FIFTY-EIGHTH
CITRUS PROCESSORS'
and
SUBTROPICAL TECHNOLOGY
CONFERENCE

OCTOBER 18, 2007

UF UNIVERSITY of FLORIDA
IFAS Extension

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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

PROCESSORS' MEETING PREVIOUS SESSION CHAIRPERSONS

Year	Person Chairing
1950-1968	Fred Wenzel acted as Moderator
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	Gary Merritt, Jose Rodriguez
2005	Jamie Goodner
2006	Dan King, Robert Kryger

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

FORWARD

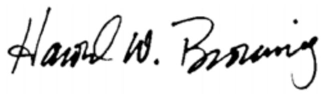
Good morning and welcome to the 58th meeting of the Citrus Processing and Technology Conference and the Subtropical Technology Conference held at the Citrus Research and Education Center. The University of Florida, IFAS is proud to co-host this meeting at CREC with the USDA, Agricultural Research Service 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.

During periods when unique challenges face businesses, research and development are more important than ever in offering opportunities and solutions. Investment in new technologies and advancing new systems for improved efficiencies and economy are vital during these periods, as are focusing on end product quality. Through biological, chemical and engineering research, and with economic assessment as an ongoing component, improvements emerge and can be tested for their adaptation to existing systems. These improvements then can be tested at commercial scale operations for their application.

We are experiencing such a time in Florida citrus, where disease issues and alternative land uses are challenging sustainable citrus fruit production and thus, impacting processing strategies. In addition, competition in the marketplace from other juice producers as well as other beverage options continues to increase. Maintaining the strong identity that Florida citrus products have in the marketplace is vital to the long-term health of the industry. We continue to encounter new challenges, and thus the focus on results of research and development is timely.

It is in this environment that we find ourselves today. UF, IFAS is proud to partner with USDA and FDOC in bringing public research, development and delivery to the citrus processing industry. Our long-term association with Florida citrus is based on exchange of ideas and new information, and we hope that the 58^{th} Conference will further illustrate the benefit of industry/research interactions.

Best wishes for a productive 2007-08 citrus processing season and please provide feedback on this conference in order for us to make the 59^{th} Conference even more useful.



Harold W. Browning
Center Director and Professor
UF, IFAS Citrus Research and Education Center, Lake Alfred

It is an honor for me, as the new Director of Scientific Research for the Florida Department of Citrus (FDOC), to welcome you on behalf of the FDOC and the Florida Citrus Commission, to the 58th Annual Citrus Processing and Technology Conference. As usual, we expect to provide you with a day of informative and timely presentations relating to current issues. At the FDOC a key marketing message is the association of phytochemicals within citrus and citrus juices to their health and wellness benefits, all delivered within a unique, 'whole food delivery system'.

Over the last few years, the FDOC has focused on research supporting these health and wellness benefits, as well as continuing its programs of monitoring citrus juices for their quality, nutritional and regulatory compliance factors. Our primary goal is to provide scientifically-based, evidence-supported information to be used within our Marketing and Public Relations programs.

Our activities have progressed from intensive literature searches for studies associating beneficial health attributes to citrus products/components, to programs emphasizing the extension and verification of *in vitro* and animal studies, with clinical (*in vivo*) studies. Our staff provides extensive support efforts associated with health, wellness and nutrition studies, including the writing of various white papers and peer-reviewed publications, internal analytical evaluations of citrus products provided by for use in clinical studies, attendance and presentations at domestic and international professional meetings, and scientific review and documentation support for public relations/marketing activities.

Research projects at several prominent research institutions have been completed or are ongoing, including clinical studies involving citrus products and their beneficial interactions with cardiovascular disease, bone health, weight management, lipid metabolism, inflammation, and immunity. In addition, a recent study evaluating nutrient densities and various popular 100% juices has shown that citrus juices demonstrate a clear advantage over other juices in the delivery of nutrients on a 'per calorie' basis.

So today, several of the areas of interest we are pursuing will be presented. Please feel free to contact any of our Staff if you have any questions or comments on our research programs. Thank you.

Dan King, 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 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. Postproduction research is important because all the resources used to plant, grow and harvest fruit is lost if the final fruit product, whether fresh or processed, is not marketable. It is important that citrus and subtropical fruits and their products compete successfully in a global market. The ability to produce juice products economically is important, but the cutting edge is in quality. We at USDA-ARS in cooperation with our colleagues at CREC and FDOC strive to conduct research that supplies the industry with information that promotes quality of processed products. We also strive to develop by-products from the citrus waste steam to expand the economic value of the citrus crop. We work with the Florida Citrus Industry Research Coordinating Council on research priorities and gaps and we recognize the many challenges facing the industry today, the most important of which is citrus greening and canker diseases. We have adapted our programs to confront these issues within the areas of our collective expertise. We report this year on issues of flavor, drug interaction, by-products and the healthful effects of orange juice. 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, 2007

58th 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, D. King & 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**
	Dan King **Director of Scientific Research** **Florida Department of Citrus** **Lake Alfred**
	Elizabeth Baldwin, Research Leader **Citrus & Subtropical Products Laboratory** **Winter Haven**

Moderator:

9:15 am **CURRENT RESEARCH IN RAPID METHODS FOR CITRUS QUALITY**
J. I. Reyes-De-Corcuera[1] and S. E. Jones[1]

9:30 am **KINETICS OF DISSOLVED OXYGEN AND VITAMIN C CONSUMPTION IN ORANGE JUICE: AN UPDATE**
R. Gracía-Torres[1] and J. I. Reyes-De-Corcuera[1]

9:45 am **QUALITY EVALUATION OF TANGERINES FROM THE CREC BREEDING PROGRAM**
P. Kerbiriou[1], A. Plotto[2], K. L. Goodner[2], E. A. Baldwin[2] and F. Gmitter[1]

10:00 am **PRESENCE OF EPOXIDE HYDROLASE ACTIVITY IN *ASPERGILLUS NIGER*: HYDROLYSIS OF 6', 7'-EPOXYBERGAMOTTIN TO 6', 7-DIHYDROXYBERGAMOTTIN**
K. Myung[2], J. A. Manthey[2] and J. A. Narciso[2]

10:15 - 10:45 ORANGE JUICE BREAK

10:45 am **ECONOMIC ANALYSIS OF ETHANOL PRODUCTION FROM CITRUS PEEL WASTE**
W. Zhou[2] and W. W. Widmer[2]

11:00 am **SOME THOUGHTS ABOUT AN UNUSUAL SET OF PHENOLS IN CALAMONDIN LEAVES AND ORANGE JUICE**
J. A. Manthey[2]

11:15 am **ION BINDING CAPACITY OF ENZYME MODIFIED CITRUS PEEL AND PECTIN**
R. G. Cameron[2], G. A. Luzio[2], W. W. Widmer[2] and M. Iqbal[4]

11:30 am **SUSPENSION PROPERTIES OF PECTINS DEESTERIFIED USING PECTIN METHYLESTERASE**
G. A. Luzio[2] and R. G. Cameron[2]

11:45 am **BIOLOGICAL CHARACTERIZATION AND HEALTH BENEFITS OF CITRUS DIETARY FIBER**
J. Zhang[3]

12:00 pm - 1:30 pm LUNCH

Moderator:

1:30 pm **NOVEL OFF-FLAVORS PRODUCED FROM THE THERMOPHILLIC BACTERIA, *ALICYCLOBACILLUS ACIDOTERRESTRIS*, IN PINEAPPLE JUICE**
C. Bousquet[1], M. Danyluk[1] and R. Rousseff[1]

1:45 pm **ANALYTICAL CONSIDERTIONS IN THE DETERMINATION OF SULFUR VOLATILES IN GRAPEFRUIT JUICE USING HEADSPACE SPME**
F. Jabalpurwala[1] and R. Rouseff[1]

2:00 pm **ROASTING EFFECTS ON TOTAL SULFUR VOLATILES AND AROMA ACTIVE COMPOUNDS IN ARABICA COFFEE USING GC-PFDP AND GC-O.**
K. Mahattanatawee[5], M. P. Ruiz Perez-Cacho[6], N. Rattanapanone[7] and R. Rouseff[1]

2:15 pm **HYDRATION, EXERCISE AND ORANGE JUICE**
F. Valim[3] and S. Barros[3]

2:30 – 2:45 BREAK

2:45 pm **BENEFICIAL ROLE OF CITRUS IN BONE HEALTH**
P. Cancalon[3]

3:00 pm **ORANGE JUICE CONSUMED REGULARLY DECREASED THE INSULIN RESISTANCE IN NORMAL SUBJECTS**
T. B. Cesar[2], L. U. Rodrigues[8], M. S. Perez[8] and N. A. P. Bonifacio[8]

3:15 pm **HEALTH AND NUTRITIONAL BENEFITS OF CITRUS JUICE**
S. Barros[3], W. S. Stinson[3], M. Azik[3], P. Cancalon[3], G. Rampersaud[9], F. Valim[3] and J. Zhang[3]

[1]Citrus Research and Education Center, UF/IFAS, Lake Alfred, Florida
[2]USDA-ARS Citrus & Subtropical Products Laboratory, Winter Haven, FL
[3]Florida Department of Citrus, Lake Alfred, FL
[4]Water and Environmental Research Center, Institute of Northern Engineering, University of Alaska, Fairbanks, AK
[5]Siam University, Dept. Food Technology, Bangkok, Thailand
[6]Institute of Fishery and Agrarian Res. And Biolog. Agric., CIFA, Cordoba, Spain
[7]Chiang Mai University, Chiang Mai, Thailand
[8]Faculty of Pharmaceutical Sciences, Sal Paulo State University, UNESP, Araraquara, SP, Brazil
[9]University of Florida, IFAS, Food Science & Human Nutrition, Gainesville
___Presenter

9:15 am

Current Research in Rapid Methods for Citrus Quality

J. I. Reyes-De-Corcuera[1] and S. E. Jones[1]

Next to soluble solid content and titratable acidity determination of oil content in orange juice is one of the most important quality parameters determined in the citrus processing industry. Current method requires extraction with isopropyl alcohol, distillation and titration of d-Limonene, the major component of citrus oil. We reported earlier our attempt to quantify oil in juice using cyclic voltammetry in sulfuric acid. While the decrease in hydrogen adsorption peaks correlated well with concentration of oil in water emulsions, when using juice, other species adsorbed as well onto the platinum electrodes. An alternative electrochemical method is the electrochemical oxidation of d-Limonene in acidified acetonitrile solutions (Figure 1). Concentration of d-Limonene solutions in hexane and hexane extracts from orange juice correlated well with oil concentration as shown in Figure 2. The main source of error is the extraction. An enzyme methanol biosensor is being developed for the determination of pectin esterase activity. In contrast to the conventional titration, the proposed assay is run in buffered solutions and the change in methanol concentration is monitored with the biosensor obviating the need of a titrant. The principle of operation of the biosensor is shown in Figure 3.

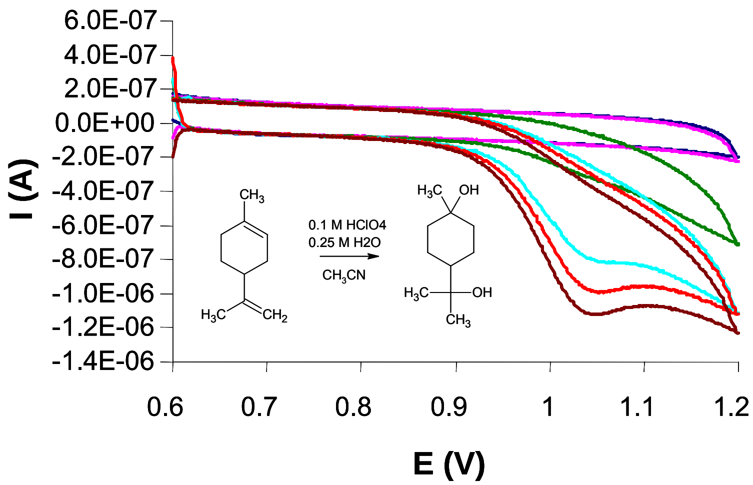


Figure 1. Cyclic voltammograms at 25 ºC in 0.1 M $HClO_4$, 0.25 H_2O in CH_3CN for (—) 0%, 0 min; (—) 0%, 30 min; (—) 0.2%, 0 min; (—) 0.2%, 30 min; (—) 0.2%, 60 min; and (—) 0.2%, 90 min. Scan rate 100 mV.s-1

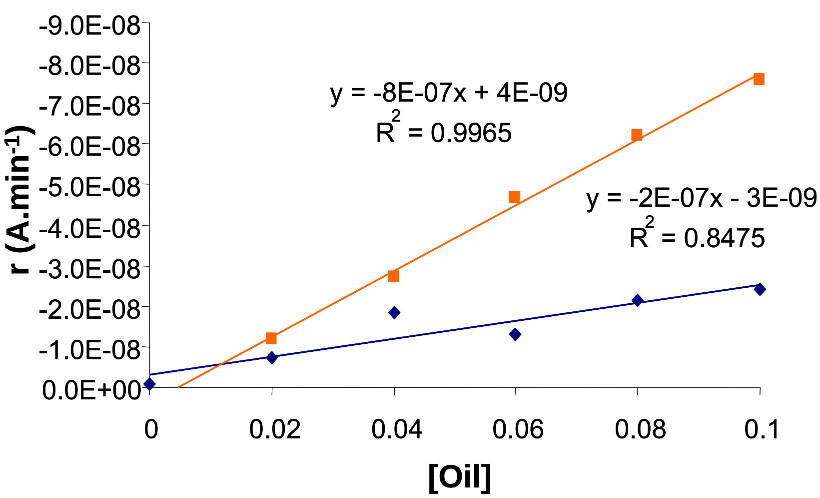


Figure 2. Rate of increase in peak current at 1.1 V *vs.* Ag/AgCl during cyclic voltammetry (1st cycle) between 0.6 and 1.4 V. every 2.5 min in 0.1 M $HClO_4$, 0.25 M H_2O in CH_3CN at 40 ºC for (■) Oil in hexane solutions and (♦) oil extracted from juice with hexane. Scan rate 100 mV.s-1

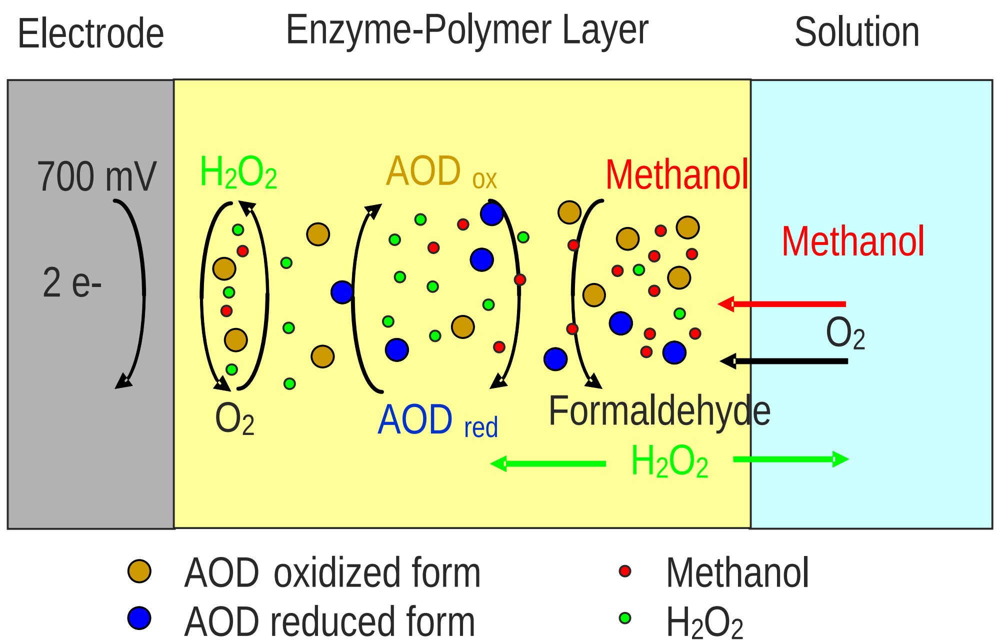


Figure 3. Schematic representation of a methanol biosensor

9:30 am

Kinetics of Dissolved Oxygen and Vitamin C Consumption in Orange Juice: An Update

R. Gracía-Torres[1] and J. I. Reyes-De-Coruera[1]

The presence of dissolved oxygen (DO) has been associated with quality deterioration of orange juice including the degradation of vitamin C. However, there is little and inconsistent information in the literature on the kinetics of dissolved oxygen consumption and the associated decrease in vitamin C in freshly squeezed orange juice. We previously reported the kinetics of oxygen consumption using fiber optic oxygen sensors. While fiber optic sensors have very low limits of detection and do not consume oxygen, we found that orange juice degrades them resulting in loss of sensitivity. This study presents the kinetics of DO and vitamin C degradation in fresh orange juice at 5, 23.7, 34 and 45 °C. Order of reaction, rate constant and activation energy were determined. 'Valencia' oranges were stored at 5°C until experiments were run. Juice was squeezed manually and filtered to obtain a low pulp juice (6 to 12%). Sodium azide was added as an antimicrobial at a concentration of 50 mg/L to prevent spoilage. pH, TA, °Brix and color were measured at the beginning of all the experiments. DO was continuously measured using a Clark type electrode (08101MD, Thermo Scientific Co., Beverly, MA) in vials completely filled with juice (no head space). Ascorbic acid was quantified using Capillary Electrophoresis. Color was measured using a Minolta colorimeter.

9:45 am

Quality Evaluation of Tangerines from the CREC Breeding Program

P. Kerbiriou[1], A. Plotto[2], K. L. Goodner[2], E. A. Baldwin[2] and F. Gmitter[1]

A quality evaluation of tangerines from the CREC breeding program was undertaken in 2006-2007. Forty-five tangerine hybrids and 10 named commercial cultivars were sampled from November 2006 to March 2007. Some samples were harvested multiple times over the season. Fruit were washed, sanitized, and carefully juiced to avoid incorporating peel oil volatiles into the juice. Juice was frozen for volatile, titratable acidity, carotenoid and sugar analyses as well as for sensory evaluation.

Data from gas chromatography mass-spectrometry revealed that while about 50 compounds were found in 75% of the samples, 96 compounds were found in less than 25% of the samples, and might be more variety specific. A cluster analysis based on presence/absence of volatiles revealed ten main clusters influenced by harvest date and/or progenitors, highlighting relationships among certain hybrids based on their volatile composition. Cluster 2 mainly grouped samples having ‘Fallglo’ and ‘Fairchild’ in their parentage, while cluster 4 mainly grouped samples having a common parent ‘Murcott’. Cluster 10 grouped samples having sweet oranges in their genetic background and being rich in esters, which are known to give a fruity note to orange juice. This method provided useful information on tangerine hybrid volatile content in relation to their genetic make-up.

Juice soluble solids, titratable acidity, and solids/acids ratio ranged 9.3-16.3% SSC, 0.119-1.675 % citric acid, and ratios 7.4-116.8, respectively, with average 12.2 °Brix, 0.932% citric acid, and 15.4 solids/acids ratio. A panel was trained and defined 17 descriptors for aroma, flavor and taste. Descriptors were sulfury aroma, tangerine, orange, grapefruit, fruity non-citrus, floral, green/fatty aroma and flavor, sweet, sour, bitter taste, and aftertaste. Panelists smelled and tasted thawed juice in a completely randomized design, 5 juices per session. A multivariate analysis showed separation among hybrids based on green/fatty, sulfury, tangerine, and [orange, fruity, floral] aroma descriptors, and on sour, tangerine, [sweet, orange, fruity, floral], and [green/fatty, grapefruit, bitter, aftertaste] flavor descriptors. Panelists could distinguish samples harvested multiple times from the same tree by increasing sweetness or decreasing sourness with later harvests. Data are being analyzed to identify correlations between instrumental and sensory data.

This study showed the wide variation in aroma and taste attributes in tangerine hybrids from the CREC breeding program. More sensory analysis will be performed on tangerine juice, but also, taste panels will be performed using whole fruit during the 2007-2008 season.

10:00 am

Presence of Epoxide Hydrolase Activity in *Aspergillus Niger*: Hydrolysis of 6’, 7’-Epoxybergamottin to 6’, 7’-Dihydroxybergamottin

K. Myung[2] J. A. Manthey[2] and J. A. Narciso[2]

The 6´, 7´-epoxybergamottin (EB) is one of major furanocoumarins in grapefruit. Previously, we have shown that *Aspergillus niger* has a capability of metabolizing EB into 6´, 7´-dihydroxybergamottin (DHB), which is further metabolized to bergaptol and bergaptol-5-sulfate *in vivo*. In this study, we attempted the biotransformation of EB into DHB *in vitro*. When EB was incubated with enzyme extract prepared from 4-day *A. niger* culture, an epoxide hydrolase activity was detected where EB was hydrolyzed into DHB within 30 min at pH 8.0 (Fig. 1). The EB incubated with boiled enzyme extract was not hydrolyzed into DHB (Fig. 1), demonstrating that the epoxide hydrolase activity is present in the fungus. Specific activity of epoxide hydrolase was approximately 28.2 nmole/hr/mg protein. Similar hydrolytic activity was obtained from enzyme extracts prepared from either *A. niger* culture with or without pre-treated EB, indicating that the hydrolytic activity is not induced by EB; rather the epoxide hydrolase activity is constitutively present. Since the hydrolytic reaction occurred in our assay system only containing a buffer, EB, and crude extract, the hydrolysis of EB by epoxide hydrolase would be accomplished in a cofactor-independent manner. Overall, our data support that the epoxide hydrolase activity is present in *A. niger*, in which EB is hydrolyzed into DHB.

10:45 am

Economic Analysis of Ethanol Production from Citrus Peel Waste

W. Zhou[2] and W. W. Widmer[2]

The Florida citrus juice industry produces about 3.5 million tons of wet peel waste per year. In current industrial practice, waste peels are dried and sold as cattle feed to offset the waste disposal cost. Profitability would be greatly improved if peel could be used to produce higher value products. Recent advances by USDA/ARS scientists and their partner Renewable Spirits, LLC have given rise to the potential of a new process for making fuel ethanol from citrus peel waste. In this paper, the economics of the process for making citrus ethanol is analyzed and discussed. The economic model for the cellulose-to-ethanol process was used as a benchmark to estimate the project cost and the fixed operating cost for the peel-to-ethanol process. The production cost of citrus ethanol is estimated to be approximately $1.23 /gallon, possibly higher than the cost of corn ethanol ($1.00/gallon), but lower than the cost of cellulose ethanol ($1.35-1.62/gallon). This study allows us to pinpoint the economics of the process for making fuel ethanol from citrus peel waste, and is useful for predicting the cost benefit of proposed research and its economic impact on the juice industry.

11:00 am

Some Thoughts About an Unusual Set of Phenols in Calamondin Leaves and Orange Juice

J. A. Manthey[2]

Antioxidant phenols are thought to be responsible for much of the anti-inflammatory properties of orange juice. In addition to this, the compounds may be active against some plant pathogenic bacteria; possibly the canker organism, *Xanthomonas axonopodis* pv *citri*. This possibility led to our investigation of these compounds in Calamondin leaf tissue. Several main classes of flavonoids and related phenols occur in Calamondin leaves, including polar hydroxycinnamates, flavone glycosides, flavanone glycosides and polymethoxylated flavones. Crude separations of these classes of compounds were made by low-pressure reversed-phase column chromatography, and cursory structural analyses were achieved by HPLC-mass spectroscopy. Among these compounds, an unusual set of seemingly related UV-absorbing compounds of intermediate polarities was detected and further isolated. HPLC analysis of this set of compounds produced a “humptogram”, and this suggested that this unusual set of compounds is composed of a very large number of minor-occurring constituents. The UV spectra of the combined compounds further suggest that they are comprised mainly of mixed flavones and hydroxycinnamates. Nothing else is known about these compounds, but it is significant that this set of compounds represents a substantial portion of the total phenolics in calamondin leaves. This is also true of the powerful anitoxidant phenols in orange juice.

It has been noted that similar “humptograms” occur in the HPLC of total phenol extracts of chocolate, pine bark, and cranberry. These plant materials contain widely varying mixtures of proanthocyanidin oligomers, which make up major portions of the humptograms in their respective HPLCs. These oligomers have been extensively studied for their potential beneficial biological activities in human cells. Thus, it is tempting to speculate that the citrus compounds might also be comprised of complex sets of mixed oligomers, not necessarily proanthocyanidins, but rather of flavones and hydroxycinnamates. Such oligomers may arise from oxidative coupling in citrus plant tissues abundant in these many diverse polyphenols.

11:15 am

Ion Binding Capacity of Enzyme Modified Citrus Peel and Pectin

R. G. Cameron[2], G. A. Luzio[2], W. W. Widmer[2] and M. Iqbal[4]

A wide ranging group of enzyme modified pectin and polygalacturonic acid (PGA) samples were tested for their biosorption potential, specifically their ability to bind Pb. Two sample sets were the pH 4.5 and 7.5 demethylated series obtained from demethylation of a model homogalacturonan produced using the Citrus salt-independent PME. Another group of samples consisted of either commercially available pectin or laboratory extracted pectin (both total pectin and non calcium sensitive pectin; peel particles still present) that were demethylated with a commercially available plant extract. A third set of samples were three different narrow range size-classes of PGA produced by differential precipitation of a limited endo polygalacturonase digest of PGA. The final sample tested was the peel hydrolysate produced during the conversion of citrus peel to ethanol.

The highest biosorption capacity was observed in the narrow range size classes produced from PGA. Although significant differences were observed among the three size-classes, all had a greater capacity than any of the other samples. Block demethylation of commercial pectin with a commercial plant extract was the closest sample to the PGA size-classes. However, all samples tested were able to remove the heavy metal ion from the solution in significant quantities.

11:30 am

Suspension Properties of Pectins Deesterified Using Pectin Methylesterase

G. A. Luzio[2] and R. G. Cameron[2]

After removal of soluble sugars and other compounds by washing, citrus peel is largely composed of pectin, cellulose and hemicellulose. In order to utilize the greatest amount of citrus peel product, it would appear reasonable that one or all three of these polysaccharides be converted to a useful material. One of the components, pectin is relatively easy to modify using enzymes and has great utility in the food industry and other applications. Thus it appears reasonable to focus on the use of pectin for the maximum utilization of fruit peel for new products from peel.

During deesterification, the ester groups on the pectin can be removed in a random or blockwise manner with enzymes. When the ester groups are removed in a sequential manner, they are referred to as being deesterified in a "blockwise manner," as blocks of unesterified galacturonic acid units are created. The unesterified galacturonic acid units formed by blockwise deesterification are highly reactive to divalent cations such as calcium and crosslink to form gels. Pectins having such blocks of unesterified galacturonic acid are said to be "calcium sensitive." Calcium sensitivity affects rheology, such as yield stress and storage modulus and is an important functional property of pectins in suspension applications such as pulp suspension in citrus drinks.

Pectins, with a high degree of esterification (DE) were demethylated with a monocomponent preparation of a pectin methylesterase PME isolated form citrus fruit tissue and with an unpurified fraction of PME from papaya to a DE of 55%. Rheology and calcium sensitivity measurements on one of these pectins had indicated that both block number and block size needed to be considered for crosslinking with calcium ion. More recent work indicates that calcium/pectin ratios are important for optimizing storage modulus and recovery of storage modulus. This data together with a discussion of requirement for recovery of storage modulus for suspension will be discussed.

11:45 am

Biological Characterization and Health Benefits of Citrus Dietary Fiber

J. Zhang[3]

Dietary fiber is a general term that refers to a wide variety of compounds found in plants that are resistant to the digestive enzymes produced by humans. It does not provide calories or energy to the body since it is resistant to digestive enzymes, and is not broken down or absorbed. Dietary fiber is often classified as soluble fiber, such as pectin, and insoluble fiber, such as cellulose and hemicellulose. Citrus fruit contain both soluble and insoluble fiber. Pectin, cellulose and hemicellulose, with small amount of lignin, are the predominant components of dietary fiber from citrus fruit. Most citrus fiber is contained in the citrus peel (albedo), membrane and juice sac. Diets rich in dietary fiber have been reported to relate to the decreased risk of developing various health problems, including coronary heart disease, diabetes, hypertension, diverticular disease, constipation, overweight/obesity, and certain cancers.

The recommended intake of fiber is 25 to 30 grams per day for adults. However, a dietary survey indicates that fiber intake among adults in the United States averages about 15 grams per day, which is approximately half the recommended amount. The citrus fruit are a good source of dietary fiber since they are rich in pectin, cellulose and hemicellulose. An increased consumption of fresh citrus fruit will not only help meet the recommended intake of dietary fiber, but also help meet the recommended intake of other essential nutrients and beneficial phytochemicals.

References:

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1:30 pm

Novel Off-Flavors Produced from the Thermophillic Bacteria, *Alicyclobacillus Acidoterrestris*, in Pineapple Juice

C. Bousquet[1], M. Danyluk[1] and R. Rouseff[1]

Alicyclobacillus spp. is known to produce off-flavors in fruit juices, especially in apple (*1-4*) and orange juices (*4, 5*). In prior studies, the primary off-flavor compounds produced by *Alicyclobacillus* spp. were guaiacol and/or halogenated compounds (2,6-dibromophenol and 2,6-dichlorophenol). Guaiacol is generally accepted as the major metabolite associated with a "medicine" off-aroma in fruit juices (*6, 7*). In this study, an unexpected "cheese" off-aroma was detected 14 days after the inoculation of pineapple juice with a five strain cocktail of this bacterium. The objective of this work was to identify compounds which might be responsible for this off-aroma, using GC-O and GC-MS.

As expected, guaiacol was identified in inoculated samples (but not in control samples) and was described as having a "medicine" odor. A few volatiles (acid compounds and sulfur compounds) were observed at appreciably higher concentrations in inoculated samples and are listed in Table 1.

Table 1. Odor-active compounds associated with the spoilage caused by *Alicyclobacillus acidoterrestris*

Compounds[a]	DB5 column			DB Wax column		
	LRI[b]	Descriptors[c]	Intensity [c]	LRI	Descriptors	Intensity
isobutyric acid	-	-	-	1558	fruity	98
3-methylbutyric acid	856	cheese	94	1664	cheese	98
2-methylbutyric acid	875	cheese	80	1664	cheese	
dihydro-2-methyl-3(H)-thiophenone	990	sulfur	96	1523	sulfur	98
methyl-3(methylthio)- propionate	1026	sulfur	98	1519	-	-
guaiacol	1091	medicine	92	1864	medicine	97
ethyl-3(methylthio)-propionate	1100	floral	97	-	-	-

[a]: compounds identified by matching mass spectra with library values; [b]: identification of compounds confirmed using linear retention indices (LRI); [c]: descriptors and odor intensity determined by a time-intensity approach in GC-O on a 0-100 scale.

As shown in Table 1, 2-methylbutyric acid and 3-methylbutyric acid were associated with a "cheese" note. These two acids seem to be primarily responsible for the overall "cheese" off-aroma described by the olfactory assessors.

Two sulfur esters, methyl-3(methylthio)-propionate and ethyl-3(methylthio)-propionate, normally present in pineapple juice in low concentrations, were detected at high concentrations in inoculated samples. Although these esters were associated with the “cheesy” off-aroma, they do not appear to cause it. Increased concentrations of these esters were associated with a corresponding decrease in methional, the major degradation product of the sulfur containing amino acid methionine. In inoculated samples, methional could undergo oxidation followed by an esterification leading to methyl-3(methylthio)-propionate and ethyl-3(methylthio)-propionate. Since these reactions occurred only in inoculated samples, the reactions were not simply chemical in nature. It appears that *Alicyclobacillus acidoterrestris* produces substrates and/or enzymes which facilitate these reactions thus explaining the elevated concentrations of these esters in inoculated samples.

Dihydro-2-methyl-3(H)-thiophenone was the third sulfur compound observed in high concentration only in inoculated samples. The “sulfur” note attributed to this compound with a high intensity could contribute to the “cheese” off-aroma in inoculated samples.

These results suggest that *Alicyclobacillus acidoterrestris* may contain enzyme systems capable of producing volatiles other than guaiacol provided the proper substrate is available. These off-flavors could be a cause for consumer rejections, so it is important to identify possible precursors/substrates for these cheesy off-flavors so as to prevent their conversion expressed off-flavors and consequently enhance the perceived quality of pineapple juice.

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1:45 pm

Analytical Considerations in the Determination of Sulfur Volatiles in Grapefruit Juice Using Headspace SPME

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The characteristic aroma of grapefruit juice (GFJ) has been attributed to presence of volatile sulfur compounds (VSC's) primarily: 1-p-menthene-8-thiol (one of the lowest food thresholds of 10^{-7} ug/l)[1] and 4-mercapto-4-methylpentan-2-one with an even lower threshold[2]. Some VSC's are extremely potent aroma compounds present at sub ppb levels, thereby requiring concentration prior to detection. In addition, the extreme reactivity of sulfur compounds may result in alterations of the overall VSC composition during sample preparation. The goal of this study was to develop an analytical procedure which would collect and concentrate the VSC's in grapefruit juice so that they could be clearly identified and processing changes monitored. Analytical parameters such as SPME-headspace atmosphere, type of fiber, extraction time and temperature were examined. This study characterized the oxidative potential of sulfur compounds during sample preparation and demonstrates the need for use of inert (nitrogen) atmosphere. The choice of fiber, extraction time and temperature was determined by whether low or high molecular weight (MW) sulfur compounds were of primary interest. In addition, increasing extraction temperature influenced both the number and relative amounts of VSC's detected in GFJ.

In order to separate and identify the sulfur volatiles in GFJ, high resolution capillary GC using 3 different (DB5, DBWax and PLOT, porous layer open tubular) columns coupled with pulsed flame photometric detection (PFPD) was employed. This enabled identification of sulfur compounds, particularly the early co-eluting sulfur peaks such as H_2S, SO_2, methanethiol (MeSH), CS_2 and Dimethyl sulfide (DMS) unambiguously. A rapid analytical method to determine the major VSC's present in GFJ has now been established. It can be used to track VSC changes during processing and storage. An example chromatogram is shown below.

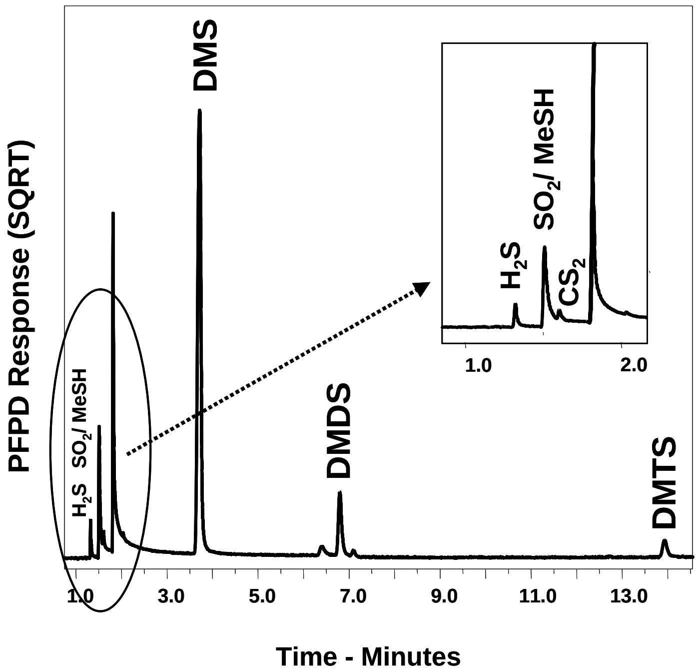

Fig 1: GC-PFPD chromatogram on PLOT column of low MW sulfur volatiles in grapefruit juice (canned juice from concentrate).

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2:00 pm

Roasting Effects on Total Sulfur Volatiles and Aroma Active Compounds in Arabica Coffee using GC-PFDP and GC-O.

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Coffee is one of the most popular drinks in the world. Considering the enormous economic importance of coffee, there are relatively few coffee gas chromatography-olfactometry, GC-O, studies and even fewer studies on coffee sulfur volatiles. Since several key coffee aroma volatiles contain sulfur, the purpose of this study was to examine how the degree of roasting affects the total number of sulfur volatiles and to determine which sulfur volatiles possess aroma activity. Many of these volatiles are formed during coffee roasting via Maillard and Strecker reactions and are major impact factors in coffee aroma. For example, 2-furfurylthiol is a major character impact compound in coffee (*1*). Other important contributors to the aroma of roasted coffee determined using GC-O include: 2-methyl-3-furanthiol, methional, 3-mercapto-3-methylbutylformate, 3-isopropyl-2-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 3-isobutyl-2-methoxypyrazine, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon), 4-ethylguaiacol, 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone, 4-vinylguaiacol, and (E)-β-damascenone (*2*). Subsequent studies (*1*) included 2,3-butanedione, 2,3-pentanedione and 3-methyl-2-butenthiol as additional aroma impact compounds.

Arabica coffee beans were obtained from the Highland Research and Education Center, Faculty of Agriculture, Chiang Mai University, Thailand. The beans were heated at 200 °C in a stainless steel rotary drum roaster for 12, 14 and 16 minutes to produce the light, medium and dark roast coffee with whole bean L values of 26.7, 20.9 and 17.6 respectively. Five grams of finely ground coffee were placed into a 40 mL glass vial. The vial headspace was purged with nitrogen before sealing with a Teflon-coated septum. The sample was equilibrated at 60°C for 15 min in a water bath and a 2 cm 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) Stable Flex fiber (Supelco, Bellefonte, PA) was exposed to the coffee volatiles for 30 min. Coffee sulfur volatiles were detected using a PFPD (pulsed flame photometric detector). Time-intensity GC-O was employed to determine which sulfur volatiles possessed aroma activity.

Forty-Five aroma compounds were observed in the raw beans, 98, 104 and 109 (aroma active volatiles were observed in the light, medium and dark roasted coffees respectively). Thirty-five of these major volatiles have been tentatively identified. GC-sulfur (PFPD) analysis of the identical samples produced only 14 sulfur peaks in the raw coffee headspace. One Hundred Forty-Five sulfur peaks were observed in the light roasted ground coffee which increased to 175 and 181 peaks in the medium and dark roast respectively. Total peak heights from the PFPD (square root mode) were greatest for the medium and dark roast. Raw and light roast total sulfur values were only 3.3 and 55 % as much as the medium roast total. Not all sulfur peaks were associated with aroma activity. However, 37 of the 109 peaks in the dark roast were associated with aroma activity. Twelve of these sulfur volatiles have been identified by comparing their retention behavior on wax and DB-5 columns with that from other studies (*3, 4*), their aroma quality and where possible their MS spectra. Relative concentration trends of key sulfur aroma active components such as 3-methyl-2-butenthiol, methional, 2-furfurylthiol and 3-mercapto-3-methylbutylformate from the raw to the light, medium and dark roast will be presented and discussed. Roasting effects on other newly identified aroma active sulfur volatiles will also be presented. Five other aroma peaks characterized with coffee aroma descriptors remain to be identified.

Table 1. The effect of roasting conditions on the total aroma active compounds,sulphur peaks and sulphur peaks with aroma activity

degree of roasting	total aroma active compounds	total sulphur peak height ($x10^3$)	number of sulphur peaks	aroma active S compounds
raw	45	106	14	11
light	98	1,756	145	39
medium	104	3,191	175	40
dark	109	3,064	181	37

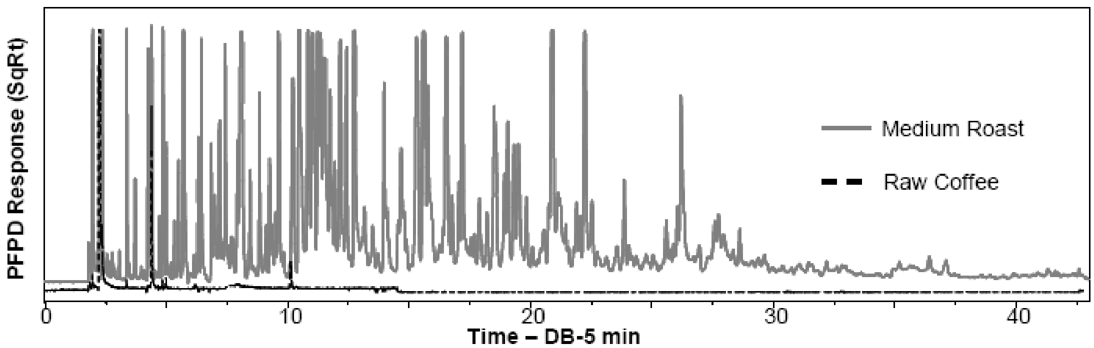

Figure 1 - Roasting effects on the number of sulphur volatiles in raw versus medium roast coffee measured by a sulphur specific PFPD detector

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2:15 pm
Hydration, Exercise and Orange Juice
F. Valim[3] and S. Barros[3]

Water balance is extremely important in day-to-day life in the maintenance of good health and the prevention of chronic disease. Water is lost from the body in varying amounts via a number of different routes. The total daily water loss is about 2.5 L, but this varies greatly between individuals and depends on environmental conditions. Dehydration is associated with a number of negative effects on health and well-being. There is increasing evidence that even mild dehydration, defined as a 1-2% loss in body mass caused by fluid loss affects the onset of various chronic diseases such as kidney stones, urinary tract infection, hypertension, pulmonary disorders and even coronary heart disease.

Given the increasing awareness of the importance of hydration for health and performance, a significant amount of research has provided new insight into several key aspects of hydration. Populations at particular risk of dehydration and its sequel include the very young and the elderly. One of the factors for the propensity of dehydration and alteration in water homeostasis in older adults is that the sense or perception of thirst is lessened with age, leading them to not drinking enough to rehydrate themselves. Healthy children may also be at risk of dehydration if there is a sudden increase in water loss for any reason, and physically active children will be at particular risk during periods of warm weather.

Adequate hydration is essential for optimum performance and to ensure that athletes do not incur heat-related illness. A primary role of ingesting carbohydrates in a fluid replacement beverage is to maintain optimal blood glucose concentration. Athletes should begin consuming fluids and carbohydrates immediately after exercise to help the body replace fluids lost in sweat and to replenish glycogen, which is the main source of muscle glucose.

The addition of carbohydrates to a fluid replacement solution can enhance intestinal absorption of water. The inclusion of glucose, sucrose and complex carbohydrates in fluid replacement solutions has equal effectiveness in increasing exogenous carbohydrate oxidation, delaying fatigue, and improving performance. Because fructose is converted slowly to blood glucose, it should not be the predominant carbohydrate, as it will not improve performance. There is little physiological basis for the presence of sodium in an oral rehydration solution for enhancing intestinal water absorption as long as sodium is sufficiently available in the gut from the previous meal or in the pancreatic secretions. In most cases, sodium losses which occur from sweating during exercise (less than 4-5 hours) can be replenished by normal dietary intake. During exercise, there is an increase in oxygen uptake in various organs, particularly in the skeleton muscle. Excess reactive oxygen species (ROS) release during exercise may have adverse implications on health and performance. Dietary antioxidants, such as vitamin C and flavonoids, may be helpful in reducing excess ROS.

Among the readily available beverages to fulfill hydration requirements orange juice is a healthy, flavorful, and nutrient-dense beverage. In addition, Florida orange juice is a convenient, fat and sodium free food composed of about 88% water plus natural vitamins, minerals and flavonoids. Orange juice contains about 10% carbohydrates, which includes one part of glucose, two parts of sucrose and only one part of fructose, and therefore should not be discounted as a fluid replacement beverage before, during and after exercise.

2:45 pm

Benefical Role of Citrus in Bone Health

P. Cancalon[3]

Several citrus compounds including vitamin C, folic acid, flavonoids such as hesperidin and naringin, carotenoids such as beta-cryptoxanthin act on bones. They act during the early part of life to increase the anabolic process and increase bone production to allow the bone to reach maximal bone mass. The same compounds act later in life during senescent and post-menopausal osteoporosis to limit bone losses. Citrus may also contribute to bone health by limiting inflammation during the entire life and may have a beneficial effect on the various forms of arthritis. Furthermore citrus potassium organic acids (citrate and malate) may limit blood acidity and slow calcium leakage**.** Studies published in the last few years have shown that several compounds in citrus may act synergistically to promote bone health during the entire life span.

3:00 pm

Orange Juice Consumed Regularly Decreased the Insulin Resistance in Normal Subjects

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Introduction: Orange juice is a natural source of citrus flavonoids, that can protect against coronary heart disease (CHD) and cancer, and it can be easily incorporated into a healthy diet. In spite of this, orange juice is also associated with high sugar intake (glucose and fructose) that can increase insulin resistance and lead to diabetes and its complications.

Objective: The objective of this study was to evaluate the effect of routine consumption of orange juice on the insulin resistance (IR) determined by Homa Index and to analyze the relation to other components of the metabolic syndrome.

Subjects and Method: We studied 30 non-diabetic individuals (15 men and 15 women), aged between 25 and 55 years, randomly selected among students and employees of a Phramaceutical School in Araraquara, SP, Brazil. They volunteered to drink 750mL/day of orange juice, without added sugar, during 60 days. The HOMA index was calculated as fasting insulin concentration (μU/mL) x fasting glucose concentration (mmol/L) / 22.5. In addition it was measured by standard methods in all subjects: anthropometric parameters and blood lipids levels.

Results: Measurements of fasting glucose and insulin in the beginning of the experiment were respectively: 4.45 mmol/L and 7.75 μU/mL, and after 60 days: 4.53 mmol/L and 6.55 μU/mL. HOMA index calculated for the first day of orange juice consumption was: 1.54 ± 0.9, and after 60 days was: 1.33 ± 0.8 ($p< 0.01$). At the end of experiment, total cholesterol, LDL-c and HDL-c decreased by 10%, 14% and 6% respectively ($p< 0.01$), while triglycerides were not changed ($p= 0.19$).

Conclusions: The significant decrease of 14% of HOMA Index was associated with a reduction of Insulin Resistance after 60 days of consumption of orange juice in non-diabetic individuals. In addition, the biochemical indicators: serum glucose, insulin, LDL-c and triglycerides (TG) showed that orange juice did not increase the risk for diabetes or coronary heart disease.

3:15 pm

Health and Nutritional Benefits of Citrus Juice

S. Barros[3], W. S. Stinson[3], M. Azik[3], P. Cancalon[3], G. Rampersaud[9], F. Valim[3] and J. Zhang[3]

Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases, such as cancer and cardiovascular disease, the major causes of death in Western countries. The link between a healthy diet and disease prevention may seem like a natural connection, however, consumer behavior does not reflect this as a very small amount of the people in the Western World consume the recommended amount of fruits and vegetables on a daily basis (5 or more servings/day).

Research into the nutrients found in citrus juices reveals that these easily obtainable juices possess both health promoting and disease preventive properties.

While the quantity of vitamins in citrus juice is very high, on a weight basis the quantity of phytochemicals is even higher. Data is growing, mostly in the form of epidemiological and animal studies, that these phytochemicals possess biological activity that promotes human health including disease prevention.