FIFTY-SEVENTH
CITRUS PROCESSORS'
and
SUBTROPICAL TECHNOLOGY
CONFERENCE

OCTOBER 18, 2006

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

STATE OF FLORIDA, DEPARTMENT OF CITRUS

AND

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

Welcome to the 57th Citrus Processing and Technology Conference and the Subtropical Technology Conference held at the Citrus Research and Education Center. The University of Florida is proud to co-host this meeting at CREC with the USDA, Agricultural Research Service and the Florida Department of Citrus. Continued interaction between the research and education institutions with citrus growers, harvesters and processors regarding this phase of the industry is a hallmark of the Florida industry history, and will offer opportunities to address the environment of change and challenge that is facing all of us today.

Today’s program represents a sampling of a much larger effort on the part of UF/IFAS, USDA/ARS, and FDOC to provide support on an ongoing basis to Florida citrus partners. Through independent research, through collaborations within Florida, and by reaching out to scientists, engineers, and economists from around the world, the research and education community attempts to bring broad perspective and tools to address industry problems.

A number of issues currently affecting citrus in Florida converge to create an environment of tremendous uncertainty and present some great challenges for the industry. All sectors are affected, and the resolve of the scientific community in concert with you, the industry will lead to development, testing and adoption of solutions. Today’s program highlights progress on a number of fronts, reflecting the areas of emphasis in citrus processing from each of the research institutions.

On behalf of UF, IFAS and the faculty and staff of CREC, I express our deep appreciation for the support, confidence, and encouragement that you provide to our programs. We hope that you enjoy and benefit from the presentations today and provide some feedback that assists us in planning future conference topics.

Harold W. Browning
Center Director and Professor
I would like to welcome you on behalf of the Florida Department of Citrus (FDOC) and the Florida Citrus Commission (FCC) to the 57th Annual Citrus Processing and Technology Conference. We look forward to an informative day of presentations relating to product and health attributes of citrus and citrus juices, new furanocoumarins identified in grapefruit juice and other areas of interest to the citrus industry.

The 2005/06 fiscal year was the first full year for the Scientific Research staff to be focused primarily on its new mission to be the go to source for health, nutrition and wellness information relating to citrus and citrus juices for the Florida citrus industry. Our key objective has been to identify sound scientific based health messages for citrus with a focus on orange juice that could be used by Marketing/Public Relations. We have made major progress this past year on building a strong foundation of scientifically supported health and nutrition information upon which marketing can build meaningful product messages.

In accomplishing this, nine White Papers were written, four peer reviewed papers were published and five papers were presented at professional meetings. In addition, eight scientific meetings on health related topics both internationally and in the US were attended. Also eight special reports were prepared on health related issues on competitive juices, book reviews and special topics of interest. Two major sponsored research projects at prominent research institutions were initiated, are on-going, and have been supported by our staff. Florida orange juices were tested for glycemic index and determined by an outside laboratory to be low glycemic index products.

Today, you will have the opportunity to hear some of the areas we are currently exploring. We would welcome any questions and comments on our new direction.

William S. Stinson, Ph.D.
Director, Scientific Research
Florida Department of Citrus, Lake Alfred
On behalf of the U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS), Citrus & Subtropical Products Laboratory, I would like to welcome you to the Subtropical Technology portion of this conference. We extend our thanks to the University of Florida, Citrus Research and Education Center for including us in theirProcessor’s Day program. We continue to work along side of the CREC and the FDOC to conduct postproduction research relevant to the citrus industry. We appreciate input from the Florida Citrus Industry Research Coordinating Council on research priorities to help advise ARS on problems and challenges to the industry. We recognize that there are many challenges at present including urbanization, global competition, disease and weather, necessitating a pooling of academic resources to confront these issues. We hope that, in the near future, design money will be allocated by Congress for co-location of our facility with CREC and FDOC. This would promote synergism and stabilization of our scientific efforts in the areas of food safety/biosecurity, postharvest physiology and food science as well as sharing of costs and equipment. Our scientific presentations will be in the area of flavor quality, grapefruit drug interaction, and value-added products from citrus processing waste. We hope you find these talks informative and relevant. We welcome any discussion 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 18, 2006

57th ANNUAL CITRUS PROCESSORS’ AND SUBTROPICAL TECHNOLOGY CONFERENCE MEETING

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

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

Program Committee: R. M. Goodrich, F. Valim & E. Baldwin

8:15 a.m. Registration and Coffee

8:45 a.m. Announcements and Welcome

Harold Browning, Director
Citrus Research and Education Center
Lake Alfred

William Stinson
Director of Scientific Research
Florida Department of Citrus
Lake Alfred

Elizabeth Baldwin, Research Leader
Citrus & Subtropical Products Laboratory
Winter Haven
Moderator: Dan King, Florida Department of Citrus

9:15 am  SULFUR VOLATILES IN HAND SQUEEZED, PASTEURIZED AND RECONSTITUTED GRAPEFRUIT JUICES  
O. Gurbuz\textsuperscript{1}, J. M. Smoot\textsuperscript{1}, J. Rouseff\textsuperscript{1} and R. Rouseff\textsuperscript{1}

9:30 am  CHARACTERIZATION OF GRAPEFRUIT SEED SPROUT OFF-FLAVOR IN GRAPEFRUIT JUICE USING GC-O AND GC-MS  
C. Emanuels\textsuperscript{1} J. M. Smoot\textsuperscript{1} and R. Rouseff\textsuperscript{1}

9:45 am  ORANGE JUICE OFF-FLAVOR COMPOUND THRESHOLDS MEASURED IN A PUMPOUT MATRIX  
A. Plotto\textsuperscript{2}, K. L. Goodner\textsuperscript{2}, J. A. Narciso\textsuperscript{2} and E. A. Baldwin\textsuperscript{2}

10:00 am  HOW DOES THE IN VITRO CHEMICAL ESTIMATION OF FOODS ANTIOXIDANT POTENTIALS REFLECT THEIR IN VIVO BENEFICIAL EFFECTS?  
P. Cancalon\textsuperscript{3}

10:15 - 10:45 ORANGE JUICE BREAK

10:45 am  COMPARISON OF SULFUR AROMA IMPACT COMPOUND FORMATION MECHANISMS IN WINE AND GRAPEFRUIT JUICE  
R. Rouseff\textsuperscript{1} and O. Gurbuz\textsuperscript{1}

11:00 am  QUANTIFICATION OF GUIACOL IN ORANGE JUICE USING GC-MS WITH SELECTED ION MONITORING  
P. R. Perez-Cacho\textsuperscript{1} and R. Rouseff\textsuperscript{1}

11:15 am  CITRUS SALT-INDEPENDENT PECTIN METHYLESTERASE DEMETHYLATION OF A MODEL HOMOGALACTURONAN: EFFECT OF PH ON DEMETHYLATED BLOCK SIZE AND DISTRIBUTION  
R. G. Cameron\textsuperscript{2}, K. L. Goodner\textsuperscript{2} and G. A. Luzio\textsuperscript{2}

11:30 am  FUNCTIONAL PROPERTIES OF PECTIN AFTER TREATMENT WITH AN ORANGE PECTIN METHYLESTERASE  
G. A. Luzio\textsuperscript{2} and R. G. Cameron\textsuperscript{2}

11:45 am  LC/MS AND NMR IDENTIFICATION OF NEW GRAPEFRUIT FURANOCOUMARINS  
J. Yu\textsuperscript{3}, B. Buslig\textsuperscript{3}, C. Haun\textsuperscript{3} and P. Cancalon\textsuperscript{3}

12:00 pm - 1:30 pm LUNCH
Moderator: Robert Kryger, Molecular Separations Specialists, LLP

1:30 pm *IN VITRO INHIBITION OF CYP3A4 ACTIVITY BY SOME NEWLY-ISOLATED MINOR-OCCURRING FURANOCOUUMARINS IN GRAPEFRUIT JUICE*

K. Myung² and J. A. Manthey²

1:45 pm *RESULTS OF SEVERAL IN VIVO STUDIES OF THE BIOLOGICAL ACTIONS OF 3,5,6,7,8,3',4'-HEPTAMETHOXYFLAVONE FROM ORANGE PEEL*

J. A. Manthey²

2:00 pm *FLAVONOIDS IN SWEET ORANGE AND JUICE*

M. Azik³

2:15 pm *ANALYSIS OF POLYMETHOXYLATED FLAVONES IN CITRUS PRODUCTS BY DIRECT INJECTION AND IN-LINE TRACE ENRICHMENT*

W. W. Widmer²

2:30 pm *EVALUATION OF HOLD TIMES FOR CLEANED FOOD TRANSPORT TANKERS*

P. Winniczuk¹ and R. Goodrich¹

2:45 - 3:00 BREAK

3:00 pm *POTENTIAL BENEFITS OF CITRUS FOR ASTHMATICS*

S. Barros³ and F. Valim³

3:15 pm *INFLUENCE OF ORANGE JUICE CONSUMPTION ON THE BLOOD LIPID PROFILE OF MEN AND MIDDLE AGED WOMEN*

T. B. Cesar⁴, N. P. Bonifacio⁴ and A. C. Garcia⁴

3:30 pm *CURRENT RESEARCH ON RAPID METHODS FOR ORANGE JUICE QUALITY*

J. Reyes De Corcuera¹

3:45 pm *ORANGE JUICE CONSUMPTION AND POSSIBLE PROTECTIVE EFFECT AGAINST ARTHRITIS*

F. Valim³ and S. Barros³
1University of Florida, IFAS, CREC
2Citrus & Subtropical Products Laboratory
3Florida Department of Citrus
4Sao Paulo State University, Brazil
___Presenter
Flavor is one of the most important determinants of food and beverage quality since the interaction of aromatic substances with the senses of smell and taste lead to consumer purchase and consumption decisions. Sulfur volatiles are surprisingly widespread in foods in terms of numbers but miniscule in terms of total amounts (1). The aroma component of grapefruit juice flavor is largely determined by the relative concentrations of three components; 1-p-menthen-8-thiol, 4-mercapto-4-methyl-2-pentanone and nootkatone. Little is known about the relative concentrations of the first two aroma components which are also thought to be the most important (2) because they exist at µg/L levels and are difficult to measure. More specifically, almost nothing is known about their distribution between various types of grapefruit juices. Therefore the objective of this study was to survey three types of grapefruit juice, fresh hand squeezed (FHS), not-from-concentrate (NFC), and reconstituted from concentrate (RFC). A major component of this study was to identify and quantify the sulfur volatiles in different types of grapefruit juice using gas chromatography with a sulfur specific, pulsed flame photometric detector, PFPD.

Static headspace procedures (SPME) were employed to concentrate grapefruit juice sulfur volatiles. High resolution capillary gas chromatography was employed with PFPD to separate and quantify individual sulfur volatiles in grapefruit juice on both DB-5 and wax columns. As many as 23 sulfur peaks were observed in freshly squeezed FHS, 26 sulfur compounds in pasteurized not-from-concentrate NFC grapefruit juice and 23 sulfur peaks in reconstituted from concentrate (FC) juice. Commercial processing techniques can strongly influence the relative distribution of sulfur volatiles in grapefruit juice as well as alter the relative amounts of sulfur volatiles. Effects on the absolute amounts of sulfur volatiles generally increased with the amount of thermal processing. Specific details will be presented and possible flavor implications discussed.
References:


The seeds in late season grapefruit begin to sprout while the fruit is still on the tree. An example of these sprouted seeds can be seen in figure 1 where some of the juice vesicles have been removed in a Ruby Red grapefruit section. These root sprouts (figure 1a) are fairly tender are often macerated during commercial juice extraction. Macerated root sprouts can alter the flavor of the resulting juice (Fellers, 1985). The object of this study was to examine the juice from seed sprouted fruit to identify what components from the roots was responsible for the altering the normal grapefruit juice aroma.

Ruby Red and Marsh grapefruit were harvested in late May and early June 2006, cut axially, sorted into sprouted and non sprouted groups and hand squeezed. The juice was analyzed for aroma compounds using GC-Olfactometry and major volatiles identified using GC-MS. The seed sprouts were excised using a scaple, ground in methanol and extracted using a Soxhlet extraction apparatus.

The MS total ion chromatogram of the root sprout extract is shown in Figure 2. One of the major volatiles in the root sprout extract was Methyl N-methylantranilate (RT = 22.7 min) whose structure is shown below along with a similar compound methylantranilate. Methyl N-methylantranilate is sometimes shortened to dimethylantranilate. This compound was found to be highly elevated in seed sprouted grapefruit juice as shown in Figure 3.
Both of these compounds possess an artificial grape or grape Kool-Aid aroma and are usually associated with mandarin rather than grapefruit juice.

GC-O and sensory studies of commercial GFJ with added Methyl N-methylantranilate confirmed that this compound has can significantly alter the aroma of late season GFJ.
Reference:
Orange Juice Off-Flavor Compound Thresholds Measured in a Pumpout Matrix
A. Plotto\textsuperscript{2}, K. L. Goodner\textsuperscript{2}, J. A. Narciso\textsuperscript{2} and E. A. Baldwin\textsuperscript{2}

The data presented is part of a continuing study to provide the industry with threshold guidelines more adequate for use of flavors in citrus juices. The Best Estimate Threshold (BET) of volatile compounds reported to generate off-flavor in orange juice (OJ) was determined in a deodorized OJ matrix (pumpout). The Three-Alternative-Forced-Choice (3-AFC) method was used (ASTM: E-679). The USDA staff, comprised of 16 to 20 experienced panelists, age 25 to 65, were presented with orange juice samples (15 ml in 30 ml capped cups) arranged in five rows of three samples corresponding to five spiking levels, each separated by a factor of 3, with a 3-AFC presentation at each level. For each compound, the test was repeated four times, adjusting for concentrations when necessary. The orange juice matrix was sampled several times during the study to verify absence of microorganisms that might induce off-flavor. Compounds with the lowest population BET were 2-methyl-3-furanthiol, 1,4-cineole, and guaiacol, with orthonasal BET of 0.64, 2.69, and 3.64 $\mu$g/L, respectively. 2-Methyl-3-furanthiol, as well as methional, furaneol, p-vinylphenol, and ethanol, are likely to be perceived as off flavors in some juices, since their thresholds could be lower than reported concentrations if the juice was thermally abused, or tainted with bacterial contamination. Other compounds, such as terpiene-4-ol and a-terpineol, which are reported off flavors, had thresholds significantly higher than reported concentrations, and, therefore, would not impart an off-favor to orange juice. Interactions between off flavor compounds with orange juice desirable flavor volatiles will be studied by analyzing the perception of fresh orange juice spiked with these compounds at subthreshold levels.
Table 1. Preliminary data\textsuperscript{a} for orthonasal (smell) and retronasal (taste) population thresholds (T) of selected off flavor compounds in an orange juice matrix, individual recognition thresholds, and reported concentration ranges of these compounds in orange juice.

| Compound\textsuperscript{a} | Origin | Detection T (ppb)
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<td>smell</td>
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<tr>
<td>2-Methyl-3-furanthiol</td>
<td>thermal degradation</td>
<td>0.64</td>
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<tr>
<td>1,4-Cineole</td>
<td>thermophilic bacteria</td>
<td>2.69</td>
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<tr>
<td>Guaiacol</td>
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<td>3.64</td>
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<tr>
<td>Methional</td>
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<td>Dimethylsulfide</td>
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<tr>
<td>Diacetyl</td>
<td>bacterial contamination</td>
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<td>1,8-Cineole</td>
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<td>Furaneol</td>
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<td>p-Vinylguaiacol</td>
<td>thermal degradation (ferulic acid)</td>
<td>174.67</td>
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<td>Thymol</td>
<td></td>
<td>392.67</td>
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<td>d-Carvone</td>
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<td>554.05</td>
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<td>4,5-Dimethyl thiazole</td>
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<td>655.41</td>
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<td>Carveol</td>
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<td>2136.33</td>
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<td>Terpinen-4-ol</td>
<td>sugar degradation</td>
<td>2549.94</td>
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<td>Furfural</td>
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<td>~Terpineol</td>
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<td>Ethanol (ppm)\textsuperscript{f}</td>
<td>bacterial contamination</td>
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\textsuperscript{a} Compounds used for spiking were obtained from Sigma, and nominal purity is used for calculation, but olfactory purity has yet to be verified.
\textsuperscript{b} Population detection thresholds (geometric mean of individual Ts)
\textsuperscript{i} Ethanol concentrations are ppm, not ppb
Disease prevention has become an important part of health maintenance programs and the interest in the health benefits of plant foods is constantly increasing. However, the attention has been focused, mainly, on the antioxidant properties of foods. In order to assess the relative values of various foods, analytical procedures have been developed to measure their antioxidant potential. The measurements are mainly performed by in vitro chemical reactions. The ORAC (Oxygen Radical Absorbance Capacity) assay is the most well known. In recent years most foods, juices and drinks have been compared by such methods. The claims state that the healthiest are those with the highest ORAC value. Generally, these comparisons have not been favorable to citrus and it has been reported that citrus have poor antioxidant values.

Equating the health benefits of phytochemicals to their in vitro antioxidant potential has now been challenged by several groups. Their arguments have been summarized by Azzi et al. (2004) and expressed in detail in numerous publications particularly at the 1st International conference on Polyphenols and Health (2004, published in 2005). They have pointed out that:

1) A phytochemical has only a health value once inside the body. Phytochemical bioavailability is of major importance to assess health benefits.

2) Once in the body compounds are transformed into metabolites which may have properties different from those of the parent chemical.

3) To be active a compound has also to penetrate a specific organ. Hesperidin (the main orange flavonoid) and vitamin C can penetrate the blood brain barrier and act on the brain.

4) The chemical antioxidant potential is only part of the health value of a phytochemical.

The health properties of polyphenols have been extensively examined in the last few years. They have long been known to act as antioxidants and radical scavengers. However, the average concentration of these compounds in plasma is very low (1 mM...
or less) and much lower than those of the endogenous antioxidants such as uric acid (150–450 mM) and it is difficult to explain that direct scavenging of free radicals by polyphenols is the key mechanism explaining their effects on biomarkers and other risk factors. It is now hypothesized that other properties of these compounds are responsible for a significant proportion of their beneficial effects. The major health properties of polyphenols can be summarized as follow:

1) Chemical Antioxidants.
   - Scavenge reactive oxygen and nitrogen species.
   - Chelate redox-active transition metal ions.
   - Spare and interact with other antioxidants.

2) Physiological antioxidants.
   - Inhibition of the redox-sensitive transcription factors (acting on genes).
   - Inhibition of pro-oxidant enzymes.
   - Induction of antioxidant enzymes.

3) Growth of atherosclerotic plaque.
   - Reduce adhesion molecule expression.
   - Anti-inflammatory.
   - Reduce the capacity of macrophages to oxidatively modify LDL (low-density lipoprotein).

4) Platelet function and homeostasis.
   - Inhibit platelet aggregation.

5) Blood pressure and vascular reactivity.
   - Promote nitric oxide-induced endothelial relaxation.

6) Plasma lipids and lipoproteins.
   - Reduce plasma cholesterol and triglycerides, LDL.

In this list only one out of six properties could be detected by a chemical evaluation of polyphenol antioxidant potential, assuming that the active compound is present in vitro. It can be concluded that the physiological properties of phytochemicals are complex and need to be examined as they interact with specific organs and cells.
References:

Comparison of Sulfur Aroma Impact Compound Formation Mechanisms in Wine and Grapefruit Juice

R. Rouseff¹ and O. Gurbuz¹

Wine is an international commodity of enormous economic impact compared to grapefruit juice. A tremendous body of research devoted to wine flavor has developed due to its economic importance. Although one would not normally think that wine and grapefruit juice have much in common, there are a surprising number of aroma impact compounds which are common to both products. The object of this study was to see if it would be possible to leverage some of the wine research to determine if some wine volatile formation mechanisms also hold true for grapefruit juice.

4-Mercapto-4-methylpentan-2-one, 4-MMP, is an extremely potent volatile thiol that was initially discovered in Sauvignon wine (1). At trace levels 4-MMP exhibits a pleasant tropical fruit/grapefruit aroma and is one of the most intense odorants in certain white wines but at higher concentrations its aroma smells like cat urine (2). 4-MMP contributes to wine aroma and not the initial grape aroma because it is liberated from its non volatile 4-MMP-cysteine conjugate through the action of yeast enzymes during fermentation (3) as diagramed in the following chemical equation.

4-MMP has also been reported in fresh, hand squeezed grapefruit juice (4). Subsequent quantitative and flavor reconstitution experiments suggested that the typical sulfurous grapefruit odor was due to 4-MMP and the terpene thiol, 1-p-menthene-8-thiol, with 4-MMP having the greater overall impact on grapefruit juice flavor. Our initial results failed to detect 4-MMP in fresh hand squeezed GFJ using pulsed flame photometric detection. However, 4-MMP could be detected in commercial GFJs. Therefore we hypothesized that 4-MMP might be initially present in a bound form and
could be released from bound forms through heating or the action of fermentation enzymes similar to what occurs in wine. This presentation will document the increased levels of 4-MMP observed from heated and fermented GFJ suggesting that some if not most of the 4-MMP found in GFJ is from the conversion of a bound precursor.

References:


Contamination of fruit products by Alicyclobacillus has been characterized by the development of medicinal and antiseptic off-odors. Guaiacol is one of the primary metabolic products of this bacterium. Recent GC-O and GC-MS studies (Gocmen, Elston et al. 2005) identified guaiacol along with two halogenated phenolics (2,6-dichlorophenol and 2,6-dibromophenol) as being responsible for the off-odors in Alicyclobacillus contaminated orange juice. As seen in Figure 1, guaiacol is the most consistent marker for the presence of Alicyclobacillus in orange juice. Previous studies with apple juice reported a sensory threshold of 2.2 µg/L (Orr, Shewfelt et al. 2000). These same authors developed a GC-MS method for the determination of guaiacol whose limit of detection was above that of the sensory threshold.

The objective of this study was to develop a method which could be used to quantify trace levels of guaiacol in orange juice as a possible indicator of Alicyclobacillus contamination. GC-MS can be a very powerful tool in identifying and quantifying trace volatiles in complex samples. However, this technique can be made even more powerful if sophisticated mass spectral sampling techniques are employed. Shown in Figure 2 is a comparison of different results from an orange juice spiked with guaiacol. This figure compares 2 µg/L guaiacol (9.8 min) responses using the most common MS output, total ion chromatogram, TIC, extracted ion chromatogram, EIC, and selected ion chromatogram, SIM. The optimum response in terms of chromatographic selectivity and highest signal to noise ratio is achieved using SIM at the molecular mass of guaiacol of m/z 124. The three major mass fragments for guaiacol occur at m/z 81, 109 and 124 (M+).
outputs employing all three fragments were found to be unsatisfactory because of interfering compounds in the matrix at m/z 81. Ion ratioing can be employed to determine peak purity from SIM plots of m/z 109 and 124. The success of this method is due to the optimization of dwell time, sampling rate and selection of unique guaiacol ion masses.

References:

Multiple forms of pectin methylesterase (PME) purified from citrus have been shown to have a differential affect on citrus juice cloud leading to the hypothesis that they have different modes of action on pectin. PME hydrolyzes methyl esters on C6 of galacturonic acid residues in the homogalacturonan regions of pectin. Both the degree of esterification and the spatial distribution of the methyl esters have been shown to affect pectin functional properties. To test the hypothesis that the citrus PMEs can be used to produce pectins with tailored functional properties, we have initiated a program to create a series of demethylated pectins with each of the PMEs and to map their structure. These pectins also are being used to determine their corresponding functional properties. Initial studies have focused on the salt-independent PME from citrus. A pectin with an initial degree of esterification (DE) of 94% was demethylated to 90, 80, 70, 60 and 50% DE at pH 7.5 and 4.5. A minimum estimate of the size of the demethylated blocks was obtained by lightly digesting the pectins with endopolygalacturonase. HPAEC coupled to an evaporative light scattering detector provided data on the distribution of sizes and amounts of the demethylated blocks. At pH 7.5 demethylated block size jumped from 19 to 47 when the DE dropped from 80% to 70%. At pH 4.5 a similar change occurred between a DE of 70% and 60%. Compared to pH 7.5 there were more small demethylated blocks present in the pH 4.5 demethylated pectins. At a DE of 70% the average block size was 11 in the pH 7.5 pectin but only 5 in the pH 4.5 pectin. The average number of blocks was higher in the pH 4.5 material. These results suggest pectin functionality can be manipulated by enzymatic treatment under controlled conditions.
Degree of esterification (DE) is a primary determinate for applications involving pectin. More recently 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. Recent work indicates that pectin structural information can be obtained and these structural changes can be matched with the functional properties. This will be important for new high-volume applications involving suspension such as using citrus peel for drilling muds or for paper additive products.

In this work the rheological data resulting from the stepwise decrease in DE of non-calcium sensitive pectin after demethylation at pH 4.5 and 7.5 with the salt-independent PME from citrus fruit peel was correlated with pectin structural properties. Yield stress measurements using an AR2000 rheometer together with calcium sensitivity values were matched with block size and number as determined by HPAEC analysis of pectin methylesterase (PME) block deesterified pectins.

Starting with high DE pectin, DE had to be reduced to a value of 70 or less before yield stress behavior was observed in the presence of calcium ion. Both block size and block number appears to be important for suspension properties. It may now be feasible to structurally characterize de-esterification patterns produced by PME and relate this to its functional properties. The goal now is to impart greater chemical stability into this citrus peel product for these new applications.
Grapefruit furanocoumarins and related compounds have been shown to induce the catabolism of the enterocyte cytochrome P450, CYP3A4 and as a result affect the bioavailability of certain drugs. Only a few grapefruit bioactive furanocoumarins have been identified so far. A systematic study of the composition of grapefruit compounds involved in drug interaction has been initiated between the USDA and the FDOC.

Crude furanocoumarins were extracted from grapefruit juice with ethyl acetate at the Citrus and Subtropical Products Laboratory in Winter Haven. They were concentrated and separated by flash chromatography using an eight step gradient consisting of hexane and ethyl acetate. The fractions containing furanocoumarins were identified by LC-MS and were further separated by semi-preparative liquid chromatography (PLC). Unknown furanocoumarin peaks were collected and analyzed by LC-MS and NMR. Finally, 5 new furanocoumarin conjugates (1-5) were identified, where 2 and 3, 4 and 5 were diastereoisomers. The compound 1 (MW=524) is the conjugate between 6',7'-DHB and an ether with linalool oxide; 2 and 3 are the conjugates between 6',7'-DHB and bergapten; 4 and 5 are the conjugates between 6',7'-DHB and furanocoumarin basic structure without side chain.
A number of furanocoumarins, which are minor phenolic constituents in plants, have been extensively studied for their biological functions in plant defense mechanisms, grapefruit-drug interactions, and therapeutic medications. In grapefruit-drug interactions, it has been shown that furanocoumarins act as natural inhibitors of cytochrome P450 (CYP) enzymes, thus interfering with the metabolism of a variety of substrates. Table 1 shows the mass spectra of eleven newly-isolated minor-occurring furanocoumarins in grapefruit juice. These furanocoumarins were tested for their inhibition of in vitro CYP3A4 activity and compared to those of known furanocoumarin standards, such as bergamottin (12), 6?, 7?dihydroxybergamottin (13), and 6?, 7?epoxybergamottin (14) (Table 1). Three unknown compounds, 1, 2, and 11, showed IC₅₀ values similar to the known standards (12-14). Two furanocoumarin dimers (3 and 7) exhibited the lowest IC₅₀ values of 2.16 and 1.68 ng ·100 µL⁻¹, respectively (Table 1). In addition, IC₅₀ values of four other unknown compounds (4, 6, 8, and 10) were significantly lower than those of 12-14. In contrast, the IC₅₀ values of two compounds (5 and 9) were higher than those of the standards. Our results support the known relationship between chemical structure and inhibition of CYP3A4 activity. Furthermore, these results suggest that minor-occurring furanocoumarins are potent inhibitors of CYP3A4, and play important roles in grapefruit-drug interactions.
Table 1. Mass spectra and IC\textsubscript{50} values of eleven newly-isolated minor-occurring furanocoumarins (1-11) and three furanocoumarins, bergamottin (12), 6\textsuperscript{H}, 7\textsuperscript{H} dihydroxybergamottin (13), and 6\textsuperscript{H}, 7\textsuperscript{H} epoxybergamottin (14), in grapefruit juice. Mass ions designated with (*) represent protonated molecular ions (M+H)+. IC\textsubscript{50} values represent means ± standard errors from triplicate experiments.

<table>
<thead>
<tr>
<th>Compound number</th>
<th>Mass Spectra</th>
<th>IC\textsubscript{50} values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>361(+Na)/339*/283/229/215/203</td>
<td>65.02 ± 8.86</td>
</tr>
<tr>
<td>2</td>
<td>377(+Na)/355*/244/203</td>
<td>96.47 ± 0.92</td>
</tr>
<tr>
<td>3</td>
<td>749(+Na)/727*/579/373/355/337/203</td>
<td>2.16 ± 0.39</td>
</tr>
<tr>
<td>4</td>
<td>637(+Na)/615*/445/373/355/337/243</td>
<td>2.38 ± 0.48</td>
</tr>
<tr>
<td>5</td>
<td>337(+Na)/315*/285/240/229</td>
<td>482.12 ± 9.00</td>
</tr>
<tr>
<td>6</td>
<td>579(+Na)/557*/355/337/215/203</td>
<td>2.99 ± 0.25</td>
</tr>
<tr>
<td>7</td>
<td>709*/507/373/355/337/215/203</td>
<td>1.68 ± 0.29</td>
</tr>
<tr>
<td>8</td>
<td>623(+Na)/601*/355/247/203</td>
<td>10.48 ± 1.52</td>
</tr>
<tr>
<td>9</td>
<td>435(+Na)/413*/355/337/153</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>10</td>
<td>420(+Na)/379*/357/339/203</td>
<td>9.87 ± 0.26</td>
</tr>
<tr>
<td>11</td>
<td>631(+Na)/609*/591/407/237</td>
<td>52.06 ± 2.09</td>
</tr>
<tr>
<td>12</td>
<td>361(+Na)/339*/244/203</td>
<td>53.45 ± 2.31</td>
</tr>
<tr>
<td>13</td>
<td>377(+Na)/355*/337/215/203</td>
<td>46.79 ± 5.15</td>
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<tr>
<td>14</td>
<td>395(+Na)/373*/355/337/215/203</td>
<td>30.3 ± 3.31</td>
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The citrus polymethoxylated flavones inhibit the production of certain proinflammatory cytokines by bacterial lipopolysaccharide (LPS) stimulated monocytes. This inhibition has been shown to be due to the polymethoxylated flavone (PMF) inhibition of the phosphodiesterase-IV of the monocytes, an action that modulates the levels of intracellular cAMP, and consequently controls the signaling for cytokine production. A mouse study showed that intraperitoneal (ip) injection of 3,5,6,7,8,3',4'-heptamethoxyflavone (HMF) significantly inhibited, in a dose-dependent manner, the production of the cytokine, tumor necrosis factor-a in LPS treated-mice. Levels of the inhibition of TNF-a production were proportional to the serum levels of free HMF and its glucuronidated metabolites. The question remained whether the PMFs, mainly HMF, would also be active when administered orally. A recent study has shown that mice administered HMF orally at 50 and 100 mg/Kg body weight experienced no modulation of blood serum TNF-a levels compared to control animals. In contrast, mice administered HMF 100 mg/Kg (ip) showed an 11% decrease in blood serum TNF-a levels. These results suggest that the presence of the nonmetabolized HMF after ip administration attenuates the cytokine production, while the glucuronidated metabolites lack this ability. This observation is consistent with the in vitro studies that showed that flavonoid glycosides were devoid of inhibitory activity in the LPS-stimulated human monocyte assay.
Flavonoids, naturally occurring polyphenolic compounds, are found in fruits, vegetables, fruit juices, teas, wines and grains (1-4). In citrus, four types of flavonoids (flavanones, flavones, flavonols, and anthocyanins, the last one only in the blood oranges) are found and more than 60 individual flavonoids have been identified. Structurally, they have a typical C6-C3-C6 carbon skeleton consisting of two aromatic rings enclosing a heterocyclic six-membered ring with oxygen (5). Most of these compounds cannot be found commercially and synthesis of them are very costly due to complex structural characteristics (6). Citrus fruits and juices are rich in flavonoids (7) and may have positive effects on human health since they have antioxidant, antiallergic, antiinflammatory, and anticarcinogenic, properties (8-9). The human body cannot synthesize these compounds. The purpose of this presentation is to introduce the review of quality and quantity of flavonoids determined in sweet oranges and juice.

References


Analysis of Polymethoxylated Flavones in Citrus Products by Direct Injection and In-Line Trace Enrichment

W. W. Widmer²

Analysis of the polymethoxylated flavones (PMFs) in citrus products has been of interest for chemotaxonomic studies and because of their biological activity. They only occur in the oil glands which are located in the peel flavedo of intact fruit. PMFs from the peel are incorporated into citrus juices during the juice extraction process and normally occur at low ppm concentrations in commercial juice products. Due to the low concentrations of PMFs present in juice, sample preparation usually consists of liquid or solid phase extraction followed by concentration prior to analysis by either normal or reverse phase liquid chromatography. Solvent extracts of citrus peel normally do not need concentration due to the higher PMF concentrations present, but also contain many other components and solid phase extraction for sample clean-up facilitates PMF analysis. A sample preparation procedure utilizing a simple extraction protocol and analysis with in-line sample clean-up and trace enrichment using an automated switching valve has been developed to facilitate PMF analysis. The sample preparation method is similar to that developed for analysis of limonin in citrus products (Widmer and Martin, 1994; Widmer and Haun, 2000) but utilizes a C-18 column for the analytical separation of the PMFs to optimize resolution. The method can be used for quantitative analysis of the PMFs in citrus juice, wet or dry peel, and citrus processing waste. Citrus juice preparation consists of simply diluting samples to contain 20% acetonitrile (v/v) while citrus solids are extracted by sonication in an appropriate amount of 20% acetonitrile. Prepared samples are then filtered and either 80-100µL juice or 5-20µL peel extract injected. Clean-up and PMF trace enrichment is performed with acetonitrile:water (20:80) to eliminate the flavanone glycosides and other polar components. The valve is then switched to elute the concentrated PMFs onto the C-18 analytical column with acetonitrile:water (40:60) and isocratic or gradient analysis depending on the desired application.
References


Evaluation of Hold Times for Cleaned Food Transport Tankers

P. Winniczuk\textsuperscript{1} and R. Goodrich\textsuperscript{1}

A study was conducted to determine if there was an effect on tanker sanitation when the washed and sanitized food grade tanker was held for a period of time after cleaning. Tankers were cleaned following the prescribed JPA wash protocol (Type 2 or Type 4). Tankers were sanitized either by the prescribed hot water method (HWS) or by a chemical sanitizer (CS). The tankers were sampled at Day 0 and closed up but not sealed for sampling either at 24 or 48 hours. Sampling sites for this study were split in half in order to not disturb a portion of the site for the next days sampling. Tankers were evaluated for APC, yeast, coliforms, fecal coliforms, \textit{E. coli}, \textit{Salmonella} spp, and \textit{Listeria} spp. Tankers were sampled over a period of 1 year.

The results indicate that when samples at 24 hours of hold time, APC, coliforms, fecal coliforms, and \textit{E. coli} were recovered in more than twice as many HWS tankers then in CS tankers. APC, coliforms, fecal coliforms and \textit{E. coli} were found in 24 hour hold tankers in HWS and CS tankers but only APC and coliforms were found in both 48 hour hold tankers. Most 48 hour hold tankers were a Type 4 wash which may explain this. Microorganism populations in the HWS tankers had a 2 log increase from the Day 0 counts while the CS tankers were kept to less than 1 log increase. In CS tankers that had a 1 log increase or greater, the sanitizer residue was less than 25% of the recommended amount. Microorganisms recovered were potentially from residual bacteria left in the tanker after cleaning, from re-contamination due to equipment handling, or from the cool down water.
According to the American Lung Association lung disease is the number three killer in America, responsible for one in seven deaths. Lung disease and other breathing problems are the number one killer of babies younger than one year old. Today, more than 35 million Americans are living with chronic lung disease such as asthma, emphysema and chronic bronchitis.

Asthma is a respiratory disease characterized by episodes or attacks of inflammation and narrowing of the small airways in response to asthma triggers.

The prevalence of asthma and associated atopy (a hereditary allergy characterized by symptoms produced upon exposure to the exciting antigen without inoculation) has increased substantially over the past 30 years in most developed countries and appears to have risen in developing countries in relation to the degree of affluence of the population.

According to the Asthma and Allergy Foundation of America, 20 million Americans have asthma. Asthma is a chronic disease and one of the most common and costly in America. It is responsible for 14.6 million missed days of school each year, making asthma the leading cause of school absenteeism. It is also responsible for 14.5 million missed days of work for adults each year, a 100 million days of restricted physical activity for children and adults each year, 1.9 million emergency room visits, 14 billion dollars in medical expenses and indirect costs and approximately 5,000 deaths annually. This presentation will focus on the links between citrus juice and asthma.
Influence of Orange Juice Consumption on the Blood Lipid Profile of Men and Middle Aged Women

T. B. Cesar, N. P. Bonifacio and A. C. Garcia

Introduction: High serum cholesterol is considered a major risk factor for coronary artery disease (CHD). Classic therapeutic lifestyle changes include dietary modifications and regular exercises, which help to lower serum cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). Recent studies have shown that the citrus flavonoids, naringin and hesperidin, found in grapefruit and orange and its juices can decrease serum LDL-C and TG. Furthermore, several studies have accounted for the preventive effect of vitamin C on the heart disease, as a potent antioxidant that can decrease atherogenesis.

Objectives: The aim of this study was to examine if the consumption of orange juice, as a source of flavonoids and vitamin C, could alter the plasma lipoprotein profile when incorporated into a regular diet in men or, when associated with aerobic exercises in middle aged women.

Methods: First experiment: 66 healthy men aged 20 to 60y, with regular consumption of orange juice, from one cup (8 oz) to 3 cups a day. Second experiment: 26 women aged 30 to 55y, previously sedentary, volunteered to participate in endurance training (controls) or endurance training plus daily consumption of orange juice (500mL/d), for 12 weeks. In both experiments blood samples were collected and analyzed for biochemical markers: total cholesterol (TC), triglyceride (TG) and HDL-C. BMI and fat mass (%) were analyzed to evaluate nutritional status. The protocols were approved by the Ethical Committee of Faculty of Pharmaceutical Sciences, University of Sao Paulo State (UNESP), Brazil.

Results: Experiment 1: Men (n=66) with TC <240mg/mL and TG <200mg/mL were divided in 3 groups related to their consumption of orange juice (OJ). ANOVA showed decrease in TC and LDL-C for the subjects that consumed 250-400mL of OJ/d (TC: -8%, LDL: -12%) and for those with consumption of >400mL of OJ/d (TC: -14%, LDL: -18%) in comparison to the control group (0 to <200mL of OJ/d). Similar analyses showed that the consumption of >400mL of OJ/d decreased LDL/HDL ratio (- 26%,
p<0.05), dropping significantly the risk for CHD. TG showed no difference among groups. A negative and significant correlation was also detected between the consumption of orange juice and BMI (r=-0.24), weight (r=-0.41) and fat mass (r=-0.24).

**Experiment 2:** After 12 weeks, women (n=13) who submitted to the aerobic exercises reduced weight (-2.4%), and fat mass (-14%), while those (n=13) treated with exercise plus OJ decreased fat mass (-11%), but did not change weight. The consumption of OJ duplicated the dietary intake of vitamin C and folic acid. Serum LDL-C was also affected by the OJ, decreasing 15%, while HDL-C increased 18%.

**Conclusions:** Regular consumption of orange juice associated with aerobic exercise is effective in reducing CHD risk factors and could be easily incorporated into a healthy lifestyle change.
Raw material, in-process and final product quality control is crucial in the very competitive market of processed orange juice. Product quality can be assured to a great extent by adequately controlling most processing parameters such as flow rate, temperature and level. However, the lack of on-line or in-line instruments to measure juice composition makes it impossible to control the process where there is a high variability in the quality of raw materials (Reyes-De-Corcuera and Cavalieri 2003). This produces the need for frequent routine off-line analyses in the quality assurance laboratory. Excluding microbiological assays, some of the most relevant analyses used in the orange juice industry include the determination of soluble solids content (SSC), titratable acidity (TA), ascorbic acid, oil and pulp content, color, cloud, bitterness and residual pectin esterase activity (Kimball 1999). SSC can be done on-line by refractometry. Gravimetric SSC and TA automated analyses exist in all processing plants for State test inspection. However, for better bin management, it would be very desirable to have an estimate of these parameters before trucks are unloaded. We have focused a great part of our research efforts on developing rapid methods for two of the most tedious assays: analysis of oil in juice and determination of pectin esterase activity. Currently, oil analysis is done using the so-called ‘Scott’ method (Scott and Veldhuis 1966) which requires extraction, distillation and titration and pectin esterase activity which requires a lengthy titration at constant pH (Rouse and Atkins 1955). We are also developing a portable reagentless sensor for rapid acid titration of fresh fruit at or before harvest time.

An electrochemical approach has been taken in all cases and preliminary results are presented. For oil analysis, cyclic voltammetry of platinum disk electrodes in acidified oil emulsions is being investigated. A decrease in hydrogen adsorption peaks shown in Figure 1 in the presence of oil has been quantified for different concentrations of oil.
Figure 1. Cyclic voltammograms of platinum in (a) 0.1 M H$_2$SO$_4$ and (b) 0.1% orange oil in 0.1 M H$_2$SO$_4$.

Coulometric titrations where the titrant is produced electrochemically are proposed for both TA and PE activity measurements. A schematic representation of our prototype is shown in Figure 2.

Figure 2. Coulometric titrator for the determination of titratable acidity in orange.

Preliminary results and future directions on these three rapid methods are presented.
References:


Arthritis refers to a disease of the joints, which can often result in joint pain, swelling, stiffness or loss of function over time. Rheumatoid arthritis (RA) is a chronic disease, mainly characterized by inflammation of the lining (synovium), which is supposed to protect and lubricate the joints. Due to the recent interest in the possible benefits of citrus or its components relating to RA, this presentation will mainly focus on this type of arthritis. RA can lead to long-term joint damage, resulting in chronic pain, loss of function and disability. Fruit and vegetable consumption has been shown to have an important role in the etiology of other chronic diseases, such as cardiovascular disease and some cancers. As cardiovascular disease shares similar inflammatory and immunologic pathways with those observed in RA, it is reasonable to hypothesize that a higher intake of fruit and vegetables may influence the etiology of inflammatory joint disease (Pattison et al., 2004).

Pattison et al. (2005) investigated the hypotheses that some dietary carotenoids are associated with reduced risk of inflammatory arthritis. Their results showed that subjects with higher intake of $\beta$-cryptoxanthin were at lower risk of developing arthritis compared to those with lower intake. These data are supported by the findings of Cerhan et al. (2003) that also demonstrated an inverse association between high $\beta$-cryptoxanthin intake and RA onset. On the basis of these findings, supplementation of diets with just one glass (8 oz.) of orange juice per day is sufficient to raise the intake of $\beta$-cryptoxanthin to the highest and most protective tertile of intake. Orange juice is also a good source of potassium and folate, which have been shown to become deficient during treatment of RA with widely used disease modifying anti-rheumatic drugs (DMARs). Orange juice is an excellent source of vitamin C, which may help iron absorption and treatment of iron deficiency anemia that may develop as a result of chronic inflammation and gastrointestinal blood loss caused by arthritis medications.
References:
