on taste, ascorbic acid loss and browning for HTST pasteurized single-strength orange juice. **Journal of Food Science** 51(4):1021-1023.

2:45 pm

Microbiological Evaluation of Mechanically Harvested Citrus Fruit

L. Friedrich⁷, T. Spann⁷, R. Ebel⁸ and M. Danyluk⁷

For Florida to continue to compete efficiently in the citrus industry, significant reductions in harvesting costs will be necessary. Mechanical harvesting (MH), a possible means of cost reduction, can be thought of a two-step process: (i) removal of fruit from the tree and (ii) collection of fruit (immediately, by a catch-frame device or during retrieval of fruit from the ground). This 4-year study evaluates the microbiology of the surface and juice of citrus fruit collected by various mechanical harvesting systems, with or without the application 5-chloro-3-methyl-4nitro-pyrazole (CMNP), an abscission compound. For each replicate, treatments groups evaluated may include: (i) hand-harvested fruit (control); (ii) ground fruit (picked up directly from ground following canopy shaking); and (iii) mechanically-harvested (MH) fruit (collected from a catch frame), or any of these groups sprayed with CMNP. Microbial analysis included a total plate count (TPC), an acidophilic organisms count (OSA), a thermophilic aciduric bacillus count (TAB) and generic *Escherichia coli* and *Salmonella* testing on pooled samples of 5 oranges. Juice samples, were subjected to the same tests, with the exception of TAB. Control fruit generally had fewer microbes on the surface of the fruit and in the juice than either ground or MH fruit on both TPC and OSA. TAB were rarely detected. Application of CMNP did not significantly alter microflora. However, no real trends can be attributed to harvest method for all runs. Generic *E. coli* was detected in ground, and MH and following CMNP application on control pooled fruit. *Salmonella* spp. was rarely detected from pooled ground fruit and not detected in any of the juice samples. These results suggest that mechanically harvested fruit are not consistently significantly higher in surface or corresponding juice microflora than the hand harvested control and factors such as weather conditions and grove floor maintenance may contribute to these results.

⁷University of Florida, Citrus Research and Education Center, Lake Alfred, FL

⁸University of Florida, Southwest Florida Research and Education Center, Immokalee, FL

3:00 pm

Mechanical Harvesting of Florida Citrus – Program Overview

S. Barros², and D. King²

During the mid to late 1950's the Florida Citrus Industry was becoming aware of the possibility of a manual labor shortage to harvest its ever increasing fruit crop. As a result of this concern, the idea of using mechanical harvesting was explored.

Due to a variety of reasons, including projected equipment costs, concerns for possible tree and fruit damage incurred during mechanical harvesting, and the devastating freezes which occurred during the 1980's the citrus industry's interest in the project faded.

However, due to continuing increases in cost for manual labor, significant competition in the international market place from foreign competitors with a significant advantage in labor costs, the interests in mechanical harvesting were revitalized in the early 1990's.

In 1995, the Florida Department of Citrus' Mechanical Harvesting Program was initiated. Since then significant progress has been made in development and evaluation of harvesting equipment prototypes and systems. In addition, an abscission chemical has been identified as both usable and economically viable. That chemical has been evaluated according to federal requirements and the research is now being compiled for submission of an application for EPA registration.

²Florida Department of Citrus, Scientific Research, Lake Alfred, FL

3:15 pm

Flavor Analysis of 'Hamlin' and 'Valencia' Juice from Greening (Huanglongbing) Trees in 2009

S. Dea¹, A. Plotto¹, F. Valim², R. Rouseff⁷, E. Baldwin¹, T. Mccollum⁵ and M. Irey⁶

Difference-from-control tests were run for "healthy" juice obtained from fruit harvested from 15 control non-HLB symptomatic trees versus "HLB" juice from asymptomatic fruit harvested from 15 HLB-symptomatic trees within the same grove (~450 fruit for healthy and HLB). A trained panel was also used to evaluate the flavor of the above juice plus juice from symptomatic fruit (small, green, lopsided) harvested from HLB-symptomatic trees (a few trees, ~200 fruit). Fruit were juiced using a JBT 391 extractor and pasteurized under simulated commercial conditions (Microthermics HTST Model 25, 90 °C/10 s).

For Hamlin samples, harvested in February, the Brix ranged from 10.33 to 11.16, acid from 0.54% to 0.58 %, ratio from 19.1 to 21.1 and oil from 0.014% to 0.019 %. For the April Valencia samples, the Brix ranged from 11.9 to 12.5 , acid from 0.65% to 1.18 % (symptomatic HLB juice being highest), ratio from 10.1 to 18.6 and oil from 0.020% to 0.037 % (symptomatic fruit juice had the highest oil content). For the June Valencia samples, the Brix ranged from 10.6 to 12.2, acid from 0.50% to 0.58 %, ratio from 20.8 to 21.2 and oil from 0.014% to 0.028 % (highest for symptomatic fruit). For the Hamlin samples, there were no major differences in Brix, acid, ratio and oil content, but for Valencia fruit from the first harvest (April), the juice from symptomatic HLB fruit was high in acid and oil content compared to the controls, and the asymptomatic HLB fruit juice was in between symptomatic and control values. For the second Valencia harvest, the main difference was that the oil content was higher in asymptomatic and symptomatic fruit juice, possibly due the action of the extractor on fruit of smaller size. Juice samples were frozen for other chemical analyses (individual sugars and acids, vitamin C, limonin, nomilin and other secondary metabolites as well as aroma volatiles) for later analysis by HPLC and GC, and samples for gas chromatography-olfactometry (GC-O) work were prepared. Some of this data will be also be presented.

Sensory Tests

Laboratory panel of 20 to 24 employees, experienced at orange juice evaluation, performed difference-from-control tests (DFC) comparing juice from fruit from control (asymptomatic for HLB) trees and asymptomatic fruit from trees symptomatic for HLB. Each panelist was served two sets of juice presented as 15 mL juice in 30 mL cups (Solo cups company, Urbana, IL) with serving temperature between 11-13 °C. Each set comprised a control labeled as "control", and a test sample labeled with a 3-digit code number. The coded sample was juice from HLB (diseased) or from healthy (control) trees. The control coded samples were presented to account for the placebo effect. Panelists were asked to compare the coded sample with the control juice, by smell, and by taste, and rate the degree of difference on an 11-point category scale, where "0 = no difference", and "10 = extremely different". They were also asked to describe the quality of the difference using their own vocabulary, although a suggested list of descriptors was provided. They were informed that some test samples were identical to the control. The two sets were presented to account for panelist variability.

Juice from control trees and from both asymptomatic and symptomatic fruit from trees symptomatic for HLB disease was also presented to a nine-member panel specifically trained (>50 hours) for descriptive analysis of citrus (different from the panel performing the DFC tests). Specific descriptors were chosen and reference standards were provided. Control juice was served as a warm-up sample and panelists were asked to calibrate themselves using the warm-up as they reviewed the reference standards. Samples were served as 50 mL juice in 110 mL cups (Solo Cups Company, Urbana, IL). Three juices (control juice from normal fruit, HLB juice from asymptomatic HLB fruit and HLB juice from symptomatic HLB fruit) were presented in a randomized order. Panelists rated nine aroma and 15 flavor/taste/mouth feel descriptors using a 16-point intensity scale where 1 = low, 7-8 = medium and 15 = high. Juice evaluation was performed in duplicate on two different days.

All taste panels took place in isolated booths equipped with positive air pressure and under red lighting. Water and crackers were provided to rinse the mouth between samples as necessary.

Data were analyzed by Analysis of variance (ANOVA) with panelists as a random variable using Senpaq v.4.1 (Qi Statistics, Reading, UK). Difference between means were performed using the LSD test, $\alpha = 0.05$.

Sensory Results

There were no differences between juices made with fruit from control or asymptomatic HLB juice by the difference-from-control test for either Hamlin, harvested in January or Valencia, harvested in April or June. This means that the average consumer would not likely find a flavor difference between these two groups of juices (healthy controls and HLB juice from asymptomatic fruit) for either of these varieties, much less detect a difference if this juice was blended with normal juice.

Differences were found, however by the trained panel, but mostly for symptomatic HLB fruit juice (from small, green, lopsided fruit) compared to healthy controls and only a few for asymptomatic HLB fruit juice compared to controls.

There were differences in Hamlin juice for orange and paint aromas as well as orange, fruity-non-citrus, fresh, sour/fermented, peppery/musty, paint, sweet, sour, salty/umami, bitter and metallic flavor descriptors between healthy controls and symptomatic HLB juice, but not between healthy control and non-symptomatic HLB juice. Fruity-non citrus was the only descriptor that was lower than control for both HLB asymptomatic fruit and symptomatic fruit. For the rest of the descriptors, symptomatic fruit were significantly lower in intensity for orange aroma and orange, fresh and sweet tastes; and higher in sour/fermented and paint aromas and sour/fermented, peppery/musty, paint, sour, salty/umami, bitter, and metallic flavors as well as a tingly mouth feeling sensation.

For the April Valencia harvest, almost all significant differences were between healthy and symptomatic HLB juice with the exception this time of the peppery/musty aroma descriptor where both HLB asymptomatic and

symptomatic fruit were rated higher than controls. For this Valencia harvest, the fresh and peppery/musty aroma descriptors showed significant differences, where they did not in the Hamlin juice, and peel oil flavor also showed a significant difference, where it did not in the Hamlin juice.

For the June Valencia harvest, there were no differences in aroma descriptors, and this time control juice was lower in orange and fresh flavor compared to asymptomatic HLB juice, and higher in the paint descriptor. Symptomatic HLB juice was higher in sour/fermented, peppery/musty and salty/umami descriptors than asymptomatic HLB juice, but not different from control juice.

In conclusion, the large tree sample size reduced the tree-to-tree variation observed in previous years. HLB juice from asymptomatic fruit was not significantly different in flavor from control juice in the difference from control tests, and even a trained panel could find little difference between these two types of juices. Juice from HLB symptomatic fruit, however, harvested from diseased HLB trees showed differences in many descriptors rated by the trained panel compared to control juice from control (asymptomatic) trees.

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

²Florida Department of Citrus, Scientific Research, Lake Alfred, FL

⁵USDA-ARS US Horticultural Research Laboratory, Ft. Pierce, FL

⁶US Sugar Corp., Clewiston, FL

⁷University of Florida, Citrus Research and Education Center, Lake Alfred, FL

3:30 pm

Use of Electronic Tongue and Nose Sensors to Differentiate Between Juice from Greening (Huanglongbing) and Healthy Fruit

A. Plotto¹, X. Bredzinski⁹, E. Baldwin¹, G. Mccollum⁵ and M. Irey⁶

In 2007 and 2008, consumer and descriptive sensory analyses were made of juice from fruit harvested from healthy and Huanglongbing (HLB) affected trees. Differences were sometimes found for flavor, mainly for juice from HLB fruit symptomatic for the disease (small, green, asymmetrical shape) with descriptors such as bitter, sour, metallic, and general off taste (Plotto et al., submitted manuscript; Goodrich-Schneider et al., 2008). Levels of the bitter compounds limonin and nomilin tended to be higher in juice from HLB symptomatic trees and fruit, although not above reported sensory thresholds (Baldwin et al., submitted). Hamlin fruit was harvested in February 2008, and again in January 2009 from a commercial grove, juiced and pasteurized under commercial conditions. The 2008 samples were processed in batches of 200-400 fruit harvested from three replicates of three trees per treatment, while the 2009 juice was processed in one unit of 200-500 fruits per treatment harvested from 15 trees. Treatments included fruit from healthy trees, non-symptomatic fruit from HLB-affected trees and HLB-symptomatic fruit. Samples were presented to a trained panel (>50 hours) for sensory evaluation and analyzed using the Astree® electronic tongue and Prometheus® electronic nose (Alpha-MOS, Toulouse, France). Correlations between sensory and electronic nose and tongue were done using AlphaSoft software V.12.

In 2008, Hamlin juice from HLB affected trees was lower in orange and fresh flavor and sweet taste and higher in bitter, metallic, pungent/peppery and astringent flavor and taste (trained panel evaluation). Differences between juice from symptomatic and non-symptomatic fruit were found for sour/fermented and musty/earthy flavor, and juice made with symptomatic fruit was perceived to have a salty/umami taste. In 2009, with a larger sample size, there were no differences between juice from healthy and non-symptomatic HLB fruit for most descriptors except fruity flavor, lower in juice from HLB-affected trees. However, juice from HLB symptomatic fruit was significantly lower in orange aroma and flavor, fresh

flavor and sweet taste, and higher for most of the off-flavors. The electronic tongue sensors could clearly differentiate between juice from HLB symptomatic fruit, and juice from healthy trees both years. Juice from non-symptomatic HLB fruit was classified between these two samples in 2008, with some overlap between healthy and HLB samples, and undifferentiated from the healthy samples in 2009 (Figure 1). There were very good correlations between electronic tongue and sensory panel data. The electronic nose could also separate juice from symptomatic fruit in 2008.

Research will be pursued evaluating sensory perception of juice blends from symptomatic, asymptomatic, and healthy fruit, and to relate the electronic nose and tongue classifications with the sensory data. Work will also be done to determine which chemical compounds are correlated with the electronic nose and tongue responses.

Reference:

1. Goodrich-Schneider, R., Sims, C.A., Spann, T., Danyluk, M.D., Rouseff, R.L. 2008. Effect of greening plant disease (Huanglongbing) on orange juice flavor and consumer acceptability. International Research Conference on Huanglongbing, Orlando, FL. Available from: http://www.doacs.state.fl.us/pi/hlb_conference/proceedings.pdf.
2. Plotto, A., Baldwin, E.A., McCollum, T.G., Manthey, J., Narciso, J.A., Irey, M. Effect of Liberibacter infection (Huanglongbing or "greening" disease) of citrus on orange juice flavor quality by sensory evaluation (**submitted to Journal of Food Science**)
3. Baldwin, E.A., Plotto, A., Manthey, J., McCollum, T.G., Bai, J., Irey, M., Cameron, R., Luzio, G. Effect of Liberibacter infection (Huanglongbing disease) of citrus on orange fruit physiology and fruit/fruit juice quality: chemical and physical analyses (**submitted to J. Agric. Food Chem.**)

¹USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL

⁵USDA-ARS US Horticultural Research Laboratory, Ft. Pierce, FL

⁶US Sugar Corp., Clewiston, FL

⁹Alpha-Mos, Toulouse, France

Figure 1. Electronic tongue analysis of Hamlin juice harvested in February 2008 and January 2009. A = juice from healthy fruit; B = juice from non-symptomatic HLB fruit, S = juice from symptomatic HLB fruit

