

# BERRY HEALTH BENEFITS SYMPOSIUM

The only conference focusing solely on berries & human health.



## **Symposium Pre-Proceedings**

**2013 Berry Health Benefits Symposium  
June 18-20, 2013 | Concord, NC- USA**



## The Berry Sessions

Tuesday, June 18<sup>th</sup> - Concord A

**8:30 Registration/Coffee** – Registration  
South Embassy Suites Hotel, Concord, NC

**9:00 - Berryology 101 -**  
Understand the terminology used in speaking about berry health and a look at the ORAC debate and how to express berry health benefits beyond antioxidants.  
**Dr. Navindra Seeram & Dr. Ronald Prior**

**10:00 - Marketing Berry Health to Social Media Outlets** – A panel discussion with large and small berry groups focusing on how they utilize the best of social media to reach consumers.

**Panel:**  
•Jodie Reinman – California Strawberry Commission  
•Darcy Kochis – Oregon Raspberry & Blackberry Commission  
•Emily Valentine – US Highbush Blueberry Council  
•Wendy Bazilian – Driscoll's Inc.

**11:00 – Break**

**11:15 - Current Research Forum** – Hear the latest in berry health research from berry groups and companies in lay terms.

**Panel:**  
•Dr. Christina Khoo – Ocean Spray Inc.  
•Dr. Eugene Woltering – Louisiana State University  
•Dr. Roger Hurst – The New Zealand Institute for Plant & Food Research

**12:00 - Lunch – Concord A**  
**Keith Mixon, Director, Dole Berry Business**

**1:00 - Current Research Forum (continued)**

**Panel:**  
•Chris Christian – California Strawberry Commission  
•Tom Krugman/Leigh Selby – Washington Red Raspberry Commission  
•Dr. Leslie Wada – US Highbush Blueberry Council

**2:00 - Berry Research Trends from the Lab to the Consumer** – Learn how scientific research moves from the lab to the table through marketing, food service and retail channels.

**Panel:**  
•Dr. Britt Burton Freeman – University of California Davis  
•Megan Lambert, RD – Johnson & Wales University  
•David A. Stuart Ph.D - Food & Nutrient Impact, LLC  
•Chef John Blumreich, Restaurant Forty Six

**3:00 - Health Claims in Labeling & Advertising** – Leslie Krasny – Partner, Keller & Heckman, LLP

**4:00 - Developing Effective Outreach with Market Research** - Dr. Michael R. Thomsen – University of Arkansas

**Evening – Dinner on your own**

# The Berry Health Benefits Symposium

Wednesday, June 19<sup>th</sup> – Concord E

**7:30am-8:45– Poster Presentations**

## **Berry Compositional Chemistry & Biological Effects**

**8:45 Current Research Review**

Chair: Dr. Navindra Seeram, University of Rhode Island

**9:00 - Bioavailability of Berry Phenolics and Potential Protective Effects**

Dr. Alan Crozier, University of Glasgow

**9:30 – Anthocyanin Absorption after long-term Blueberry Feeding**

Dr. Wilhelmina Kalt, Agriculture and Agri-Food Canada

**10:00 - Italy Strawberry and Human Health: Effects Beyond Antioxidant Activity**

Dr. Maurizio Battino, Università Politecnica delle Marche

## **Berries and Cancer**

**10:45 - Current Research Review**

Chair: Dr. Gary Stoner, Medical College of Wisconsin

**11:00 - Evaluating cranberry constituents as inhibitors of esophageal adenocarcinoma utilizing in vitro assays and in vivo models**

Dr. Laura Kresty, Associate Professor of Medicine - Medical College of Wisconsin

**11:30 – The Role Of Black Raspberries and Fruit Phenolics on Inflammation and colorectal Neoplasia**

Dr. Christine Sardo, University of Arizona

**12:00 - Lunch**

**1:00 – The Emerging ‘Colored’ Compounds in Blueberry for Prevention and Treatment of Cancers**

Dr. Ramesh Gupta, University of Louisville

## **Berries and Brain Aging**

**1:30 - Current Research Review**

Chair: Dr. Barbara Shukitt Hale, USDA/Tufts University

**1:45 - Berry Effects on Cognition and Motor Function in Aging**

Dr. Barbara Shukitt Hale, USDA/Tufts University

**2:15 – Long-Term Berry Intake and Cognitive Health In Older Women**

Dr. Elizabeth Devore, Brigham and Children’s Hospital/Harvard Medical School

**2:45 - Berry Fruit Intervention and Human Memory**

Dr. Robert Krikorian, University of Cincinnati

**3:30 – Break**

## **Berries and Metabolism –**

**3:45 – Current Research Review**

Chair: Dr. Ronald Prior, University of Arkansas

**4:00 – Berry Source and Secondary Phenolic Acid Metabolites**

Dr. Ronald Prior, University of Arkansas

**4:30 – Bilberry Anthocyanin Inhibits Atherosclerosis Development by Affecting Expression of Genes Involved in Early Stages of Disease Development**

Dr. Dragan Milenkovic, INRA/centre de Recherche de Clermont- Ferrand/Theix, France

**5:00 – Berries and Early ‘Nutritional’ Experiences in the Prevention of Chronic Diseases**

Dr. Rosalia Simmen, University of Arkansas

**5:30 - 6:30 PM – Poster Presentations**

## Wednesday, June 19<sup>th</sup> – Concord A - cont.

7 PM – Dole Keynote Dinner – Concord A

*New Breakthroughs on Berries and Human Health*  
(but..... your ancestors already knew this)

**Keynote Speaker- Dr. Mary Ann Lila**

David H. Murdock Distinguished Professor,  
Director North Carolina State University Plants for  
Human Health Institute, North Carolina Research  
Campus Kannapolis



## Thursday, June 20<sup>th</sup>- Concord E

### **Berries and Heart Health**

**8:30 - Current Research Review**

Chair: Dr. Britt Burton Freeman, University  
of California, Davis

**8:45 – Strawberry polyphenols on risk  
factors for diabetes and cardiovascular  
disease: a look at the clinical data**

Dr. Britt Burton Freeman, University of  
California, Davis

**9:15 - Effects of Blueberry Polyphenols on  
Vascular Function in Healthy Men**

Dr. Ana Rodriguez-Mateos, University of  
Reading, UK

**9:45 - Berry Intake and Cardiovascular  
Health Through Epidemiologic and  
Clinical Studies**

Dr. Howard Sesso, Harvard University

**10:15 - Break**

### **Berries and Gut Health/Gut Microflora**

**10:30 – Current Research Review**

Chair: Dr. Jess Reed, University of Wisconsin

**10:45 – Berry Polyphenols and Gut Health**

Dr. Jess Reed, University of Wisconsin, Madison

**11:15 - Polyphenolics and the Gastrointestinal  
Immune System**

Dr. Dhanansayan (Dhanu) Shanmuganayagam,  
University of Wisconsin, Madison

**11:45 –Alterations in Lipid Metabolism by  
Black Raspberry Intervention in Colorectal  
Cancer Patients**

Dr. Li Shu Wang, Medical College of Wisconsin

**12:00 – Box Lunch – Lobby**

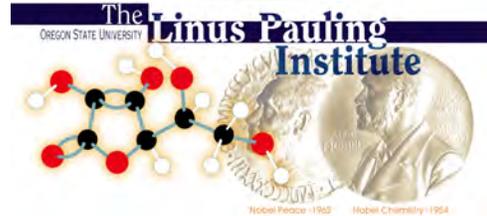
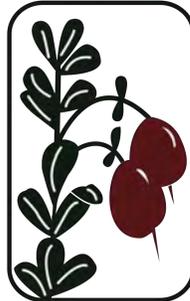
**1pm - North Carolina Research Tour (Buses  
Depart at 1:00 from the main lobby)**



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# Thank you Sponsors



Cranberry  
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# Tuesday, June 18<sup>th</sup>

## The Berry Sessions



**Navindra Seeram Ph. D.**

**University of Rhode Island**

**Berryology 101**

Navindra P. Seeram, Ph.D., is an Assistant Professor of Pharmacognosy in the Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island. Prior to this, he was the Assistant Director of the Center for Human Nutrition in the Department of Medicine, University of California, Los Angeles (UCLA), and an Assistant Adjunct Professor in the David Geffen School of Medicine at UCLA. His research group, the Bioactive Botanical Research Laboratory, investigates medicinal plants, including plant foods, as potential sources of botanical drugs, phytopharmaceuticals and phytomedicines. Targeted diseases of interest include cancer, diabetes and inflammation.

Dr. Seeram has more than 15 years of experience in botanical research. His work on berry phytochemicals, as both singly purified and combined (extract or food) forms, has included examining their bioavailability, metabolism, and mechanisms of action using in vivo systems. He has co-authored more than sixty peer-reviewed research articles, thirteen book chapters, two patents, and co-edited one book.

He currently reviews scientific articles for over two dozen peer-reviewed journals and serves on the editorial board for Recent Patents on Anticancer Drug Discovery by Bentham Science Publishers Ltd. He is the series editor for a recently launched CRC Press/Taylor and Francis book series entitled Clinical Pharmacognosy. Dr. Seeram did his doctoral and postdoctoral research at the University of the West Indies, Jamaica, and at Michigan State University, respectively.



**Ronald L. Prior, Ph.D.**

**University of Arkansas**

**Berryology 101**

Dr. Prior received his Ph.D. in Nutrition with minors in biochemistry and physiology from Cornell University. His graduate training was followed by two years of post-doctoral training in Comparative Gastroenterology through the College of Veterinary Medicine at Cornell University. Dr. Prior was with the Agricultural Research Service of the USDA for 35 years. Following 13 years at the USDA Human Nutrition Research Center on Aging at Tufts, Dr. Prior moved in 2000 to the USDA Arkansas Children's Nutrition Center in Little Rock, AR where he provided leadership for their phytochemical and health research program. In May of 2010 Dr. Prior retired from the USDA, but he continues to serve as adjunct professor in the Dept of Food Science at the Univ. of Arkansas, Fayetteville and to consult with organizations on matters related to phytochemicals and nutrition. Dr. Prior has published more than 220 articles in peer reviewed scientific journals. Dr. Prior received the Alex Wetherbee Award from the North American Blueberry Council for his contributions to the blueberry industry. In 2006, was ranked as the top-cited author in agricultural sciences by Science Watch.

Dr. Prior has published more than 200 articles in peer reviewed scientific journals. Dr. Prior received the Alex Wetherbee Award from the North American Blueberry Council for his contributions to the blueberry industry resulting from research on the antioxidant components and health benefits of blueberries. Dr. Prior's research efforts have focused on assessing antioxidant capacity of fruits and vegetables and understanding the absorption and metabolism of antioxidant phytochemicals in fruits and vegetables. Dr. Prior's laboratory has provided the data on anthocyanins, proanthocyanidins and antioxidant capacity of fruits and vegetables for the flavonoid database in the USDA food nutrient database. Dr. Prior's recent research focus has been on effects of foods high in anthocyanins on the development of obesity and metabolic syndrome.



## **Marketing Berry Health to Social Media**

**Jodi Reinman**

**Public Relations & Social Media Manager  
California Strawberry Commission**

As a fifteen year public relations expert, Ms. Reinman began her career building positive awareness for top Silicon Valley companies, including Hitachi, Cisco and Intuit. After a decade of media tours with top executives, garnering prized cover stories in the highly competitive tech industry, she transitioned to agriculture and used her experience to build a vibrant social media presence to connect consumers with California Strawberries. A key strategist, she creates innovative programs and forges relationships with top food and lifestyle bloggers that promote the fruit, the farmers, and the health benefits.

Jodi holds a B.S. in Public Relations from San Jose State University. In her spare time she authors a personal food blog, Garlic Girl, where she shares recipe creations and food photography. A mother of three, she enjoys running, hiking and family activities.



**Darcy Kochis**

**Marketing & PR  
Food First Marketing / Oregon Raspberry &  
Blackberry Commission  
[darcy@foodfirstmarketing.com](mailto:darcy@foodfirstmarketing.com)**

Darcy Kochis is co owner of Food First Marketing based in Oregon. Food First Marketing has a focus on marketing and PR for Oregon berries as well as event coordination of berry centric events. The Oregon Berry Festival draws national attention to spot light Oregon berries, farms and products in July. Darcy is a graduate of the University of Oregon in 2000. After college she worked as a reporter for the Meredith TV stations, PDX 49 and FOX 12. The time that she spent in TV was during the rise of social media. A combination of social media and traditional networking is the approach she finds most effective.



**Emily Valentine**

**US Highbush Blueberry Council**

As a senior account executive at CRT/Tanaka, Emily provides brand marketing and public relations counsel to clients including the U.S. Highbush Blueberry Council (USHBC). Since 2009, she has worked with the Blueberry Council to guide the development of its little blue dynamos brand platform and manage strategy for the brand's implementation, including spokesperson engagement, national print and web advertising, website redesign (standard + mobile), media relations, social media marketing, foodservice relations and grower communications. Emily graduated from University of Virginia and currently resides in Charlottesville, Va., where she loves hiking, running and exploring local food and wine.



**Dr. Wendy Bazilian**

**Writer, researcher, educator, food enthusiast and award-winning journalist**

Dr. Wendy Bazilian is a registered dietitian and an American College of Sports Medicine-certified Health and Fitness Specialist, and Dr. Bazilian's skills in integrating nutrition, fitness and wellness practices help clients and readers who are motivated to reach their health and wellness goals. Her expertise covers a wide range of subjects in nutrition and health that include optimal eating for active individuals, weight management and healthy aging. Dr. Bazilian has appeared nationally as a spokesperson on healthy living using nutrition and exercise; she has published over 200 articles for corporate and other media. She is author of *The SuperFoodsRx Diet: Lose Weight with the Power of SuperNutrients* (Rodale Publishers). She graduated summa cum laude, Phi Beta Kappa with a Bachelor's Degree from Tufts University, then received her Masters Degree from the University of California, San Diego. She completed her Doctoral Degree in Public Health and Nutrition from Loma Linda University, graduating summa cum laude and receiving the highest academic honor, the prestigious Chancellor's Award.



### **Current Berry Research Forum**

**Christina Khoo Ph. D.**

**Senior Manager, Ocean Spray  
Cranberries, Inc**

Christina received her M.S.n Nutritional Sciences (1992) and Ph.D. in Nutritional Biochemistry (1995) at the University of Florida. She was a postdoctoral Associate/Project Coordinator at Harvard School of Public Health from 1996 to 2001 conducting basic and clinical study in triglyceride metabolism using a novel immunoaffinity chromatography method to purify and isolate lipoproteins based on their different apolipoproteinE and C3 contents and obtained material for a kinetic modeling project of lipoprotein metabolism using the SAAM II kinetic modeling program and GC-mass spectrometry data. Christina is currently Senior Manager of Research Sciences at OCEAN SPRAY CRANBERRIES, INC., managing the research sciences group to develop strategy for health platform to support cranberry health benefits and strengthen the Ocean Spray Brand. Her current focus is on urinary tract health, maintaining heart health and improving lipid profile, obesity, inflammation and immune function.



**Eugene A Woltering, MD, FACS**

**The James D. Rives Professor of Surgery and Neuroscience;  
Division Chief of Surgical Oncology and Endocrine Surgery;  
Director of Surgical Research ; Louisiana State University  
Health Science Center**

Dr. Eugene Woltering attended medical school at The Ohio State University School of Medicine and completed his internship and residency at Vanderbilt University Affiliated Hospitals in Nashville Tennessee. Dr. Woltering has also completed fellowships in surgical oncology at The Ohio State University School of Medicine and the National Cancer Institute. Dr. Woltering specializes in the diagnosis and management of all types of neuroendocrine tumors (NETs). His laboratories have produced over 150 peer reviewed publications and have 16 patents, most of which apply directly to neuroendocrine tumors.



**Roger Hurst Ph. D.**

**Science Group Leader, Food & Wellness  
Food Innovation Plant and Food Research, New Zealand**

BSc (Hons), Applied Life Sciences, University of Wales, United Kingdom  
PhD, Biochemistry, University of Keele, United Kingdom  
Research Interests & Activities  
Research expertise in the health and food area with an emphasis on oxidative stress/antioxidant/inflammatory mechanisms. Heavily involved in programmes investigating the health benefits of fruit-derived compounds. This work involves cell culture modelling of various tissues, animal and human intervention trials, determination and monitoring of inflammatory signals, evaluation of antioxidant levels, enzymatic antioxidant systems and modulatory pathways, evaluation of oxidative stress status, analysis and identification of fruit-derived compounds.



## **Lunch Speaker**

**Keith Mixon – President of Dole Berry Company**

Keith Mixon is President of Dole Berry Company, a subsidiary of Dole Food Company. Dole Berry Company is a major grower and distributor of fresh berries to the wholesale, food service and retail markets throughout North America. In addition to growing on their own farms, Dole Berry packages and distributes blueberries, blackberries, raspberries and strawberries for independent growers located in North, Central and South America.

Dole Berry Company was formed in October 2011 as a result of Dole's acquisition of SunnyRidge Farm. SunnyRidge Farm was a family business established in 1933 by the Mixon Family and was a major producer and marketer of blueberries and blackberries throughout most major growing regions. Keith served as President and CEO from 2004 until the Dole acquisition. Dole Berry Company represents a significant share of the berry industry and is very active in the promotion of health research. Keith brings insight into the berry industry as well as into Dole's commitment to health research for its consumers.

Keith serves a Representative on the US Highbush Blueberry Council and is Chairman of the Florida Fruit and Vegetable Association. He is also a member of the Board of Directors for Farm Credit of Central Florida.

Keith graduated from Georgia Tech with a degree in Electrical Engineering and received a Masters degree in Business Administration from the University of South Florida.



**Chris Christian**

**Vice President, Marketing California Strawberry Commission**

A produce industry professional with over 25 years experience, Chris Christian serves as vice president of marketing at the California Strawberry Commission where she leads consumer and trade marketing and nutrition research programs. In addition to leading the Commission's marketing programs, Chris also supports the management of finance and general operations.

Prior to her ten years at the Commission, Chris worked in management positions in the food and agriculture industries, including ten years at Fresh Express Inc., where she held key roles in business management. Throughout her career, Chris has gained credibility and established relationships with produce professionals in the retail and food service industries, world class researchers, and food safety professionals.

Chris holds an M.S. in Food Science and B.S. in Microbiology from Pennsylvania State University. She serves on several produce trade association leadership councils and is a member of the Institute of Food Technologists (past Chair of Fruit & Vegetable Products Division), and the Academy of Nutrition and Dietetics.



**Leigh Hays Selby**

**Washington Red Raspberry Commission**

Born in Washington DC and reared in DC, Afghanistan, France and Haiti, Leigh attended the University of California, Santa Cruz where she completed degrees in economics and language studies.

Leigh's first career was in international development in the fields of tourism and international public health. Subsequently, she worked in health and human services with an emphasis on nutritional programs for preventive health as well as geriatric care, particularly with respect to Alzheimer's disease and related dementia.

Since 2006, she has worked with whole food commodity boards to educate influencers about the health and nutrition benefits of various foods, including tomato products, raspberries, onions, pistachios, olive oil and table olives. She has a facility for translating complex scientific research for multiple audiences.

Leigh began working with the Washington Red Raspberry Commission (WRRC) in 2006 to facilitate its developing a strategic plan for communicating the health and wellness attributes of raspberries. After the adoption of the plan by the board of directors in 2006, she has worked in conjunction with Tom Krugman to implement the strategies, tactics, programs and projects outlined within the strategic plan.

After working in Washington DC, Leigh relocated to California's Central Coast where she is the owner of Firehorse. She serves as the WRRC's Health and Wellness Consultant.



**Thomas Krugman**

**Washington Red Raspberry Commission**

Born and raised in Southern California, Tom attended the University of California, San Diego, where he completed his degree in Biochemistry. He later attended and graduated from the Santa Clara University with an MBA, Agribusiness.

The first stop on Krugman's professional career was the California Cling Peach Advisory Board. This position provided the opportunity for him to experience the challenges of working with a voluntary elected board of directors composed of growers and processors.

His next position was with Tri Valley Growers, a fruit and tomato processing and marketing cooperative. Here, Krugman was responsible for customer and government relations, and bridging the gap between sales, marketing, and operations.

Tom returned to the public sector with the Almond Board of California. A senior management position found him developing and directing the industry's food safety program, taking a lead role in crisis management and strategic planning, and coordinating government procurement programs.

After spending his professional career in California's Central Valley, he next moved to the Monterey Peninsula where he joined the California Strawberry Commission, again in a senior management position. Strategic planning and development, and reorganizing the Commission's marketing direction were the immediate tasks at hand.

Krugman then relocated to the Pacific Northwest where he lives today. As the owner of Central Consulting, Tom currently serves as the Marketing Director for the Washington Red Raspberry Commission.



**Leslie Wada, Ph.D., RD**

**US Highbush Blueberry Council**

Leslie Wada is the Research Administrator for the US Highbush Blueberry Council (USHBC). She has worked with the USHBC for the past 7 years where she coordinates and oversees all of the research projects that are supported by the Council.

Leslie is a registered dietitian and has an undergraduate degree in nutrition and dietetics from the University of California at Davis. She earned her PhD in nutritional sciences from the University of California at Berkeley where she worked as a research scientist on nutrition studies and taught classes in dietetics. She left academia to continue her career working with companies that developed functional foods and ingredients. For the past 16 years she has worked as an independent consultant and currently, in addition to working with the USHBC, she consults for companies in the food and agriculture industry.

## **Berry Research Trends from the Lab to the Consumer**



**Britt Burton Freeman, Ph.D.**

**University of California, Davis**

Dr. Freeman's current research interests are in mitigating disease process through dietary approaches focused on the health promoting properties of whole foods. Specific disease targets are vascular disease and obesity, including food intake regulation. In her current appointment, she leads a public health initiative with FDA/CFSAN to develop and provide underpinning science for comprehensive approaches using innovative processing solutions to support the availability of safe food with health opportunities.

Dr. Freeman is an active member of multiple professional societies dedicated to health and disease abatement including the American Society for Nutrition, the Obesity Society and the Society for the Study of Ingestive Behavior. Dr. Freeman earned a M.S. and Ph.D. in Nutrition Science with an emphasis in Endocrinology and Physiological Chemistry at the University of California, Davis.



**Megan Lambert, RD**

**Johnson & Wales University**

D. Megan Lambert, RD, CHE, CB, CEPC is an Associate Instructor in the International Baking & Pastry Program at Johnson & Wales University, Charlotte. This is her ninth year of teaching a variety of baking and pastry classes. Prior to joining the faculty at Johnson & Wales, Chef Lambert was the co-owner/production manager of The Flour Shop Bakery in Morrisville, NC. Her previous cooking experience includes baking at a small café/wine bar, plated desserts in a fine dining restaurant, and baking at a

large university bakeshop. Chef Lambert graduated from The Pennsylvania State University with degrees in Nutrition and Hotel Restaurant Management, and then completed a Dietetic Internship at Syracuse University. Chef Lambert has a particular interest in Mexican cuisine and baking, as well as sustainability and local food issues. She is currently pursuing a Master's of Science Degree in Nutrition at East Carolina University and loves to spend what little free time she has in her garden. Chef Lambert is the co-author of "The Organic Gardener's Cookbook – Easy Growing Tips and Delicious Recipes for Your Home Grown Vegetables" as well as the co-owner of Garnet Gals, Local Jams, Jellies, and Preserves.



**DAVID A. STUART, Ph.D**

**Food & Nutrient Impact, LLC  
University of California, Berkeley**

**BIOGRAPHY: DAVID A. STUART, Ph.D.**

David A. Stuart Ph.D. is Founder and Principal of “Food & Nutrient Impact, LLC”, a located in Hershey PA focusing on research and advice on healthy foods, on cacao and on agricultural sustainability.

He holds a B.S. in Biology and minor in Chemistry from California State University, Sacramento. He earned his M.A. and Ph.D. in Botany and Plant Physiology from the University of California, Berkeley where he studied the biophysics of plant cell growth.

David earned an Executive Program degree in Agribusiness from the Smeal School of Business at The Pennsylvania State University in 1999. David worked for Plant Genetics, Inc. in Davis CA for eight years where he was leader of their cell biology, plant pathology and the genetic improvement programs. Work there was done in association with some familiar companies such as Kirin Breweries, McCormick Spices and Best Foods (now part of Unilever).

David began work at The Hershey Company in 1989 where he was a manager or director the Research and Development Department. His career there literally spanned *Cocoa Bean to Chocolate Bar*. He began as Manager of Biochemistry where he immediately became involved with cocoa, its genetics, biochemistry and field production. He was active as a leader in the Chocolate Industry’s Cocoa Biotechnology programs which have led to advances in variety cloning, new variety development and the Cocoa Genome which was published in 2010. Eight years ago, he developed the proposal leading to the Hershey Center for Health and Nutrition becoming Center Director. He led a group of nine Ph.D. scientists responsible for the characterization of the flavanol chemistry of cocoa, flavanols levels of market basket surveys, flavanol loss during processing, shelf stability of flavanols, clinical trials using commercially available dark chocolate and cocoa, product labeling and web-site development. He worked with national and international groups to set the standard testing protocols for these naturally occurring bioactive compounds. His area was also responsible for the development of new cocoa and high-flavanol ingredients for use in product development as well as studies on the absorption of flavanols into the blood stream and on the impact of these compounds on human metabolism. He was recently awarded the life-time achievement award by the National Confectioners Association for his contributions to the industry.

David has authored more than 35 publications, an inventor on eight patents, including 17 papers and published presentations since the Center was formed. He has also participated in numerous lectures at universities, presentations at meetings and at scientific conferences.

*Dave Berry Meeting Bio m5 d31 y13*



**Chef John Blumreich**

**Restaurant 46**

Chef John Blumreich is American born, and raised in Europe where he was exposed to many cultures and quality cuisines, giving rise to his passion for flavor and variety.

After attending the “University of South Florida” he graduated from “New England Culinary Institute” in Montpelier, Vermont, receiving a degree in Culinary Arts.

Chef John has worked in Fine Dining establishments since 1992, and is currently the executive Chef at "The Club at Irish Creek" in Kannapolis, NC. His Prior experience includes working with the prestigious “Bern’s Steak House” in Tampa FL, “Tanglewoods” in Waterbury Center, VT, “Red Hen Baking Company” in Middlesex VT, ten years as Executive Chef at “Carpe Diem” in Charlotte, NC and two years as Executive Chef at “Forty Six” in Kannapolis NC, before joining "The Club at Irish Creek.”

Chef John is committed to guaranteeing that each event or meal meets the highest standards of freshness and quality, underscoring the importance of flavor, texture and presentation.



## **Health Claims in Labeling & Advertising**



**Leslie Krasny**

**Partner, Keller & Heckman, LLP**

Leslie Krasny is a partner with Keller and Heckman LLP and manages the San Francisco office. Ms. Krasny practices regulatory law, focusing on food and dietary supplements, including safety, labeling, advertising, recalls, ingredient evaluation, claim substantiation, organic requirements, country of origin, California's Proposition 65, pesticides, and biotechnology. She also works with litigators and scientists at Keller and Heckman to defend consumer class action lawsuits for food marketing claims filed under state consumer protection laws. Ms. Krasny has specialized in food law for 25 years, with law firms and in-house, as Vice President and General Counsel of Dole Packaged Foods Company. Her clients are growers, processors, distributors, retailers, foodservice companies and trade associations, and she serves as General Counsel to the Produce Marketing Association. She is a frequent speaker and writer on food law issues, is a member of the Food and Drug Law Institute's Board of Directors and its Food and Dietary Supplements Committee, and serves on the Editorial Advisory Board of Food Processing Magazine.

## **Developing Effective Outreach with Market Research**



**Michael R. Thomsen Ph.D**

**University of Arkansas**

Mike Thomsen is an Associate Professor of Agricultural Economics in University of Arkansas Division of Agriculture. Mike's research, teaching, and outreach programs emphasize food market behavior, food policy, and the economics of horticultural food crop production. His recent research projects have addressed food safety, value-added opportunities for regionally produced fruit and vegetable crops, and the economics of regional vineyard and winery operations. Mike grew up on a small farm in the Intermountain West. He completed BS and MS degrees in agricultural economics, both from Utah State University. His PhD is from the University of Minnesota in agricultural and applied economics with a minor in business administration.

# Berry Health Benefits Symposium Speaker Bios & Abstracts

## Berry Compositional Chemistry & Biological Effects



**Navindra P. Seeram, Ph.D.**

**Session Chair  
University of Rhode Island**

*Current Research Review*

Navindra P. Seeram, Ph.D., is an Assistant Professor of Pharmacognosy in the Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island. Prior to this, he was the Assistant Director of the Center for Human Nutrition in the Department of Medicine, University of California, Los Angeles (UCLA), and an Assistant Adjunct Professor in the David Geffen School of Medicine at UCLA. His research group, the Bioactive Botanical Research Laboratory, investigates medicinal plants, including plant foods, as potential sources of botanical drugs, phytopharmaceuticals and phytomedicines. Targeted diseases of interest include cancer, diabetes and inflammation.

Dr. Seeram has more than 15 years of experience in botanical research. His work on berry phytochemicals, as both singly purified and combined (extract or food) forms, has included examining their bioavailability, metabolism, and mechanisms of action using in vivo systems. He has co-authored more than sixty peer-reviewed research articles, thirteen book chapters, two patents, and co-edited one book.

He currently reviews scientific articles for over two dozen peer-reviewed journals and serves on the editorial board for Recent Patents on Anticancer Drug Discovery by Bentham Science Publishers Ltd. He is the series editor for a recently launched CRC Press/Taylor and Francis book series entitled Clinical Pharmacognosy. Dr. Seeram did his doctoral and postdoctoral research at the University of the West Indies, Jamaica, and at Michigan State University, respectively.



**Alan Crozier Ph.D**

**University of Glasgow**

***Bioavailability of berry phenolics  
and potential protective effects***

Alan Crozier is a graduate of the University of Durham in the UK and carried out PhD studies at the University of London. He held positions in Canada and New Zealand before moving to the University of Glasgow where he is currently an Emeritus Professor in the School of Medicine. Among his research interests is the biosynthesis of purine alkaloids, such as caffeine and theobromine in coffees and teas. The current activities of his research group are focussed principally on the fate of potentially protective dietary flavonoids and phenolic compounds in fruits and beverages as they pass through the body following ingestion. He has published extensively in this area and has recently edited four books on the topic.

### **The bioavailability of (poly)phenolic compounds in red raspberries and other fruits and their potential protective effects following consumption.**

**Alan Crozier, School of Medicine, University of Glasgow, United Kingdom**

The principal (poly)phenolic compounds in red raspberries are anthocyanins, with cyanidin-3-*O*-sophoroside predominating, along with ellagitannins. Human feeding studies with raspberries have been carried out with healthy subjects and ileostomist who have had their colon surgically removed. This has provided information on the restricted absorption of anthocyanins in the small intestine and their breakdown to low molecular weight phenolic and aromatic acid in the large intestine that is mediated by the colonic microflora. Substantial amounts of phenolic acids produced in this way are absorbed into the portal vein prior to passage through the circulatory system and excretion in urine. The high molecular weight ellagitannins, such as sanguin H6, are not absorbed in the proximal gastrointestinal tract but enter the colon where *in vitro* incubations with fecal slurries under anaerobic conditions show that they are converted to ellagic acid which is then very efficiently converted to urolithins by the microbiota. During passage through the wall of the colon the urolithins are glucuronidated and it is these conjugates rather than the aglycone that enter the circulatory system.

Similar studies have been carried out with other berries and purple grape juice and research has begun with animal models and *in vitro* test systems to investigate the potential protective effects of these products. It is important when using *in vitro* models, to test not the parent compounds that are ingested but their *in vivo* metabolites which enter the circulatory system and at physiological concentrations initiate events leading to protective effects.



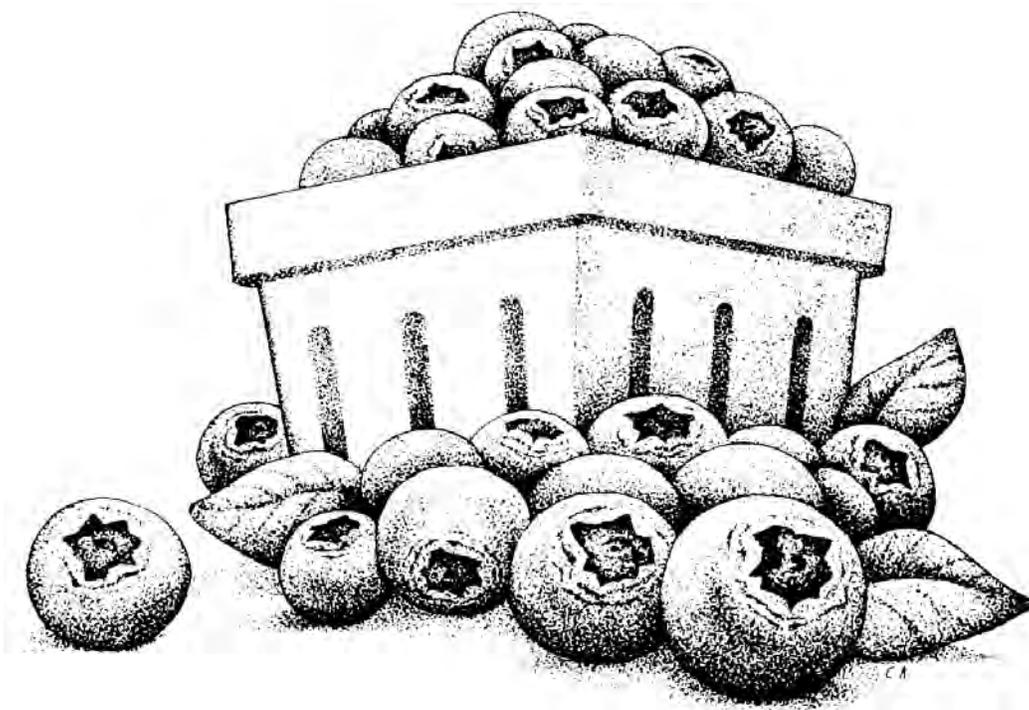
**Wilhelmina Kalt Ph.D.**

**Agriculture and Agri-Food Canada**

***Anthocyanin Absorption after Long-term  
Blueberry Feeding***

Wilhelmina Kalt obtained her Ph.D. degree from North Carolina State University and is currently employed with the Canadian federal agriculture department, Agriculture and Agri-Food Canada in the province of Nova Scotia. Dr. Kalt's research on the health benefits of berries has focused on the anthocyanins of blueberry species and other berry crops. She's characterized the impact of horticulture and food factors on the antioxidant phenolics in berries and continues to work on the separation of berry phenolic mixtures for use in vitro and in vivo. Dr. Kalt has conducted animal and human studies to assess the bioavailability and functional bioactivity of blueberry flavonoids, with particular emphasis in vision physiology.

Dr. Kalt is currently the P.I. on a 3 year multi-site initiative than encompasses research in diabetes, vision and cardiovascular disease. One of her specific goals in this work is to develop tools and information for human clinical research using blueberries. Willy works closely with industry groups and in particular the blueberry industries, to support the development of their health sector.



# ANTHOCYANIN ABSORPTION DURING LONG-TERM BLUEBERRY JUICE INGESTION

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*Introduction.* Anthocyanins (Acn) are the most widely distributed and often the most abundant flavonoid in commercial berry crops. They are thought to contribute significantly to the health benefits of berry consumption, despite knowledge that Acn are poorly absorbed and rapidly eliminated by the body. The notions that orally administered Acn are poorly absorbed and rapidly eliminated arise from pharmacokinetic studies examining Acn metabolism and clearance after a single dose. Very few studies examine Acn absorption after more than one day of intake. In contrast, clinical biomedical research on berry crops examining health or disease outcomes are typically conducted over weeks or even months. Since these biomedical studies rarely also examine Acn bioavailability, the nature of Acn absorption and metabolism over time frames similar to clinical trials is essentially unknown. The clinical trial reported here is an attempt to address this gap in our knowledge of Acn bioavailability.

*Study Design and Analysis.* The trial included 17 normal, healthy volunteers (age 24-60, 13 women, 4 men) who abstained from all Acn-containing foods for 5 days before, and during the 37-day intervention. For 30 days, volunteers consumed daily 250 ml single-strength wild blueberry (*Vaccinium angustifolium*) juice (BJ) containing 216 mg cyanidin 3 glucoside equivalents (C3g eq). Eight volunteers consumed BJ in three 83 ml doses over a maximum of a 12 h period, while the other nine consumed BJ in a single 250 ml dose before 10 am. At days 1, 8, 15 and 29 volunteers collected over a 24 h period a sample of urine from each void and recorded the total urine volume. As a 'washout', after day 29 and until day 36 volunteers abstained from BJ and other Acn-containing foods. Then at day 36 volunteers consumed BJ and collected urine samples for the next 24h. Acn and Acn metabolites in urine were analysed using LC-MS/MS (ABSciex QTrap 4000) following SPE cleanup using multiple reaction monitoring (MRM). Sixty MRMs were tracked including cyanidin, delphinidin, peonidin, petunidin, malvidin aglcones and monoglycosides of arabinose, galactose and glucose and their methylated, glucuronidated and sulfated metabolites. Quantification was carried out using pure standards of the glucosides of cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin.

*Results.* To date the approximately 500 urine samples have yielded more than 100 distinct MRM/retention-time combinations. Two major findings, based on preliminary results, suggest that Acn absorption may be modified by long term ingestion. First, after long term ingestion, Acn were not as effectively cleared from circulation, compared to after a single dose. Second, a more complex suite of Acn metabolites were found in urine after long term BJ ingestion. This suggests that a higher concentration of Acn metabolites may be in circulation during long-term biomedical studies, prompting a re-evaluation of the role of Acn in human health.

## *Key Words.*

anthocyanin; bioavailability; long-term; urine; LC-MS

## *References.*

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**Maurizio Battino Ph. D.**

**Universita Politecnica delle Marche, Italy**

***Strawberry and human health: effects beyond antioxidant activity***

Maurizio Battino, PhD, Associate Professor of Biochemistry in the Dept of Clinical Sciences, Faculty of Medicine, Università Politecnica delle Marche (Italy) is the Director of Nutrition & Health projects and Master courses at FUNIBER platform (Barcelona-Spain). His research group, the Bioenergetics Group, investigates the way of mitigating disease processes through the correct use of specific foods (mainly berries and dietary fats) and of their bioactive compounds. Targeted diseases are those related directly with mitochondrial impairment (e.g., fibromyalgia) and/or inflammation processes and oxidative stress including metabolic syndrome, cancer, atherosclerosis and periodontal diseases.

Dr. Battino has more than 25 years of experience in bioenergetics and in food research with special emphasis on the role of natural antioxidants and his studies are documented in more than 140 peer-reviewed research articles with h-index = 32 according to Google Scholar MyCitations or h-index = 29 according to Scopus and ISI Web of Science; he has also co-edited several books and special issues.

He was BSc in Bologna, PhD in Catania and post-doc in Granada (Spain); he obtained a MS in International Communication Technology in Medicine (Ancona) and was awarded with a Doctor Honoris Causa degree by the University of Medicine and Pharmacy “Carol Davila” Bucharest (Romania). He currently reviews scientific articles for over three dozen peer-reviewed journals, serves as Editor-in-Chief of Journal of Berry Research (IOS Press) and in the editorial board of Mediterranean Journal of Nutrition & Metabolism, Plant Food for Human Nutrition (both from Springer), Nutrition and Aging (IOS Press), Antioxidants, Diseases (both from MDPI).

## Strawberry and human health: effects beyond antioxidant activity

•Maurizio Battino Università Politecnica delle Marche, Italy

**Background:** Many epidemiological studies show that fruit and vegetables consumption is often associated with a lower incidence of several chronic pathologies. Among edible fruits, berries of the family of Rosaceae (Rubus and Fragaria) represent healthy foods rich in antioxidant compounds, as reported from several systematic analyses of a large number of dietary plants. Strawberry contains many important dietary components including vitamins and minerals, and are a rich source of phytochemical compounds (Battino M. et al., 2009; Giampieri F. et al., 2012a). These substances possess strong anti-inflammatory, antioxidant, anticarcinogenic and photo-protective properties and are able to modulate enzymatic pathways; thus, they can prevent human diseases related to oxidative stress. In recent years, our research group studied the effective protection of strawberry extracts or strawberry consumption against oxidative damage on several models: (i) *in vitro* on human dermal fibroblasts, stressed through the exposure to UV-A radiation or chemical substances, (ii) *in vivo* on animals, stressed with ethanol administration, and (iii) *in vivo* in healthy humans (Tulipani S. et al., 2008, 2009, 2011; Alvarez-Suarez J. et al., 2011; Giampieri F. et al. 2012b). We found that strawberry bioactive compounds were able to protect human fibroblasts, counteracting the intracellular ROS production and resulting effective in an increase of cell viability and in a reduction of oxidative damage on membrane lipid and DNA. With regard to the *in vivo* studies, rats fed with strawberry crude extract for 10 days showed an increase in the antioxidant enzyme activities (SOD and catalase), a decrease in gastric lipid peroxidation and a concomitantly inhibition of the development of ethanol-induced gastric lesions. In human healthy volunteers, acute and medium-term strawberry intake led to significant increases in plasma total antioxidant capacity and in folate and in vitamin C serum concentrations as well as to significant improvements of erythrocyte and lymphocyte resistance to *ex vivo* induced oxidative damage.

**New findings:** recently, we carried out an *in vivo* feeding trial on young and old rats fed for two months with a diet supplemented with strawberry fruit (15%) and stressed with Doxorubicin, a well known model for inducing oxidative stress. We evaluated the potential changes in plasma, liver and cellular antioxidant status. We observed a reduction of the physiological oxidative damage in animals underwent Doxorubicin injection and fed with strawberry diet. Indeed, strawberry supplementation increased total antioxidant capacity and improved antioxidant status of plasma by raising GSH level, decreasing thiobarbituric acids, hydroperoxides and protein carbonyl contents and ameliorating the lipid profile. We observed the beneficial effect of strawberries also in liver, where, as for plasma, a decrease in ROS concentration and in thiobarbituric acids, hydroperoxides and in the protein carbonyl contents was outlined, as well as an increase in the GSH contents and in the antioxidant enzymes (GPx, GR, GST, SOD, Catalase). At cellular level, we found that strawberry diet results in a significantly decrease of intracellular ROS in lymphocytes and hepatocytes, especially in animals subjected to oxidative stress. Finally, strawberry fruit enriched-diet led to the reduction of oxidative stress also in liver mitochondria, counteracting the ROS increase and ameliorating their functionality.

**New perspective:** The results obtained by our groups during these years confirm the potential health benefit of strawberry fruit *in vitro* and *in vivo* against oxidative stress, in physiological conditions as aging or in presence of xenobiotic treatment characterized by a higher level of oxidative stress. More in-depth studies are needed to understand the genetic mechanisms by which the bioactive compounds present in strawberry can exert their protective capacity, focusing especially on the signal pathways of AMPK $\alpha$  and Nfr-2 activation, antioxidant enzymes and mitochondrial/nuclear sirtuins.

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# Berries & Cancer



**Gary Stoner, PhD**

**Session Chair  
Professor of Medicine  
Medical College of Wisconsin**

*Current Research Review*

Dr. Gary Stoner is Professor of Medicine at the Medical College of Wisconsin (MCW) Division of Hematology and Oncology, specializing in the fields of chemical carcinogenesis and cancer chemoprevention. He serves as Director of the Molecular Carcinogenesis and Chemoprevention Program in the newly developing Cancer Center.

Dr. Stoner received his PhD in microbiology from the University of Michigan in 1970 and became involved in cancer research as a post-doctoral fellow and research scientist at the University of California-San Diego (UCSD). While at UCSD, his research was focused on the development of a mouse model of lung cancer for the identification of environmental carcinogens and for mechanistic studies of lung carcinogenesis. He then joined the Laboratory of Human Carcinogenesis at the National Cancer Institute where he conducted research on the metabolism of tobacco carcinogens in human lung tissues and developed human lung cell culture systems for investigations of carcinogen/oncogene-induced cell transformation. He became involved in chemoprevention research in the early 1980's while at the Medical College of Ohio, initially investigating the chemopreventive potential of naturally-occurring ellagitannins and isothiocyanates in the rodent lung and esophagus. As an extension of research with ellagic acid, Dr. Stoner's laboratory developed a "food-based" approach to the prevention of esophagus and colon cancers in rodents and in humans using freeze-dried black raspberries. His research is documented in more than 350 peer-reviewed publications and book chapters, and he has edited several books.

Dr. Stoner joined MCW after nearly 20 years at the Ohio State University College of Medicine where he held the positions of Lucius Wing Endowed Chair in Cancer Research and Therapy, Associate Director for Basic Research and Director of the Chemoprevention Program in the Cancer Center, and Chair of the Division of Environmental Health Sciences and Associate Dean for Research in the College of Public Health. In addition, he served as Director of the Laboratory of Cancer Etiology and Chemoprevention in the Arthur James Cancer Hospital and Richard Solove Research Institute.

Dr. Stoner has served on several grant and contract review committees including the NIH Chemical Pathology Study Section, the NCI Cancer Biology and Immunology Contract Review Committee, and as Chair of the NIH Chemo/Dietary Prevention Study Section and the American Cancer Society Advisory Committee on Carcinogenesis, Environment and Nutrition. He has also served as President of the Carcinogenesis and Molecular Biology Specialty Sections of the American Society of Toxicology and of the Ohio Valley Society of Toxicology. He has received numerous awards including the NIH MERIT award, and the Distinguished Alumni Award and Honorary Doctorate from Montana State University. He is also a Fellow in the American Association for the Advancement of Science.

## Berries, anthocyanins and protocatechuic acid for prevention of esophageal cancer

<sup>1</sup>Gary D. Stoner, <sup>1</sup>Li-Shu Wang, <sup>1</sup>Dan Peiffer, <sup>1</sup>Chieh-Ti Kuo, <sup>2</sup>Yi-Wen Huang, <sup>3</sup>Ben Ransom, <sup>3</sup>Steven Carmella and <sup>3</sup>Stephen S. Hecht

<sup>1</sup>Department of Medicine, Division of Hematology and Oncology, and <sup>2</sup>Department of Obstetrics and Gynecology, Medical College of Wisconsin, Milwaukee, WI, USA and <sup>3</sup>University of Minnesota Cancer Center, Minneapolis, MN, USA

Our laboratories have been evaluating the ability of freeze-dried berries to prevent gastrointestinal tract cancers in animals and in humans. Most studies have used black raspberries (BRBs), due to their high antioxidant potential and their high content of anthocyanins and fiber. In rodent studies, the consumption of BRB powder, at concentrations of 2.5, 5 and 10% (w/w) of a synthetic diet, results in a 40-70% inhibition of carcinogen-induced cancer in the rat esophagus and colon (1, 2). BRBs also inhibit the spontaneous development of intestinal tumors in Apc1638+/- and Muc2-/- mouse models of colorectal cancer, and AOM/DSS-induced ulcerative colitis and tumorigenesis in mice (3, 4). Mechanistically, BRBs exhibit a broad range of chemopreventive effects on a cellular level including inhibition of cell proliferation, inflammation, angiogenesis, and tissue invasion, and stimulation of apoptosis, cell-cell communication, cell adhesion, and differentiation (5, 6). They protectively modulate the expression levels of genes in multiple cell signaling pathways such as P13K/Akt, p38/Erk1/2, NFAT, mTor, NF-κB, COX-2, iNOS, and VEGF, as well as apoptosis and differentiation genes (5-7).

Bio-fractionation studies suggest that the most active chemopreventive constituents in BRBs are the anthocyanins (ACNs) and fiber (7, 8). Metabolic studies have shown that protocatechuic acid (PCA) is the major metabolite of ACNs produced by colonic microflora both *in vitro* (9) and in animals (10). Notably, the total amount of plasma and fecal PCA in humans who ingested ACN-containing juices accounted for more than 70% of the ingested ACNs (11). With this information, in the past two years, we have compared the ability of whole BRB powder, an anthocyanin-enriched fraction of BRBs, and of PCA, to inhibit chemically-induced cancer in the rat esophagus. Our results revealed that all three BRB constituents were effective in reducing the number of chemically-induced tumors in the esophagus and their inhibitory effects were not significantly different. In addition, all three BRB constituents down-regulated the mRNA and protein expression levels of COX-2, iNOS, p65 NF-κB, and soluble epoxide hydrolase, indicating their ability to protectively modulate genes associated with inflammation and proliferation. In addition, all three constituents were about equally active in up-regulating the protein expression levels of PTX3, a tumor suppressor gene that is down-regulated in most human esophageal squamous cell carcinomas. These results suggest that the anthocyanins in BRBs and their major metabolite, PCA, may account for much of the chemopreventive effect of whole BRBs in the rat esophagus.

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**Laura Kresty Ph. D.**

**Associate Professor of Medicine –  
Medical College of Wisconsin**

***Evaluating cranberry constituents as inhibitors of esophageal adenocarcinoma utilizing in vitro assays and in vivo models***

Laura Kresty, Ph.D., M.S. is an Associate Professor of Medicine at the Medical College of Wisconsin Division of Hematology and Oncology, specializing in Cancer Prevention. Dr. Kresty received her PhD in Public Health from The Ohio State University in 2000 with a major in Cancer Chemoprevention and Epidemiology and minor in Health Promotion and Disease Prevention. Dr. Kresty remained at The Ohio State University to complete a NCI-Sponsored Post-Doctoral Fellowship in Molecular Oncology, followed by a faculty appointment in Internal Medicine. In 2008 Dr. Kresty joined the University of Miami Miller School of Medicine and Sylvester Cancer where she continued her research focused on the inhibition of esophageal adenocarcinoma and head and neck cancers. Dr. Kresty also served as Director for the Doctorate in Epidemiology Program and was a Cancer Biology Steering Committee Member at the University of Miami. Dr. Kresty joined the Medical College of Wisconsin in January 2013 where she will continue research focused on the molecular biology, etiology, and inhibition of cancers of the esophagus and head and neck. Dr. Kresty serves as a peer reviewer for multiple journals in her field, has over 40 peer-reviewed research articles and book chapters, has delivered more than 35 invited talks throughout the world and frequently serves on NIH/NCI review and special emphasis panels. Her ongoing research focus is on evaluating the cancer inhibitory potential of cranberry constituents, a novel HDAC inhibitor, vitamin D and investigating energy excess as it relates to esophageal adenocarcinoma risk. Her laboratory is also collaborating on investigations focused on novel imaging technologies to detect early epithelial and sub-epithelial esophageal changes for more rapid evaluation of chemopreventive agents.



## **Abstract - Evaluating cranberry constituents as inhibitors of esophageal adenocarcinoma utilizing *in vitro* assays and *in vivo* models**

**Dr. Laura Kresty**

**Associate Professor of Medicine – Medical College of Wisconsin**

Esophageal adenocarcinoma (EAC) rates have increased 500% over the last three decades resulting in EAC being identified as the fastest increasing of all cancer types in the US. Esophageal cancer is an extremely deadly malignancy with 5-year survival rates consistently under 20%. The precise reasons for increasing rates of EAC are an active area of investigation. Persistent, symptomatic, reflux of gastric and duodenal contents, known as gastroesophageal disease (GERD), strongly correlate with EAC development and it's only known precursor lesion Barrett's esophagus via stimulation of cellular proliferation and apoptosis resistance. Other EAC risk factors include obesity, animal-based diets and to a lesser extent tobacco and alcohol use. Plant-based diets have generally been associated with a reduction of risk for EAC. **Thus, the long-term goal of this research is to develop efficacious strategies for the prevention of esophageal adenocarcinoma using a standardized proanthocyanidin rich cranberry extract (C-PAC)**

We are investigating the mechanisms by which cranberry constituent's induce cancer cell death by first, utilizing a diverse panel of human esophageal cell lines that differ based on pathology, tumor suppressor gene status and acid sensitivity and second, employing various animal models to evaluate the *in vivo* efficacy of C-PAC as an inhibitor of EAC. To date, results show that C-PAC decreases EAC cell viability by inducing cell death via apoptosis, autophagy and necrosis when apoptotic and autophagy machinery is defective. C-PAC treatment also induces an S-phase delay, causes G2-M cell cycle arrest, alters global gene expression profiling as well as miRNAs and significantly inhibits the growth of OE19 EAC xenografts in nude mice.

Despite promising results supporting the chemopreventive application of C-PAC, substantial knowledge gaps remain with regard to the mechanisms of PAC-induced cell death and *in vivo* efficacy. Further studies are ongoing to inform the signaling mechanisms involved in C-PACs cell death-inducing capacity and to determine the *in vivo* efficacy of PAC for the prevention of esophageal adenocarcinoma utilizing a clinically relevant reflux-induced rodent model of EAC. Positive outcomes of the proposed research may lead to improved dietary recommendations and will lay the foundation to rapidly translate these preclinical findings to clinical interventions in cohorts at increased risk for EAC, such as Barrett's esophagus patients.



**Christine Sardo Ph. D.**

**University of Arizona**

***The Role of Black Raspberries and Fruit Phenolics on Inflammation And Colorectal Neoplasia***

Christine's research has focused on the effects of fruit phenolics, physical activity, and inflammation on colorectal neoplasia. Related to this research, she has also studied the post-prandial effects of black raspberries on post-prandial inflammation in older overweight and obese men.

Christine is a Teaching Assistant at the University of Arizona Mel and Enid Zuckerman College of Public Health and a Clinical Nutritionist at Canyon Ranch Health Resort and Spa in Tucson, Arizona.

Prior to her current positions, Christine was the Partnerships and Policies Director at Canyon Ranch Institute, in addition to holding positions as the program manager for Canyon Ranch Institute's partnerships with the Lance Armstrong Foundation and the Cleveland Clinic. Canyon Ranch Institute is a 501(c)3 non-profit organization. Canyon Ranch Institute (CRI) catalyzes the possibility of optimal health for all people by translating the best practices of Canyon Ranch and CRI's partners to help educate, inspire, and empower every person to prevent disease and choose a life of wellness.

Prior to joining Canyon Ranch Institute in February 2009, Christine managed the Cancer Chemoprevention Clinical Trials with black raspberries at The Ohio State University Comprehensive Cancer Center in Columbus, Ohio. She has also served as a research and planning analyst for the Leo Burnett Company in Chicago and a senior pharmaceutical representative for SmithKline Beecham and Janssen Pharmaceutica (Johnson & Johnson) in Columbus, Ohio. Christine has also worked as a research assistant at the National Institutes of Health – National Heart, Lung and Blood Institute, in the laboratory of Dr. Theodor Kolobow, investigating novel lung ventilation devices. Collaborating with the public and her professional colleagues, Christine has developed educational seminars, articles, videos, and cooking demonstrations to improve health literacy about how we can all achieve optimal wellness. Christine also served as the nutrition and cancer prevention and survivorship expert for LIVESTRONG.com and writes a bimonthly article on cancer prevention and survivorship for The Wellness Community in Columbus, Ohio.

In addition, Christine has taught cancer prevention and survivorship seminars and led educational sessions in the United States and internationally. Among those numerous presentations, Christine is particularly pleased to have had the opportunity to present at The Ohio State University Lance Armstrong Center of Excellence; Dr. Andrew Weil's Nutrition and Health: State of the Science and Clinical Applications conference; Canyon Ranch; the American Dietetic Association's National Conference; and Peking University Health Sciences Center in Beijing, China. Christine has authored and co-authored several nutrition and chemoprevention articles for the public and for peer-reviewed publications, including the Journal of Clinical Pharmacology, Seminars in Cancer Biology, Nutrition and Cancer, and Cancer Epidemiology Biomarkers and Prevention. She was also a contributor to the National Call to Action (NCTA) on Cancer Prevention and Survivorship.

Christine earned a bachelor's of science degree in pre-medicine and nutrition from The Ohio State University, and a master's in public health degree from the University of Minnesota. She completed a National Institutes of Health Fellowship in Patient-Based Clinical Research and is a registered dietitian. She is also a board member of The Wellness Community, an active member of the American Dietetic Association, and past-president of the Columbus Dietetic Association.

**STUDY 1: Pharmacodynamics of polyphenols: a clinical trial investigating the inflammomodulatory effects of freeze-dried black raspberries fed to older overweight or obese males in the postprandial state**

**STUDY 2: The biologic effects of fruit phenolics on adenoma recurrence: a pooled analysis of two large phase III, double-blind, placebo controlled clinical trials**

**BACKGROUND**

Risk of colorectal cancer is significantly increased in obese individuals [1, 2] and overweight and obesity have been associated with increased risk for adenoma occurrence<sup>[3-5]</sup>. In addition, obesity leads to substantial metabolic dysregulation creating a physiologic environment characterized by chronic activation of inflammatory mediators<sup>[6]</sup> including pro-inflammatory cytokines such as interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor alpha (TNF- $\alpha$ )<sup>[7]</sup>. Importantly, this dysregulation is exacerbated by the post-prandial state<sup>[8]</sup>, suggesting that diet may have an important impact on colorectal neoplasia. One specific group of dietary components of interest is fruit polyphenols. Few epidemiological studies have specifically determined the role of fruit polyphenols (bioflavonoids, phenolic acids) on colorectal adenoma recurrence<sup>[9]</sup> although dietary-based fruit phenolic compounds have demonstrated effectiveness in numerous chemoprevention studies of colorectal and esophageal cancer<sup>[10]</sup>, possibly due to their role in attenuating inflammatory pathways<sup>[11]</sup>. Polyphenols are found at high concentrations in berries, as are carotenoids (B-carotene, zeaxanthin, lutein) and phytosterols (B-sitosterol, campesterol)<sup>[11]</sup>. A subclass of very active polyphenols, called anthocyanins, are responsible for the pigmentation seen in many dark purple and red-colored fruits and vegetables such as black raspberries, red cabbage, red and black grapes, and watermelon<sup>[12 13]</sup>. With several potential mechanisms of action for black raspberries now established<sup>[11]</sup>, two studies were conducted. The first study objective was to continue experimental research the effect of powdered black raspberries (BRB) on post-prandial measures of inflammation in overweight and obese male adults. The objective of the second study was to further elucidate the role of plant-based foodstuffs on the recurrence of colorectal adenomas. The possibility of effect modification by body size will be investigated, since several studies have indicated a significant direct association between body size and elevated risk of colorectal adenomas; specifically in men compared to women<sup>[14]</sup>.

The relationship between recurrence of colorectal adenomas and fruit phenolics, obesity, and inflammation were investigated by: (i) a pooled analysis of data from the Wheat Bran Fiber study (WBF)<sup>[15]</sup> and Ursodeoxycholic Acid Trial (UDCA)<sup>[16]</sup> and, (ii) a post-prandial pilot study to investigate the effects of black raspberries on the modulation of inflammatory markers in obese and overweight men.

An understanding of the interactions between these various parameters will help guide us toward the goal of colorectal cancer prevention in the general population or in high-risk subpopulations.

## **Study 1: Pharmacodynamics of polyphenols: a clinical trial investigating the inflammomodulatory effects of freeze-dried black raspberries fed to older overweight or obese males in the postprandial state**

### **ABSTRACT**

**Background:** Excess adiposity and the postprandial state are associated with inflammation. Polyphenols may attenuate this response.

**Objective:** To determine whether polyphenol-rich black raspberries (BRBs) modulate postprandial inflammation in ten overweight and obese males.

**Design:** Subjects consumed 45 g/day of BRBs x 4 days, followed by a high-fat-high-calorie (HFHC) breakfast plus BRBs on Day 6 or consumed the HFHC breakfast on Day 6 without prior consumption of BRBs, then crossed over to the other treatment after a two-day washout. Blood samples were obtained before and 1, 2, 4, 8, and 12 hours after the HFHC meal. The primary study outcomes were changes in areas under the curves (AUC) of interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor alpha (TNF- $\alpha$ ). The secondary outcome was tolerability of BRBs.

**Results:** The mean AUC of serum IL-6 was significantly lower ( $p=0.03$ ) with BRBs ( $34.3\pm 7.6$  pg/mL; mean $\pm$ SD), compared to HFHC meal alone ( $42.4\pm 17.9$  pg/mL). No statistically significant differences were observed in the mean AUC of serum TNF- $\alpha$  or CRP.

**Conclusions:** Polyphenol exposure via BRBs significantly decreased postprandial IL-6 in older overweight and obese men. Additional studies should be undertaken to evaluate the anti-inflammatory effects of BRBs.

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**Ramesh Gupta Ph. D.**

**University of Louisville**

*The emerging 'colored' compounds in blueberry for prevention and treatment of cancers*

Dr. Ramesh C. Gupta received PhD in Chemistry from the Roorkee University (now Indian Institute of Technology), India, and then moved to Baylor College of Medicine, Houston for postdoctoral training in 1973. He grew to Associate Professor at Baylor prior to moving to University of Kentucky in 1989 as Professor. In 2003, he was recruited by James Graham Brown Cancer Center, University of Louisville and was appointed as Professor, Distinguished University Scholar and Agnes Brown Duggan Chair in Oncological Research. He has always worked at the cutting edge technology pioneering sensitive methods to sequence tRNAs, followed by ultrasensitive  $^{32}\text{P}$ -postlabeling to measure DNA damage by environmental carcinogens. These works have received several thousand citations.

Last year, Dr. Gupta's laboratory reported the development of novel polymeric implants for continuously ("24/7") delivering natural compounds for long duration for prevention and treatment of cancer. This technology has been filed for patents by the University of Louisville - part of the patent issued in March 2012. His recent focus has been to identify natural compounds and extracts which attack multiple targets for preventing lung, breast and cervical cancers. His laboratory was the first to report the inhibition of breast cancer and lung cancer by blueberry 'colored' compounds, and cervical cancer by withaferin A isolated from the ancient Indian herb "ashwagandha". The blueberry compounds have also elicited enhanced response of chemotherapeutic drugs which led to a lung cancer clinical trial.

The laboratory's thrust is to develop simple and effective strategies for prevention and treatment of cancer recurrence and metastasis using blueberry bioactives and other natural compounds and novel drug delivery systems. He has been fortunate to have a qualified team of researchers, continuous funding from NIH and State grants, the Duggan endowment and James Graham Brown Cancer Center.

## Therapeutic Potential of Blueberry Anthos ('Color' Therapy)

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Early detection and discovery of new targeted drugs has resulted in increased survival for patients with several cancer types. However, no significant progress has been made for lung cancer and pancreatic cancer where the 5-year survival still hovers around 15% and 5%, respectively. Furthermore, there are essentially no cancer management strategies to delay or prevent the recurrence and metastasis of the disease. It is highly timely that unconventional and user-friendly alternate approaches be developed. What is needed is a multi-pronged approach in which non-toxic natural compounds attack distinct molecular targets associated with the tumor growth, and keep the disease progression halted, if not cure it. Colored pigments present particularly in berries are gaining high visibility for their chemopreventive and chemotherapeutic potential. Several papers published from this laboratory have shown significant chemopreventive and chemotherapeutic activities of colored pigments and other polyphenolics in blueberry and black raspberry<sup>1-5</sup>. Our more recent data reveal potent therapeutic activity of anthocyanidins (Anthos) from blueberry (Figure) against lung, breast, and pancreatic cancers in cell culture. In fact, the data revealed that blueberry Anthos act in concert to produce synergistic effects. This synergism presumably resulted from attack of the individual Anthos on distinct and overlapping molecular targets associated with cell proliferation, apoptosis, inflammation and invasion. Detection of all the five Anthos present in blueberry in the lung tissue of mice and rats dosed with either dietary blueberry or isolated Anthos by HPLC and LC-MS/MS combined with their chemopreventive/chemotherapeutic activities against breast cancer and lung cancer *in vivo* clearly indicate that these compounds elicit response beyond the GI tract. Furthermore, blueberry Anthos when combined with standard chemotherapeutic (chemo) drugs produced enhanced response of the drug against lung cancer cells both in cell culture and nude mouse xenograft model. The enhanced response of blueberry Anthos was also observed in conjunction with gemcitabine against pancreatic cancer cells, with paclitaxel and doxorubicin against both ER-positive and triple-negative breast cancer cells. The data discussed here suggest that blueberry Anthos can be developed as a potent therapeutic drug *per se* to kill a variety of human cancer cells, as well as enhance the therapeutic response of chemo drugs. (Work supported by USPHS grants CA118114 and CA-125152, Kentucky Lung Cancer Research Program, Agnes Brown Duggan Endowment, and Helmsley Trust Funds).

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# Berries & Brain Aging



**Barbara Shukitt-Hale Ph. D.  
Tufts University  
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**Session Chair**

***Berry Effects on Cognition and Motor  
Function in Aging***

Dr. Barbara Shukitt-Hale is a USDA Staff Scientist in the Laboratory of Neuroscience, USDA-ARS, Human Nutrition Research Center on Aging (HNRCA) at Tufts University in Boston, MA. Currently, she is the Acting Lead Scientist of the Neuroscience Lab. Additionally, she serves as an Affiliate Faculty member in the Psychology Department at Tufts University. She received her Ph.D. in Experimental Psychology from Boston University in 1993.

In 1996, Dr. Shukitt-Hale was awarded the Glenn Post-Doctoral Award, presented by the American Aging Association. She is a member of the Society for Neuroscience and has served as a board member and secretary of the American Aging Association. Dr. Shukitt-Hale has been involved in research for almost 30 years, beginning when she was an undergraduate student at Boston University; this work earned her the Research Award, given at graduation to the best student researcher in the Psychology Department. Before coming to the HNRCA, she worked as a Research Psychologist in the Division of Health and Performance and as a Neuroscientist in the Military Performance and Neuroscience Division at the U.S. Army Research Institute of Environmental Medicine (USARIEM).

Dr. Shukitt-Hale's current work involves researching the behavioral and neurochemical effects of aging in rodents, specifically investigating motor and cognitive performance changes due to oxidative stress, using the free-radical theory of aging as a working model. Her work includes determining the factors responsible for age-related behavioral changes and possible amelioration of these effects with various nutritional treatments. Her work showing that a diet supplemented with blueberry extract could reverse functional age-related deficits in motor and cognitive behavior has had a tremendous impact in the popular press. She continues to research the mechanisms behind the berry fruit's positive effects, and has found that they 1) have direct effects on signaling to enhance neuronal communication, 2) have the ability to buffer against excess calcium, 3) enhance neuroprotective stress shock proteins, and 4) reduce stress signals and increase neurogenesis. She has published more than 166 articles and selected papers.

## Berry Effects on Cognition and Motor Function in Aging

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In the last century, the lifespan of humans has almost doubled. Consequently, the percent of the population that is over the age of 65 years has markedly increased, making age-related pathologies a growing concern. Research has demonstrated, in both human and animals, that psychomotor and cognitive functioning decrease with age, even in the absence of neurodegenerative diseases such as Alzheimer's or Parkinson's Disease. The central and peripheral nervous systems are responsible for conscious control of movement, and alterations in motor function may include decreases in balance, muscle strength and coordination, while cognitive deficits are seen in processing speed, executive function, spatial learning, and memory. The cause of these functional declines is not entirely understood; however, neuronal losses and the associated changes in the activity of neurotransmitters, secondary messengers, and their receptors may be caused by long term increases in and susceptibility to oxidative stress and inflammation. One approach to improving neuronal functioning might be to alter the neuronal environment to reduce the impact of the oxidative and inflammatory stressors. Research conducted in our laboratory has shown that consumption of berry fruit, nuts, and other foods can improve cognition and mobility in aged rodents. The polyphenolic compounds found in these foods may exert their beneficial effects indirectly, through their ability to lower oxidative stress and inflammation, or directly, by altering neuronal structure and signaling involved in neuronal communication. Therefore, dietary interventions may be one strategy to forestall or even reverse age-related neuronal deficits. A recent study in our laboratory established a methodology for assessing the effects of dietary interventions on age-related declines in mobility and cognition among older adults. Importantly, this study used methodology which parallels behavioral tasks employed in our rodent model. Results indicate increases in postural sway and declines in gait speed, spatial navigation, and executive function with age. These tests are currently being used in a study investigating the effects of a dietary blueberry intervention on healthy older adults (60-75 years of age). Participants in this study consume freeze-dried blueberry powder (or a placebo powder) equivalent to 1 cup of blueberries each day for 3 months and tests of cognition and coordination are administered before, during, and after the supplementation period. We hypothesize that older adults whose diet is supplemented with berries will show improvements in motor and cognitive ability similar to those previously observed in our rodent model of aging.

Key words: Inflammation, aging, oxidative stress, cognition, mobility, berry fruits

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**Elizabeth Devore Ph. D.**

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***Long-Term Berry Intake and Cognitive Health  
In Older Women***

Elizabeth E. Devore, ScD, conducts epidemiologic research primarily on diet and lifestyle factors that are associated with cognitive aging. Her ultimate goal is to identify targeted strategies that can help alleviate the burden of cognitive impairment and dementia in older adults. The majority of her research is based in the large, prospective Nurses' Health Study cohort, although she collaborates with international cohorts as well. She currently holds positions as Associate Epidemiologist at Channing Division of Network Medicine, Brigham and Women's Hospital, and Instructor in Medicine, Harvard Medical School, in Boston. She completed her doctoral training at the Harvard School of Public Health in 2007, and her postdoctoral training jointly at Brigham and Women's Hospital and Erasmus Medical Center in Rotterdam, The Netherlands.

**Abstract**

Berries are high in flavonoids, especially anthocyanidins, and improve cognition in experimental studies<sup>1-4</sup>; therefore, we prospectively evaluated whether greater long-term intakes of berries and flavonoids are associated with slower rates of cognitive decline in older women. Beginning in 1980, a semi-quantitative food frequency questionnaire was administered every four years to Nurses' Health Study participants. In 1995-2001, we began measuring cognitive function in 16,010 participants, aged  $\geq 70$  years; follow-up assessments were conducted twice, at two-year intervals. To ascertain long-term diet, we averaged dietary variables from 1980 through the initial cognitive interview. Using multivariable-adjusted, mixed linear regression, we estimated mean differences in slopes of cognitive decline by long-term berry and flavonoid intakes. Our results indicated that greater intakes of blueberries and strawberries were associated with slower rates of cognitive decline (e.g., for a global score averaging all six cognitive tests, for blueberries: p-trend=0.014 and mean difference=0.04 [95% CI=0.01, 0.07] comparing extreme categories of intake; for strawberries: p-trend=0.022 and mean difference=0.03 [95% CI=0.00, 0.06] comparing extreme categories of intake), after adjusting for multiple potential confounders<sup>5</sup>. These effect estimates were equivalent to those we find for approximately 1.5 to 2.5 years of age in our cohort, indicating that berry intake appears to delay cognitive aging by up to 2.5 years. Additionally, in further supporting evidence, greater intakes of anthocyanidins and total flavonoids were associated with slower rates of cognitive decline (p-trends=0.015 and 0.053, respectively, for the global score). Thus, higher intake of flavonoids, particularly from berries, appears to reduce rates of cognitive decline in older adults.

**Key words:** Blueberries; strawberries; cognition; epidemiology

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**Robert Krikorian, Ph.D.**

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***Berry Fruit Intervention and Human Memory***

Dr. Krikorian is Associate Professor in the Department of Psychiatry at the University of Cincinnati Academic Health Center and Director of the Cognitive Disorders Center. His clinical and research interests include the influence of lifestyle factors - diet, exercise, and stress exposure – on age-related memory decline and risk for Alzheimer’s disease. His current research involves investigations of non-pharmaceutical interventions that may prevent or reverse age-related memory changes.

### **Abstract - Berry Fruit Intervention and Human Memory**

Alzheimer’s disease (AD) is a major public health concern that will cause increasing human and financial burden for the next several decades. Currently, there are 5.3 million cases of AD in the US with projections of as many as 16 million cases by the year 2050. Available and investigational treatments have provided marginal benefit, and it is not clear if or when effective therapy for AD might be available. However, early intervention before dementia develops is a promising, albeit under-developed approach. It is now recognized that there is a preclinical phase during which pathophysiological changes contributing to AD begin many years before dementia is apparent. From the point of view of prevention, this period is particularly important for identification of early cognitive decline and initiating interventions that might forestall or prevent progressive neurodegeneration.

Polyphenols found in berry fruits such as blueberries and grapes have been associated with several health benefits. Preclinical studies have implicated berry-derived flavonoids in moderation of oxidative stress and inflammation, increased neuronal signaling, and improved metabolic function among other effects. In addition, there are indications that flavonoid compounds may improve mitochondrial biogenesis. In animal studies, anthocyanins have been shown to cross the blood-brain barrier, to accumulate in a number of brain regions including those essential to cognitive function, and to enhance memory performance. Also, animal studies and human trials have demonstrated that flavanols improve cardiovascular function.

We will discuss the findings of recent clinical trials from our program that involve human berry fruit supplementation in the context of predementia conditions with age-related memory decline [1-3]. These studies, along with other human research, provide preliminary evidence of neurocognitive benefit. The initial human findings and the considerable basic science evidence comprise an emerging translational database demonstrating that moderate-term berry fruit supplementation can improve cognitive performance and enhance cerebral function. Clearly, these approaches have potential as preventive interventions with respect to cognitive decline with aging. Continued investigation of functional effects, intervention methodologies, and putative mechanisms will be essential to establish effective interventions.

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## **Berries & Metabolism**



**Ronald L. Prior, Ph.D.**

**Session Chair  
University of Arkansas**

***Current Research Review***

***Berry Source and Secondary  
Phenolic Acid Metabolites***

Dr. Prior received his Ph.D. in Nutrition with minors in biochemistry and physiology from Cornell University. His graduate training was followed by two years of post-doctoral training in Comparative Gastroenterology through the College of Veterinary Medicine at Cornell University. Dr. Prior was with the Agricultural Research Service of the USDA for 35 years. Following 13 years at the USDA Human Nutrition Research Center on Aging at Tufts, Dr. Prior moved in 2000 to the USDA Arkansas Children's Nutrition Center in Little Rock, AR where he provided leadership for their phytochemical and health research program. In May of 2010 Dr. Prior retired from the USDA, but he continues to serve as adjunct professor in the Dept of Food Science at the Univ. of Arkansas, Fayetteville and to consult with organizations on matters related to phytochemicals and nutrition. Dr. Prior has published more than 220 articles in peer reviewed scientific journals. Dr. Prior received the Alex Wetherbee Award from the North American Blueberry Council for his contributions to the blueberry industry. In 2006, was ranked as the top-cited author in agricultural sciences by Science Watch.

Dr. Prior has published more than 200 articles in peer reviewed scientific journals. Dr. Prior received the Alex Wetherbee Award from the North American Blueberry Council for his contributions to the blueberry industry resulting from research on the antioxidant components and health benefits of blueberries. Dr. Prior's research efforts have focused on assessing antioxidant capacity of fruits and vegetables and understanding the absorption and metabolism of antioxidant phytochemicals in fruits and vegetables. Dr. Prior's laboratory has provided the data on anthocyanins, proanthocyanidins and antioxidant capacity of fruits and vegetables for the flavonoid database in the USDA food nutrient database. Dr. Prior's recent research focus has been on effects of foods high in anthocyanins on the development of obesity and metabolic syndrome.

Dr. Mallery's research interests include evaluation of the contributions of oxidative and nitrosative stress in cancer initiation and promotion, study of AIDS-related Kaposi's sarcoma as a model of pathological angiogenesis, evaluation of local delivery formulations, and chemoprevention using both in vitro and in vivo models. Her current focus lies with the use of natural products, including black raspberries, as chemopreventive agents to prevent progression of premalignant oral epithelial lesions to oral cancer. She is also investigating use of locally injectable polymer vehicles as secondary chemopreventive agents to prevent local recurrence of excised head and neck cancer.

Ongoing berry-relevant laboratory studies in the Mallery lab include evaluation of the effects of bioactivating metabolic and phase II enzymes on the chemopreventive effects of black raspberry anthocyanins. Dr. Mallery also serves as the PI on a NCI funded multicenter, placebo-controlled clinical trial to evaluate the chemopreventive efficacy of a bioadhesive black raspberry gel on lesions of oral epithelial dysplasia.

## Berry Source and Secondary Phenolic Acid Metabolites

R.L. Prior, Ramesh Khanal and Luke Howard

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Plant polyphenols are known for their positive effects on human health. Considerable amounts of flavonoids, which are rather large molecules and present in higher amounts in many fruits and other foods of plant origin, are present in berries, including cranberry (CB), blueberry (BB), and black raspberry (BRB). Although absorption of many of the flavonoids may be limited, they also undergo transformation by intestinal microflora resulting in the production of phenolic acids (PA), which are much smaller and simpler in structure than flavonoids. The presence of large amounts of monophenolic acids has been demonstrated previously in the colon of healthy humans (1). While the absorption of flavonoids and other large molecular weight polyphenols may be poor, PA can be absorbed into the circulation and may contribute to the health-promoting effects of the diet. Moreover, PAs or their metabolic products may actually be the active compounds responsible for at least some of the health-promoting effects associated with their parent compounds (2-5). However, the extent and importance of absorption of many of these PAs is not known primarily because of the difficulty in assessing all the breakdown products of the parent polyphenols.

The urinary excretion of 18 phenolic acids and their conjugates were studied, using HPLC/MS/MS, in rats fed a control starch based diet, a control high fructose (HF) diet, or HF with 5% (dry weight basis) of cranberry (CB), blueberry (BB), or black raspberry (BRB) in the diet. Irrespective of the dietary treatments, hippuric acid (HA), 4-hydroxyphenyl acetic acid (4HPAA), 3-methoxy, 4-hydroxyphenylacetic acid, and 4-hydroxybenzoic acid (4HBA) were excreted in the greatest quantity in the urine over a 24 h period. The greatest increase in the excretion of PAs with berry feeding was observed with HA, 4-hydroxycinnamic acid, (4HCA) for CB; chlorogenic acid, 3,4-dihydroxycinnamic acid, and ferulic acid for BB; and 3-hydroxyphenyl propionic acid, 3-hydroxybenzoic acid and 3-hydroxycinnamic acid for BRB. Urinary excretion of a majority of the PAs analyzed in the current study was increased by the inclusion of berries in the diet, with a slight reduction in 4HPAA excretion. PAs were also detected in conjugated form with cinnamic acid derivatives being 50-70% conjugated and phenylacetic acid derivatives conjugated less than 10%. Conjugated PAs, not just the free PAs, need to be taken into account while determining their excretory pattern. Furthermore, studies on bioavailability and bioactivity effects of polyphenols need to consider more than just the parent compounds in the food to fully understand the potential mechanisms of the health benefits associated with polyphenols.

**KEYWORDS:** Cranberry, blueberry, black raspberry, polyphenols, procyanidins, anthocyanins, phenolic acids, urine

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**Dragan Milenkovic Ph. D.**

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***Bilberry anthocyanin inhibits atherosclerosis  
development by affecting expression of genes involved  
in early stages of disease development***

Dr Dragan Milenkovic received his PhD from University of Versailles (France) in molecular genetics. He is currently research scientist at the French National Institute for Agricultural Research (INRA) at Clermont-Ferrand. The aim of his research is identification of molecular and cellular mechanisms underlying cardiovascular protective effect of bioactive plant compounds, particularly polyphenols. This research is based on nutrigenomic analyses of polyphenols in animal models of atherosclerosis as well in clinical trials in humans. Recently he has been studying the role of polyphenol metabolites on endothelial cell function and underlying mechanisms of action, including the research on miRNA and cell signalling pathways.

### **Bilberry anthocyanin-rich extract exerts atheroprotective property through complex molecular mechanism of action**

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**Background:** Anthocyanins are water-soluble plant pigments that belong to the large group of polyphenols and more specifically to the subclass of flavonoids. They are abundant in the human diet due to their wide occurrence in fruits, such as berries, and fruit-based beverages. Bilberry is one of the richest sources of anthocyanins, with an anthocyanin glycoside content of 300–600 mg/100 g of fresh weight. Once ingested, anthocyanin glycosides are rapidly absorbed in both the stomach and small intestine and appear in blood and urine as intact, methylated, glucurono- and/or sulfoconjugated forms. Dietary intake of anthocyanin-rich foods has been associated with a reduced risk of coronary heart disease in the Iowa Women's Health Study, a prospective study of postmenopausal women (Mink et al., AJCN 2007.) Reduction of atherosclerotic lesions has been previously reported after supplementation of apolipoprotein E-deficient (apo E<sup>-/-</sup>) mice with anthocyanin-rich extracts from black rice and purple sweet potato (Miyazak et al., JAFC 2000). However, little is known about the molecular mechanisms underlying the cardiovascular protective effect of anthocyanins. In vitro experiments suggest that anthocyanins may affect the expression of genes in endothelial cells or macrophages, such as those encoding the cholesterol transporter ABCA-1, the pro-inflammatory enzyme COX-2 or the scavenger receptor CD36.

**Aim:** The aim of our studies was (1) to evaluate the effects of a bilberry anthocyanin-rich extract, when supplemented in the diet at a nutritional dose, on the development of atherosclerosis in apo E<sup>-/-</sup> mice and (2) to explore the *in vivo* mechanisms of action using a global transcriptomic approach.

**Methods:** Male apo E<sup>-/-</sup> mice at 8 weeks of age received a diet supplemented with 0.02% of anthocyanin-rich extract from bilberry. The atherosclerotic plaque was quantified in the aortic sinus by histomorphometry. Total cholesterol, triglyceride and anti-oxidant capacity were measured in plasma and liver. Impact of anthocyanins on the expression of genes has been performed using pangenomic microarrays for both liver and aorta.

**Results:** Bilberry extract is a purified anthocyanin-rich extract and the supplementation of the diet with 0.02% of this extract may correspond to an equivalent human intake of about 30 mg of anthocyanins per day; intake estimated to vary from 12.5 mg/day in the United States to 47 mg/day in Finland. After a 16-week supplementation period, a significant reduction of atherosclerotic plaques was observed as compared to the control one (-15 %). Consumption of the bilberry extracts did not modify the plasma antioxidant capacity, measured by the ORAC assay, nor the levels of markers of lipid oxidation (aortic F<sub>2</sub>-isoprostanes, hepatic TBARS). All these data support the hypothesis that the antiatherogenic effects of bilberry extracts are independent of their antioxidant capacity in this animal model.

Microarray analyses performed on the aortas of apoE<sup>-/-</sup> mice revealed that the bilberry extract-supplemented diet affected the expression of 1261 genes, with 554 genes up-regulated and 707 down-regulated. Bioinformatic analyses indicated that these differentially expressed genes are involved in the regulation of processes underlying atherosclerosis development, such as cell adhesion, migration, communication, inflammation, as well as angiogenesis and cell proliferation through vascular endothelial growth factor (VEGF) and WNT signalling pathways. The obtained gene expression profile could be related to increased inter-cellular adhesion, and decreased monocyte recruitment, cellular contractility and their regulating signalling pathways, thereby improving endothelial function and consequently lesser atherosclerosis development.

In the liver, the anthocyanin-rich extract affected the expression of 2,289 genes with 1,331 genes identified as up-regulated and 958 down-regulated. These genes are involved in various molecular and cellular pathways, such as cholesterol metabolism, VLDL removal, reverse cholesterol transport, but also inflammatory responses in the liver known to play a role in atherogenesis through the production of the pro-inflammatory cytokines. The expression profile of these genes after bilberry extract supplementation could explain the observed reduction of plasma cholesterol level via an increased elimination as bile acids, and the reduction of triglycerides in the liver via a decrease in hepatic lipogenesis. Furthermore this gene expression profile suggests a lower inflammatory status via a decrease in the expression of pro-inflammatory genes.

### **Conclusion:**

Supplementation of the diet with bilberry anthocyanin-rich extract led to a significant inhibition of plaque development in apolipoprotein E deficient mice without an effect on oxidative stress parameters, suggesting the implication of other mechanisms of action. The use of holistic transcriptomic approach provided new data and a global integrative view of molecular mechanisms involved in the preventive action of bilberry anthocyanin-rich extract against atherosclerosis. Globally, bilberry extract induced changes in gene expression to an anti-inflammatory profile, which could be related to its anti-atherogenic properties.



**Rosalia Simmen, Ph.D.**

**University of Arkansas**

***Berries and Early 'Nutritional' Experiences in the Prevention of Chronic Diseases***

Rosie Simmen is Professor, University of Arkansas for Medical Sciences and Senior Investigator in Developmental Biology, Arkansas Children's Nutrition Center (Little Rock, AR). She completed her doctoral studies in Biochemistry at the University of Hawaii under the mentorship of Drs. Fred and Gillian Greenwood and her postdoctoral training in Molecular and Cell Biology was obtained at Baylor College of Medicine with Dr. Anthony Means as mentor. Her research interests focus on women's health. As an independent investigator over the last 25 years, she has conducted studies on the biology of female reproductive tissues (uterus and mammary gland), with emphasis on mechanisms that regulate their normal biology and pathobiology. Currently, her laboratory studies how early 'nutritional' experiences influence the development of chronic diseases, specifically breast cancer, using rodent models of the disease. These investigations center on the effects of in utero, postnatal and early pubertal exposure to foods with recognized health benefits, including berries, and associated bioactive components on carcinogenesis and chemoprevention. Functional outcomes investigated include metabolic parameters, stem cells and their biology and transcriptional signatures.

Dr. Simmen is the author of more than 150 peer-reviewed publications and book chapters. She serves (has served) on the Editorial Boards of Endocrinology, Biology of Reproduction, Journal of Endocrinology and Journal of Molecular Endocrinology and on multiple NIH and USDA Study Sections. Funding for research in her laboratory comes from the USDA (through the Arkansas Children's Nutrition Center), NIH, and the Department of Defense Breast Cancer Research Program. She enjoys the opportunity to provide training and mentorship to undergraduate and pre-doctoral students, post-doctoral fellows, and junior faculty as well as productive research collaborations with many wonderful colleagues.

## Berries and Early ‘Nutritional’ Experiences in the Prevention of Chronic Diseases

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Poor maternal health as a causative factor for increased risk of chronic diseases in adult offspring has gained much ground since Professor David Barker initially advanced the concept of ‘fetal origin of adult diseases’ based on epidemiological data on coronary heart disease (1). This has led to the notion that the maternal womb is a viable target for disease prevention (2), especially in light of the dramatic rise in the worldwide prevalence of obesity, a condition associated with significant health complications including cancer, cardiovascular disease and type 2 diabetes. However, the mechanistic basis for the long-range effects of the maternal environment on the developing fetus, leading to disease susceptibility or resistance at adulthood, remains elusive. Breast cancer is the second-leading cause of cancer-related deaths and the most common malignancy among women worldwide (3). Studies have suggested that breast cancer may have early beginnings (4, 5). Given that the metabolic state dictates cancer susceptibility and diet is a modifiable risk for breast cancer, it follows that maternal diet can contribute to a metabolic state during early life that is permissive to disease susceptibility at later life. Berries are considered part of a healthy diet due to high polyphenol levels (6). Polyphenols have been demonstrated to oppose inflammatory events and attenuate oxidative stress that underlie metabolic dysfunctions and which are implicated in chronic diseases (7, 8). To explore the role of maternal diet in breast cancer outcome of adult progeny, we evaluated the effects of whole blueberry powder (BB) consumed by pregnant and lactating dams on mammary tumor incidence and progression in their female offspring. A mouse model of human breast cancer [MMTV-Wnt1-transgenic (Tg) mice] wherein 50% of females spontaneously develop mammary tumors at 5-6 months of age, was used. Female pups were exposed only to BB-fortified casein diet (3% by weight of feed) through their dams and were weaned to casein (control) diet thereafter; tumor parameters were followed until 8 months of age. BB-exposed offspring did not differ from those exposed to control diet in tumor incidence and latency (9). However, tumor weights and tumor volumes were significantly reduced in offspring of BB- versus control-fed dams. Tumors derived from BB-exposed offspring showed higher tumor suppressors PTEN and E-cadherin and lower pro-proliferative Cyclin D1, expression levels and reduced microvessel density, the latter indicative of attenuated angiogenesis. Isolated epithelial cells from pre-neoplastic mammary tissues of Tg offspring exposed to maternal control and BB diets were analyzed for percentage of basal (CD29<sup>high</sup>CD24<sup>+</sup>) and luminal (CD29<sup>low</sup>CD24<sup>+</sup>) populations. Maternal BB exposure (relative to control) resulted in lower percentage of the luminal subpopulation (6.4% vs 21%), which comprises the bulk of mammary tumors. Dams at weaning were evaluated for growth and metabolic parameters. BB-fed dams had lower abdominal and retroperitoneal fat pad weights than control-fed dams, although their body weights did not differ. BB dams also displayed increased insulin sensitivity (lower blood glucose and serum insulin) and higher serum glutathione and methionine levels. Relative to control offspring, BB-exposed offspring showed higher systemic anti-oxidative markers (cysteine, glutathione) and higher mammary PTEN and E-cadherin transcript levels prior to tumor formation and lower levels of serum insulin at sacrifice. Our findings indicate that early nutritional intervention with BB results in a favorable breast cancer outcome and suggest that this is a consequence of changes in metabolic parameters in the maternal environment that program a tumor-suppressive metabolic state and/or create a tumor-resistant mammary phenotype in progeny. Additional studies to address these potential mechanisms may have profound translational value for pregnant and would-be pregnant mothers and the well-being of the future generation.

**Key words:** berries, breast cancer, metabolic program, MMTV-Wnt1-transgenic; PTEN

**Funding:** United States Department of Agriculture (CRIS-6251-510002-06S; Arkansas Children’s Nutrition Center); Department of Defense Breast Cancer Research Program Pre-Doctoral Fellowship (W81XWH-10-1-0047); and University of Arkansas for Translational Research Institute/Arkansas Breast Cancer Research Program (1UL1RR029884).

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## Keynote Speaker



**Mary Ann Lila Ph. D.**

**David H. Murdock Distinguished Professor  
Director, North Carolina State University**

Dr. Mary Ann Lila, a David H. Murdock Distinguished Professor, is the Director of North Carolina State University's Plants for Human Health Institute on the North Carolina Research Campus in Kannapolis. Dr. Lila, who was named to direct the institute in 2008, is an internationally known scientist. Her research focuses on three areas: studying health-enhancing compounds in blueberries and other berries, isolating phytochemicals that counteract malaria, and working with scientists and students from around the world to explore natural products for biomedical use.

Working with the Global Institute for BioExploration, or GIBEX, a research and development network she helped start while she was on the University of Illinois at Urbana-Champaign faculty, Lila works with scientists, students and traditional healers in developing nations and with Native Americans to identify plants that hold promise for human health. Lila's work has taken her to Central and South Asia, New Zealand and Australia, Alaska and the Dakotas, Central and South America, and Africa. A professor in N.C. State's Department of Food, Bioprocessing and Nutrition Sciences, Lila is one of seven N.C. State faculty members now working in the institute, which is expected to grow to 15 faculty members.

### **Abstract**

The link between environmental or climatic stressors (elicitors) and deposition of health-protective secondary phytochemicals in plants is well established, and in the arctic tundra of Alaska, these stresses are taken to extreme limits. Surrounding many of the Alaska Native (AN) communities north of the Arctic Circle, wild berries are the only terrestrial wild edible plants which thrive in the harsh environment. Traditional AN diets have featured wild game, seafood, and a plethora of these wild berry species including salmonberries, mossberries, blue huckleberries and bog blueberries. In recent years, as native communities have shifted towards more Western diets and away from traditions, the incidence of diabetes and obesity has skyrocketed. Using a hands-on, field-deployable Screens-to-Nature (S2N) approach, we have partnered with AN elders and youth from three geographically distinct Alaskan villages to assess the health protective (and in particular, anti-diabetic) properties of local berries. Accumulation of bioactive flavonoid compounds (including proanthocyanidins and anthocyanin pigments) within a berry species varied according to geographic location. Berry extracts proved capable of inhibiting adipogenesis, and in particular, proanthocyanidin-rich fractions reduced lipid accumulation in 3T3-L1 adipocytes. The complex phytochemical composition of these berries was able to modulate specific cellular targets relating to metabolic syndrome and obesity. Research supported by EPA STAR Research Grant No. EPA RD-83370701.



## **Berries & Heart Health**



**Britt Burton Freeman, Ph. D.**

**Session Chair  
University of California, Davis**

### ***Current Research Review***

Dr. Freeman's current research interests are in mitigating disease process through dietary approaches focused on the health promoting properties of whole foods. Specific disease targets are vascular disease and obesity, including food intake regulation. In her current appointment, she leads a public health initiative with FDA/CFSAN to develop and provide underpinning science for comprehensive approaches using innovative processing solutions to support the availability of safe food with health opportunities.

Dr. Freeman is an active member of multiple professional societies dedicated to health and disease abatement including the American Society for Nutrition, the Obesity Society and the Society for the Study of Ingestive Behavior. Dr. Freeman earned a M.S. and Ph.D. in Nutrition Science with an emphasis in Endocrinology and Physiological Chemistry at the University of California, Davis.

## ***Strawberry polyphenols on risk factors for diabetes and cardiovascular disease: a look at the clinical data.***

Traditional risk factors of cardiovascular disease include smoking, high blood pressure, dyslipidemia, obesity, diabetes, poor diet, sedentary lifestyle, male, and older age (1, 2). In recent years, several emerging risk factors have been identified including markers of inflammation, oxidative stress, insulin resistance, endothelial dysfunction as well as lipoprotein size and density (2, 3). For many of the risk factors, behavioral changes can make a remarkable impact on overall risk. Among the most important changes are improved dietary patterns, with increased consumption of fruits and vegetables at the center of recommendations (4, 5). In addition to the essential micronutrients and fiber, fruits and vegetables also contribute intake of a variety of phenolic compounds (6). Plant foods differ in their (poly)phenolic composition and content and accordingly can exert different biological effects at different intake amounts. For years, (poly)phenols were known only for their anti-oxidant properties; however, now they are known to exert a number of other biological activities related to changes in cell signaling pathways, including paths leading to altered gene expression (6, 7).

Anthocyanins are polyphenolic flavonoid compounds commonly associated with berry intake (6). Strawberries contain appreciable amounts of the pelargonidin type anthocyanins and lesser amounts of cyanidin anthocyanins (6, 8). Strawberries have been studied in both chronic and acute feeding paradigms (7, 9). Study paradigms have generally “added” strawberries to subjects’ diets and included a control arm or meal to allow for assessment of strawberry (or the polyphenol component of strawberries) on disease endpoints of interest. Chronic feeding studies have ranged from ~3 weeks to 12 weeks in duration measuring changes in fasting clinical biomarker variables or after fat challenges (10). Acute studies on the other hand have been conducted to investigate bioavailability/metabolite patterns of target anthocyanins and other flavonoid components (6, 11, 12) and to understand strawberry effects in different physiological states, including fed conditions (13). Diets that characterize western eating patterns are comprised of meals that lead to fed-state (postprandial) “stress”, such as hyperglycemia, oxidative and inflammatory stress. This “stress” repeated daily over years is thought to play a causative role in the pathogenesis of metabolic regulatory dysfunctions resulting in metabolic syndrome and type-II diabetes; both of which are risk factors of life-threatening athero-thrombotic cardiovascular complications, such as a heart attack and stroke. Minimizing the imbalances that occur during the postprandial period may be an important function of strawberries and other berries in the diet, particularly in modern times. Health professionals often ask about how much to eat for maximum strawberry benefits; another important question may be when is the best time(s) to eat strawberries during the day. This presentation will discuss current work addressing the efficacy of strawberries in both acute and chronic settings including dose responses and suggest future areas of research relevant to berries in general.

Key Words: Cardiovascular, Inflammation, Insulin resistance, Pelargonidin, lipids, Postprandial

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Review



**Ana Rodriguez-Mateos Ph. D**

**University of Reading, UK**

***Effects of blueberry polyphenols on vascular function  
in healthy men***

Dr. Rodriguez-Mateos is a Junior Research Group Leader at the Division of Cardiology, Pulmunology and Vascular Medicine of the University of Dusseldorf, Germany. She received her PhD in Food Chemistry from the University of Reading, UK, in 2006 and then joined Professor Jeremy Spencer's group as a postdoctoral research fellow in the Hugh Sinclair Unit of Human Nutrition at the Department of Food and Nutritional Sciences of the University of Reading. She has considerable experience with the analysis of phytochemicals, in particular flavonoids, in human biofluids, and in conducting human intervention studies designed to understand the bioavailability and biological activity of dietary flavonoids. She is currently investigating the factors that affect the absorption, metabolism and efficacy of dietary polyphenols in the context of cardiovascular function, using human intervention trials and experimental models.

**Effects of blueberry polyphenols on vascular function in healthy men**

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Blueberries are a rich source of polyphenols, in particular anthocyanins, procyanidins and hydroxycinnamic acids (1). Although blueberry-enriched diets have been shown to improve vascular function in animal models (2-7), currently very limited data exists regarding the effects of blueberry consumption on human vascular function. The aim of this work was to investigate whether: 1) acute blueberry consumption improves endothelial function in healthy men; 2) blueberry polyphenols-mediated effects on endothelial function follow a dose-dependency; 3) plasma polyphenol metabolites are correlated with the effects on endothelial function; 4) food processing affects the blueberry polyphenol-mediated vascular effects. Three randomized, controlled, double blind, cross-over human intervention trials were conducted in 21 healthy male subjects where they consume a blueberry drink (319, 637, 766, 1466 and 1791 mg of total polyphenols (TP)), a blueberry baked product or macro- and micro-nutrient controls. Measurements were taken at baseline after overnight fast and at 1, 2, 4 and 6 h post consumption. The primary endpoint was flow-mediated dilation (FMD). Secondary endpoints were plasma polyphenol metabolites and other markers of vascular function.

Our results show that there were significant increases in FMD at 1,2 and 6 hour after ingestion of blueberry polyphenols taken as a blueberry drink using freeze-dried blueberry dissolved in water or as a blueberry-containing baked product. At 1 hour post-consumption, endothelial function increased in a linear fashion up to the 766 mg intake level, after which the vascular response plateau and decreased slightly at the higher intake levels. A significant increase in plasma levels of polyphenol metabolites was observed after blueberry consumption.

In conclusion, **blueberry polyphenol intake improves endothelial function in healthy men, and** the vascular improvements correlate in time with changes in plasma polyphenol metabolites. A linear dose-dependency was observed at the lower range tested but not at the highest concentrations. Food processing did not seem to affect the improvements in endothelial function exerted by blueberry consumption.

Key words: Blueberries, polyphenols, endothelial function, flow-mediated dilation, food processing

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**Howard D. Sesso, ScD, MPH,  
FAHA**

**Harvard University**

***Berry Intake and Cardiovascular Health Through  
Epidemiologic and Clinical Studies***

Dr. Howard D. Sesso is an Associate Epidemiologist at the Divisions of Preventive Medicine and Aging at Brigham and Women's Hospital (BWH), and an Associate Professor of Medicine at Harvard Medical School. He received his BA in Human Biology from Stanford University, an MPH in Epidemiology from The George Washington University, and a ScD in Epidemiology from the Harvard School of Public Health. Dr. Sesso specializes in the epidemiology and prevention of cardiovascular disease (CVD), focusing on the roles of hypertension, physical activity, obesity, and dietary factors such as antioxidant vitamins, lycopene, flavonoids, and alcohol, as well as the role of novel biomarkers that underlie these associations. He is also interested in the role of diet and lifestyle factors in the prevention of cancer. Dr. Sesso is Director of Nutrition Research and Co-Director of Hypertension Research at the Division of Preventive Medicine. Dr. Sesso is also interested in the design, methodology, and conduct of epidemiologic studies and randomized clinical trials. He leads the Physicians' Health Study II, a recently completed randomized trial that tested whether a multivitamin, vitamin E, and vitamin C have any effect on cardiovascular disease, cancer, and other chronic diseases in 14,641 men aged  $\geq 50$  years. Dr. Sesso is also currently testing the effects of vitamin D and fish oil supplements on ambulatory blood pressure and the risk of developing hypertension in an ancillary study from the large-scale VITamin D and OmegA-3 Trial (VITAL) trial.

**Berry Intake and Cardiovascular Health Through Epidemiologic and Clinical Studies**

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There are promising, albeit limited, epidemiologic data on the intake of berries and a lower risk of cardiovascular disease (CVD). The types of berries most frequently examined in relation to either coronary risk factors or the risk of developing CVD have been strawberries and blueberries. Based on limited longitudinal dietary data, berry intake has either remained steady or modestly increased over time. Limited studies have examined the determinants of berry intake, and have largely focused more broadly on fruit and vegetable intake. Perceived health benefits are an important motivator; less understood is the impact of pre-existing or newly developed medical conditions. Given the continued increases in the prevalence of obesity and its associated health effects, berries offer a viable option as part of a healthy dietary pattern to positively influence cardiovascular health.

Most of the research on berry intake and CVD has been confined to basic research studies, cross-sectional studies, and shorter-term intervention trials. These studies have provided a number of plausible cardiovascular mechanisms through which berry intake may lead to improvements in coronary risk factors, including insulin resistance, diabetes, hypertension, hypercholesterolemia, metabolic syndrome, and other intermediate vascular endpoints. Whether these promising short-term effects lead to long-term reductions in incident CVD remains to be seen. This is especially important given the increasing validity of findings from cross-sectional studies, case-control studies, prospective cohort studies, and clinical trials.

Large epidemiologic studies of berry intake and CVD have particular advantages, including excellent statistical power, dietary data typically collected long before the development of CVD, and the ability to generate hypotheses for targeted clinical trials. Disadvantages of large epidemiologic studies include the potential for measurement error, confounding by the "healthy user" effect of other associated factors on berries and CVD, and the lower levels of berry intake in the population at large versus doses tested in clinical trials. Food frequency questionnaires typically used in large cohort studies only ask about strawberry and blueberry intake, without differentiation between fresh and frozen berries. Yet this dietary assessment tool still adequately differentiates between high and low levels of berry intake.

Berries are an excellent food source of fiber, folate, vitamin C, potassium, flavonoids (including anthocyanins), and other key nutrients. Several plausible mechanisms have been identified through which these nutrients – apart from berry intake – may reduce CVD risk, including improvements in intermediate cardiovascular biomarkers and CVD endpoints, with direct clinical interpretability and utility. This raises the important question of whether the berry itself, or its particular nutrients, are responsible for any cardiovascular benefits. It is also important to understand how berries fit in the context of overall fruit and vegetable intake.

As for the epidemiologic evidence on berry intake and the risk of developing CVD, limited data are available from large cohort studies, including the Women's Health Study (WHS), Iowa Women's Health Study (IWHS), the Kuopio Ischemic Heart Disease Risk Factor Study. Results are preliminary and inconsistent, but still suggest modest cardiovascular benefits for higher levels of berry intake. In addition, the WHS reported a cross-sectional association between higher strawberry intake and a lower likelihood of having an elevated level of C-reactive protein. More recent data from large prospective cohort studies of berry intake in the WHS, Nurse's Health Study (NHS), and Health Professionals Follow-up Study (HPFS) report that blueberries may be more strongly and inversely associated with the risk of developing hypertension than strawberries. WHS, NHS, and HPFS data also indicate that strawberries and blueberries may be inversely associated with the risk of developing diabetes, while cross-sectional WHS data also suggest that strawberry intake is associated with a lower likelihood of having an elevated hemoglobin A<sub>1c</sub> level ( $\geq 6\%$ ).

Complementary epidemiologic studies and clinical trials must continue to identify the potential cardiovascular effects of berry intake, to select the most appropriate, generalizable interventions, to identify mechanistically relevant scientific aims, and to maximize statistical power for the detection of clinically meaningful results. Ultimately, any future for berry intake in the prevention of CVD will hinge upon complementary, and not competitive, nutrient- and food-based strategies. As additional well-conducted basic science, epidemiologic, and clinical studies continue to emerge, we hope to gather additional insights on the role of berries on CVD prevention.

**Key Words:** berries, strawberries, blueberries, cardiovascular disease, clinical trials, cohort studies, epidemiology, biomarkers, diet.

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# Berries and Gut Health/Gut Microflora



**Chair: Jess Reed Ph. D.  
University of Wisconsin-Madison**

*Current Research Review*

*Berry Polyphenols and Gut Health*

Dr. Jess Reed is Professor of Animal Nutrition at the University of Wisconsin-Madison. He received a PhD from Cornell in 1983. His 25 years of research has focused on the effects of phytochemicals in foods and forages on human and animal health and nutrition, including 6 years at the International Livestock Center for Africa where he studied the phytochemistry of tropical legume forages.

Starting in 1996, he began researching the effects of flavonoids in foods on human health, including cardiovascular disease, urinary tract infections and cancer. Reed has over 90 research publications in his field and a successful research program funded through competitive grants from NIH and USDA along with collaborative projects with the food and nutritional supplements industry. Dr. Reed also maintains an active outreach program in agricultural development with project experience in 20 countries.

## **Berry polyphenols and gut health: Overview of Gut Metabolism**

**Jess D. Reed, Professor, Dept. of Animal Sciences, University of Wisconsin-Madison, and Chief Scientific Officer, Complete Phytochemical Solutions, LLC**

Polyphenols are the most abundant class of phytochemicals in berries that are associated with health benefits. The diversity and abundance of polyphenols in fruits is indeed a “wonder of nature” and our understanding of their chemistry, metabolism, bioavailability and health benefits remains an enigma of modern nutritional science. The total number of different polyphenolic compounds in berries is probably around 4 to 5 thousand. This diversity makes research on structure/function relationships in regards to health benefits difficult. However, progress in understanding nutrition has depended on the characterization of nutrients and their role in metabolism and health. On the other hand, to ascribe structure/function relationships to each of the polyphenolic compounds that are present in berries is virtually impossible. Fortunately, as in all aspects of natural product chemistry, the berry polyphenols can be classified according to structural patterns that are characterized by modern methods of phytochemistry and therefore the metabolism of representative compounds may be useful in understanding health benefits. A word of caution though, “the devil is in the details” because small differences in structure within a class of polyphenolic compound may lead to large differences in bioavailability, metabolism and bioactivity.

The main classes of polyphenolic compounds in berries are: hydroxycinnamic acids, flavonoids and tannins. The hydroxycinnamic acids and flavonoids are monomeric structures whereas tannins are oligomers. Hydroxycinnamic acids consist of phenolic acids linked to non phenolic moieties such as other organic acids and sugars. For instance, chlorogenic acid, a common hydroxyphenolic acid found in strawberries, is the ester of caffeic acid and quinic acid.

Although there are 14 subclasses of flavonoids, the anthocyanins and flavonols are the most abundant flavonoids found in berries. These flavonoids are usually glycosylated. For instance cranberries contain 6 anthocyanins, cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, peonidin-3-galactoside, peonidin-3-glucoside and peonidin-3-arabinoside and more than 20 flavonol glycosides consisting of different sugar substitutions on three flavonol aglycones, kaempferol, quercetin and myricetin. The two main classes of tannins are hydrolysable tannins (gallotannins and ellagitannins) and proanthocyanidins (which are a class of oligomeric flavonoid). Each berry, and to some extent, each berry variety, has their own unique composition of these and other polyphenolic compounds that contribute to a wonderful diversity of colors, tastes and textures. The metabolism and bioavailability of these polyphenolic compounds in the gut is an important aspect of their putative health benefits.

All of the main classes of polyphenolic compounds that are present in berries undergo extensive metabolism in the gastrointestinal tract, through enterohepatic circulation and metabolism by the gut epithelium, liver and the gut microbiota in the distal small intestine and colon. This metabolism creates another level of diversity that influences structure/function activity because the polyphenolic metabolites may be the bioactive compound associated with a health benefit.

Hydroxycinnamic acids, flavonoids and hydrolysable tannins undergo extensive gut metabolism and have relatively high bioavailability in comparison to proanthocyanidins. In general, metabolism of hydroxycinnamic acids and monomeric flavonoids by enzymes of the gut epithelium and liver leads to cleavage of sugar and acid substitutions on the native plant compound followed by glucuronidation, sulfonation and methylation by phase II detoxification enzymes. Microbial metabolism of hydroxycinnamic acids and monomeric flavonoids in the gut often leads to highly modified structures. For instance, microbial metabolism of flavonoids leads to cleavage across the C ring. The carbons present in the A ring end up in volatile fatty acids whereas the B ring carbons remain in simple phenolic acids which are absorbed from the intestine and excreted in the urine.

The gut microbiota is capable of hydrolyzing the glucose esters in hydrolysable tannins to glucose and gallate in the case of gallotannins and to hexahydroxydiphenate (HHDP) and glucose in the case of ellagitannins. Gallate is further metabolized to pyrogallol and other simple phenolic compounds. The gut microbiota metabolize HHDP to a group of phenolic compounds referred to as urolithins.

In contrast to the other classes of berry polyphenolic compounds, the bioavailability of proanthocyanidins is low. The monomeric units of the proanthocyanidins are flavan-3-ols of which the catechins (catechin/epicatechin, gallic catechin/epigallocatechin, and epigallocatechin gallate/gallic catechin gallate) are the most common. These monomeric flavan-3-ols are present in foods and beverages such as green tea and chocolate and have high bioavailability. Catechins are extensively metabolized in a similar way to other monomeric flavonoids. However, the intermolecular bond between the monomeric flavan-3-ols in proanthocyanidins is not easily susceptible to enzymatic cleavage and is not susceptible to hydrolysis. Therefore, proanthocyanidins have low degradation in the intestines. Their absorption is also low because they form strong complexes with proteins and polysaccharides. Published research suggests that there is low absorption of dimers and perhaps trimers but there is no evidence for absorption of higher oligomer. There is also some suggestion that the interflavan bond may be cleaved in the gut through the action of stomach acids or through enzymatic cleavage by the gut microbiota. However, even if these two processes occur, the preponderance of research suggests that greater than 95% of dietary proanthocyanidins are excreted in the feces in complexes with proteins and polysaccharides. The low bioavailability of proanthocyanidins does not preclude them from having health benefits that are related to their effects in the lumen of the gut and on the gut mucosa. Recent research in our laboratory suggests that proanthocyanidins exert significant effects on the gut mucosal immune system and the interaction between the gut microbiota and mucosal immunity.



**Dhanansayan Shanmuganayagam, Ph.D.**

**University of Wisconsin**

***Polyphenolics and the gastrointestinal immune system***

Dr. Shanmuganayagam is currently the Director of Research of the Reed Research Group at the University of Wisconsin – Madison. He has a diverse research background that spans the fields of cardiovascular disease, aging, immune dysfunction and nutrition. His research has included the exploration of how polyphenolic compounds in fruits regulate various physiological processes that impact the development of diseases in animals and humans.

**Polyphenolics and the Gastrointestinal Immune System**

Dr. Dhanansayan (Dhanu) Shanmuganayagam, Director of Research, Reed Research Group, University of Wisconsin – Madison.

**Pre-Proceeding Abstract**

A significant portion of the body's immune system is concentrated in the gastrointestinal (GI) tract,<sup>1</sup> and thus the modulation of the GI immune system by dietary and environmental factors can influence systemic processes. The innate and acquired immune components of the GI immune system is highly regulated to preserve the integrity of the mucosal barrier that is critical for maintaining the physical and chemical barrier against food and environmental antigens, including microbes.

Enteral nutrition or enteral formulas (e.g., elemental enteral nutrition (EEN) solutions) are widely used to treat gastrointestinal disorders (e.g., Crohn's disease), inflammatory bowel disease and to maintain appropriate nutrition in the patients with acute (e.g., trauma, burns) and chronic illnesses.<sup>2</sup> However, the absence of normal solid food consumption suppresses the intestinal immune system and dramatically increases the risk of infections, related complications and mortality.<sup>3-6</sup> For example, risk of complications (e.g., pneumonia and abscess) have been shown to increase 20-40% in hospitalized patients when they are not on oral solid food consumption, resulting in tremendous emotional, medical and economic burden.

In a series of studies in a mouse model of EEN-induced mucosal immune dysfunction, we explored whether the addition of cranberry proanthocyanidins (PACs), which are unabsorbed and have complex interactions with biological components, to EEN solution would stimulate the mucosal immune system and preserve the integrity of the mucosal barrier.

The addition of PACs to EEN solution significantly protected against the impairment of intestinal barrier function following EEN by stimulating lamina propria Th2 cytokines, interleukin (IL)-4 and IL-13 that in turn induced goblet cell (GC) proliferation and mucin-2 (MUC2) production.<sup>7</sup> The PACs stimulated Th2 cytokines without any significant effect on Th1 cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$ . Furthermore, the addition of PACs to EEN solution also preserved gut-associated lymphoid tissue (GALT) function by maintaining the Peyer Patch lymphocyte population and the secretion of luminal secretory immunoglobulin A (sIgA).<sup>8</sup> We believe that the preservation of luminal sIgA was in part due to the observed preservation of the expression of polymeric immunoglobulin receptor (pIgR) on the GI epithelium that is required for the transport of sIgA into the lumen. Our studies suggest that the preservation of ileal IL-4 by PACs is involved in the maintained modulation of pIgR expression. This notion is supported by the observation that phosphorylation of nuclear factor STAT-6 (a member of the JAK/STAT signaling) at phosphorylation sites Tyrosine 641 (Tyr641) and Threonine 645 (Thr645), a process which modulates pIgR expression, is preserved by the addition of PACs to EEN.

In conclusion, our work suggests that the absence of normal solid food consumption, for example during the administration of EEN solutions, suppresses the intestinal immune system, and that the addition of cranberry PACs to such diets counteracts the observed dysfunction. We believe that a formulation of EEN solutions that includes cranberry PACs has great potential for use as prevention or therapy to preserve or improve intestinal immune function in hospital and home-care settings.

**Keywords:** enteral nutrition; cranberry; proanthocyanidins; gut-associated lymphoid tissue; goblet cells; mucin; cytokines, secretory IgA; JAK-STAT; polymeric immunoglobulin receptor

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**Li Shu Wang, Ph.D.**

**Medical college of Wisconsin**  
***Alternations in lipid metabolism by black raspberry***  
***intervention in colorectal cancer patients***

Dr. Wang is Assistant Professor at Department of Medicine, Medical College of Wisconsin. She received her Ph.D. in Veterinary Biosciences in the College of Veterinary Medicine at Ohio State in June, 2006, and immediately joined Dr. Gary Stoner's laboratory as a Post-doctoral researcher. She has experience in evaluating the effects of chemopreventive agents, including black raspberries, on gene expression in vitro (in mammary and colon cell culture systems) and in vivo (in the rat esophagus and in human colon). Using bio-directed fractionation, she showed that the anthocyanins in black raspberries are important for their chemopreventive effects and, recently, she provided evidence that the ellagitannins may be less important. Using DNA microarray, Dr. Wang investigated the effects of a black raspberry diet on gene expression during the early and late stages of rat esophageal carcinogenesis and has shown that the berries exhibit a genome-wide effect on the expression of genes associated with multiple cellular processes including proliferation, apoptosis, inflammation, angiogenesis, cell cycling and cell adhesion, carbohydrate metabolism and cell differentiation. Recently, she has evidence that berries cause demethylation of tumor suppressor genes in rodent and human colon leading to their enhanced expression. Currently, she has 37 peer-reviewed publications.

**Title: Metabolomic profiling reveals a protective modulation on fatty acid metabolism in colorectal cancer patients following consumption of freeze-dried black raspberries**

**Authors: Li-Shu Wang, Matthew Young, Chieh-Ti Kuo, Christine Sardo, Mark Arnold, Edward Martin, Gary Stoner**

Abstract:

Metabolic reprogramming which refers to altered nutrient uptake and use is thought to be essential for rapid cancer cell proliferation. Accelerated phospholipid biosynthesis is another metabolic signature of cancer because proliferating cells have a significant need for membrane production. Fatty acids that compose the hydrophobic tails of membrane phospholipids can regulate gene transcription through enzyme-mediated pathways, e.g., cyclooxygenase, lipoxygenase, and changing lipid raft composition that affect receptor-mediated signalings. We previously showed that dietary intervention with freeze-dried black raspberries (BRBs) decreased cell proliferation in colorectal tumors and IL-8, a pro-inflammatory cytokine, in plasma in colorectal cancer patients.

The goal of the current study was to determine if BRBs affect fatty acid metabolism which may contribute to their anti-proliferative and anti-inflammatory activities. Plasma samples were collected from 28 colorectal cancer patients before and after oral consumption of BRB powder (60g/day) for 1-to-9 wks for metabolomic profiling analysis. 421 biochemicals were analyzed using UHPLC/Gas chromatography and mass spectrometry. When data from all 28 patients were combined, the top 30 ranking biochemicals suggest that berry intervention led to alterations mostly in lipids, following by carbohydrates, amino acids, and cofactors and vitamins. Both monounsaturated fatty acids (MUFAs), e.g., eicosenoate (20:1n9 or 11), and polyunsaturated fatty acids (PUFAs), e.g., linoleate (18:2n6), arachidonate (20:4n6), were lower in the post plasma samples. PUFAs can be synthesized from linoleate and they also can be released by phospholipase A from phospholipid membrane. Therefore, berry intervention might alter activities of phospholipase A, elongases, and desaturases which in turn reduce levels of PUFAs. Alternatively, berry intervention increased secondary bile acids, e.g., glycodeoxycholate, produced by the action of enzymes existing in the microbial flora of the colonic environment, suggesting dietary BRBs could alter colonic microflora. Berry intervention associated alternations in bile acid metabolism could affect fat absorption and subsequently impact fatty acid metabolism. In conclusion, our results suggest that dietary berry consumption protectively modulates enzymes associated with fatty acid metabolism in the host as well as in gut microflora leading to decreased proliferation and inflammation in colorectal cancer patients. Supported by R01 CA148818 to L-S Wang

**Key words:** Black raspberries, Colorectal cancer, Metabolomic profiling, Gut microflora.

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# Poster Presentation Abstracts



# **Development of a functional confection for cancer prevention: A phase I evaluation of strawberry confections on urinary urolithin profiles in smokers and non-smokers**

Jennifer H. Ahn-Jarvis, Steven K. Clinton, Erica L. Fisher, Kenneth M. Riedl, Steven J. Schwartz, Matthew D. Teegarden, Christopher M. Weghorst, and Yael Vodovotz

**Background:** Strawberries (*Fragaria x ananassa*) are rich in phytonutrients having demonstrated anti-proliferative, anti-inflammatory, and apoptotic activity in mechanistic cell and rodent studies. Although limited, dietary intervention trials have shown that compounds in strawberries when delivered locally have great potential as a chemoprevention strategy for oral or esophageal cancer. The predominant compounds typically ingested from strawberries are anthocyanins, ellagitannins, and flavonols. However, the compounds that appear in the blood or are excreted in urine are very different from those ingested from the strawberry fruit and very difficult to quantify. These series of studies are important first steps in identifying specific biomarkers of strawberry consumption that will eventually be used in future large-scale trials investigating the role of berry compounds on cancer prevention. Using a crop's to clinic approach, a standardized mix of strawberry cultivars were used to develop a functional confection designed to evaluate ellagitannin metabolism and assess the safety and toxicity for future large-scale phase II clinical trials. We hypothesize that the strawberry functional confection compared to a placebo will serve as an excellent source of strawberry ellagitannins for cancer prevention trials.

**Objective:** A strawberry confection with freeze-dried strawberry powder was used in a 6 week phase I randomized placebo-controlled, crossover trial involving healthy smokers (n=12) and non-smokers (n=13). The following objectives were addressed:

1. Develop and select a palatable functional confection using freeze-dried strawberry powder.
2. Compare the compliance, safety, and toxicity of strawberry functional confections over 6 weeks between smokers and non-smokers.

**Methods:** Three strawberry varieties (Albion, Wel-Pict, and Driscoll proprietary variety) were provided by the California Strawberry Commission (Watsonville, CA). Fully-ripened strawberries were harvested late spring/early summer and freeze-dried at Van Drunen Farms (Mokena, IL). A single lot (20 lb) of freeze-dried strawberry powder (LSP) was used for production of the functional confection. Sensory evaluation and textural parameters were used to characterize and select a palatable functional confection with and without strawberry powder (placebo) for the clinical trial. HPLC was used to identify and quantify compounds in LSP and functional confections. Ellagitannin metabolites (urolithin A1, A2, B, and C) from 24 hour urine collections were profiled and quantified using HPLC with mass spectroscopy.

**Results:** A highly palatable functional confection with 3g of LSP and strawberry placebo confection was developed and selected for use in clinical trials. Compliance to functional confection intervention (strawberry 96 ± 16% and placebo 94 ± 17%), low ellagitannin diet (92.4 ± 11%), and other study related activities were excellent. Moreover, no signs of toxicity to the placebo or LSP functional confections were observed. Significant increase in urolithin A2 was observed after strawberry confection intervention (24 g LSP/day) compared to washout following a low ellagitannin diet and placebo intervention (p=0.005). Urolithin A2 was found in the urine of 92% (23/25) of our cohort and excretion from non-smokers (mean ± SE, 5.76 ± 1.77 μM) differed greatly than smokers (mean ± SE, 2.63 ± 0.90 μM). In our cohort, urolithin B was found in 12% (3/25) and urolithin C in 84% (21/25) of the subjects.

**Conclusion:** A palatable functional confection containing substantial amounts of strawberry powder was developed and demonstrated excellent compliance with no signs of toxicity (NIH criteria). Ellagitannin metabolites specifically urolithin A2 and C may be used as urinary biomarkers of strawberry intake. Differences in ellagitannin metabolite excretion between smokers and non-smokers warrant further studies.

# Use of Wild Genotypes in Breeding Program Increase Strawberry Fruit Sensorial and Nutritional Quality.

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Keywords: breeding, antioxidant capacity, anthocyanins, polyphenols, *Fragaria virginiana* subsp. *glauca*

## Abstract.

**Introduction:** Increasing the bioactive compounds in fruit through breeding and biotechnology is an important option to support a higher antioxidant intake even when the consumption of fruit is low. If nutritional components are combined with a high standard of sensorial fruit quality, consumer health can be further improved by encouraging more fruit consumption.

Wild species are valued by strawberry breeders as sources of novel traits, especially pest resistance and abiotic stress tolerance. Previous investigations have shown that in breeding material originating from *Fragaria virginiana* subsp. *glauca* (FVG) inter-specific crosses improves fruit nutritional quality. Recent *F. x ananassa* commercial varieties also express an interesting variability of fruit nutritional quality.

Strawberry fruit sensorial and nutritional quality generated by *Fragaria* inter and intra-specific crosses was evaluated on 78 offspring derived from 8 families: two originating from *F. x ananassa* intra specific crossing; three from back crossing of F1 – FVG x *F. x ananassa*; three originating from back crossing of F2 – FVG x *F. x ananassa*.

The genetic variability of three types of cross combinations (F2 backcross, F3 backcross and intra-specific cross) was analysed by calculating the correlations among fruit sensorial and nutritional parameters.

**Methods:** Strawberry fruit agronomic, sensorial and nutritional quality generated by *Fragaria* inter and intra-specific crosses was evaluated on 29 advanced selections derived from 8 families: two originating from *F. x ananassa* intra specific; three from back crossing of BC1 – FVG x *F. x ananassa*; three from back crossing of BC2 – FVG x *F. x ananassa*. Selections and their respective parents has been evaluated for fruit weight, commercial yield, acidity and sugar content, antioxidant capacity, phenol and anthocyanin content, folate and ascorbic acid content.

**Results:** the advanced selections evaluated in this research have shown substantial improvement either for agronomic than for quality parameters, both sensorial and nutritional parameters. The influence of wild genotypes and their genetic background confirm the importance of germplasm resources to produce new genotypes with wider genetic background and enhanced quality.

# Doxorubicin-induced oxidative stress in rats (*Rattus norvegicus*) is efficiently counteracted by strawberry (*Fragaria x ananassa* Duch.) long term rich diet

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## Abstract-

**Background:** Doxorubicin (DOX) is a widely antitumor anthracycline antibiotic employed for more than 30 years for the treatment of many neoplastic diseases. However, its clinical use is limited by the toxic side effects it produces, acting by altering DNA and by producing free radicals and oxidative stress. In this study, the protective role of strawberry against DOX-induced toxicity has been evaluated in rats. The effect of strawberry fruit was tested by comparing two strawberry cultivars: Adria, with an average values of antioxidant capacity, but with high anthocyanin content and Sveva, with high values of total antioxidant capacity related to high content of phenol compounds.

**Material and Methods:** The total antioxidant capacity (TAC), the total phenol (TPH) and anthocyanin (ACY) contents of the strawberry fruits were measured spectrophotometrically, while vitamin C was quantified by reversed-phase HPLC. The HPLC–DAD/ESI-MS-driven analysis of anthocyanins was also performed. Rats were randomly divided into four groups. The first group received no medication and was regarded as the control group (C group); the second group was injected with two doses of DOX (C-DOX group); the third and the fourth groups were fed respectively with freeze-dried Adria and freeze-dried Sveva fruits for 16 weeks before DOX injections (A-DOX group and S-DOX group respectively). After decapitation, plasma were isolated to analyze lypophilic antioxidant contents with reversed-phase HPLC, while peripheral blood lymphocytes were obtained to determine DNA damage by Comet assay; liver was immediately removed to isolate mitochondria, to verify the ROS concentration using the fluorescent probe DCF and mitochondria functionality through XF-24 Extracellular Flux Analyzer.

**Results and conclusion:** As regard the nutritional composition, Sveva cultivar presented a higher TAC value than Adria fruits, differing also for the higher phenols and Vitamin C levels, while Adria presented higher ACY contents. The same resulted was also confirmed by HPLC–DAD/ESI-MS-driven analysis. Data showed also that DOX drastically increased the DNA damage in lymphocytes, the mitochondrial ROS content and considerably decreased the plasma levels of retinol and the mitochondrial functionality in rats subjected to DOX injection. Co-treatment with srawberry counteracted almost all DOX effects, protecting DNA from oxidative damage, buffering the decrease of retinol, diminishing ROS concentration and improving mitochondrial functionality. The best health benefits enhancement was found for A-DOX group: it means animals fed with Adria strawberry fruit rich in anthocyanins, while S-DOX group showed less health benefits even if Sveva posseses higher TAC, TPH and vit C content than to Adria strawberry fruit. Additional studies are necessary to characterize the bioactive compounds which play a fundamental role against oxidative stress and by which mechanisms strawberry fruits and their phytochemicals (anthocyanins and polyphenols) can improve antioxidant defenses.

# HPLC-MS/MS analysis of flavonoids and their metabolites in urine and plasma from human volunteers administered saskatoon berries

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Saskatoon (*Amelanchier alnifolia* Nutt., Rosaceae) is a fruit-producing shrub or small tree of the Rosaceae family native to the North American Great Plains. Although saskatoon berry and blueberry (*Vaccinium corymbosum* L., Ericaceae) belong to different species, they share many similar characteristics that are important to consumers, including fruit color, shape, size, texture and nutrition.

Two high performance liquid chromatography/ tandem mass spectrometry (HPLC-MS/MS) methods were developed for the analysis of anthocyanins (in positive ion mode) and their phenolic acid metabolites (in negative ion mode), respectively. A comprehensive characterization of anthocyanins and their metabolites was conducted on urine and plasma samples from volunteers administered saskatoon berries. Five major saskatoon berry flavonoids were identified in the urine and plasma samples, i.e., cyanidin-3-galactoside, cyanidin-3-glucoside, cyanidin-3-arabinoside, quercetin-3-galactoside, and cyanidin-3-xyloside. The following metabolites were identified in urine of volunteers administered saskatoon berries: cyanidin-glucuronide, quercetin-3-galactoside-glucuronide, isorhamnetin-3-galactoside (O-methylation product of quercetin-3-galactoside), peonidin-3-glucoside (O-methylation product of cyanidin-3-glucoside), peonidin-3-galactoside, peonidin-3-arabinoside, peonidin-3-xyloside and protocatechuic acid. Cyanidin-glucuronide and protocatechuic acid were identified as major metabolites in plasma from volunteers administered saskatoon berries. (This research is funded by Agriculture Development Fund, Saskatchewan Ministry of Agriculture, Canada)

## Anthocyanins can be efficiently absorbed across the gastrointestinal wall -J. Fang

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Anthocyanins constitute the largest group of water-soluble pigments in plants, being responsible for the blue, purple, and red color of many fruits, flowers, and leaves. The absolute bioavailabilities of anthocyanins were found to be only 0.6% to 1.8% in rat and mice. Percentages of intact anthocyanins excreted in urine were found to be less than 0.1% in human studies. This is in contrast to the accumulating evidences supporting the health benefits of anthocyanins.

This article summarizes three lines of evidences supporting the efficient absorption of anthocyanins across the gastro-intestinal wall. Firstly, high plasma metabolite concentrations have been found following administration of anthocyanins. As much as 30-56% cyanidin 3-glucoside and pelargonidin 3-glucoside were found as phenolic acid metabolites in plasma following oral administration of blood orange juice [1], black raspberries [2], and strawberries [3]. Secondly, anthocyanins can be efficiently absorbed (11-37%) following in situ gastric [4] and intestinal [5] perfusion in rat. Thirdly, high “total urinary recoveries” were found following oral ingestion of [<sup>14</sup>C]-labeled anthocyanins [6, 7]. Therefore, the permeability of anthocyanins across the gastrointestinal mucosa is much better than previously thought. Up to half of anthocyanins could be absorbed from the gastrointestinal system and enter the systemic circulation as metabolites. The observed low apparent bioavailabilities of anthocyanins are primarily due to their extensive pre-systemic metabolism either within the gastrointestinal wall or in the liver. Efficient absorption of anthocyanins indicates that metabolites such as phenolic acids can be responsible for the health effects of anthocyanins. (This work is supported by Agriculture Development Fund, Saskatchewan Ministry of Agriculture, Canada)

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# Effect of cranberry proanthocyanidins' structural features on inhibiting epithelial cell invasion by *Escherichia coli*

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## ABSTRACT

Proanthocyanidins (PAC) are oligomeric flavan-3-ols that have bioactivity related to prophylaxis and treatment of diseases in humans. As an example, cranberry products may prevent urinary tract infections and promote urinary tract health. Cranberry PAC that have “A-type” interflavan bonds are the putative active components, because research shows that “A-type” PAC inhibit adherence of P-fimbriated uropathogenic *Escherichia coli* (*E.coli*) to uroepithelial cells. The “A-type” interflavan bond is associated with greater anti-adherence activity than PAC from other foods such as grapes, apples and chocolate which have “B-type” interflavan bonds.<sup>1</sup>

Research on the role of PAC in health and nutrition is constrained by the lack of analytical methods that relate the structural complexity of PAC found in foods and beverages to their biomedical effects, creating a lacuna in terms of structure-activity relationship of these compounds.<sup>2</sup> Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is considered the mass spectral method of choice for analysis of PAC which exhibit large structural heterogeneity. The application of a recently developed MALDI-TOF MS deconvolution method<sup>3</sup> to cranberry extracts generated with conventional chemical extraction techniques and supercritical fluid extraction (SFE) showed differences in terms of percentages of “A-type” and “B-type” linkages within the same degree of polymerization (DP).

Preliminary data shows that the use of SFE to extract lipophilic components before fractionation of cranberry phenolics on Sephadex LH-20 dramatically alters the “A-type” to “B-type” ratios of PAC when compared to cranberry phenolics that were directly extracted with 70% acetone.

For cranberry **press cake** extracts, the proportion of “2A-type” bonds in PAC with DP between 3 and 8 and “1A-type” bonds in PAC with DP=7 were significantly increased with SFE in comparison to no SFE prior to extraction with 70% acetone. For cranberry **fruit** extracts, the proportion of “2A-type” bonds in PAC with DP between 3 and 5 and “1A-type” bonds in PAC with DP=7 were significantly increased with SFE.

In order to investigate if these differences were relevant from a biological standpoint, PAC fractions that were generated with and without SFE were tested in a Caco-2 cell culture model of *E.coli* invasion. This model simulates the ability of extra-intestinal *E.coli* to colonize gut epithelial cells.

Preliminary results showed that cranberry PAC fractions that were generated using SFE had greater inhibition of *E.coli* Caco-2 cell invasion, probably due to the higher proportion of “2A-type” interflavan linkages in these cranberry PAC fractions.

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# Safety and efficacy of a dried whole cranberry powder (*Vaccinium macrocarpon*) for lower urinary tract symptoms in men

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**Background:** Cranberry has been widely described for its effects on prevention of urinary symptoms in women. However, there is a gap in knowledge regarding the efficiency of cranberry in preventing urinary symptoms in men, who become more susceptible to lower urinary tract symptoms (LUTS) with age. This randomized controlled trial was aimed at evaluating the efficacy and tolerability of a whole cranberry powder (*Vaccinium macrocarpon*) in men with LUTS.

**Methodology:** Forty-two participants aged 45 to 70, with LUTS, elevated prostate-specific antigen (PSA), negative prostate biopsy and clinically confirmed chronic non-bacterial prostatitis were selected. 21 participants received 1500 mg of the whole cranberry powder per day for 6 months while the 21 others did not receive any supplement (control group). The efficacy and safety parameters evaluated at baseline, 3 and 6 months included: physical examination, International Prostate Symptom Score (IPSS), basic clinical chemistry parameters, haematology, testosterone, PSA (free and total), C-reactive protein (CRP), antioxidant status, urinary flow rate, ultrasound-estimated post-void residual urine volume.

**Results:** The efficacy parameters, including IPSS, QoL and urination parameters (rate of urine flow, average flow, total volume and post-void residual urine volume), were statistically improved versus baseline for the participants taking the whole cranberry powder, at the 6-month visit. The safety was demonstrated through a lack of influence of whole cranberry powder intake on blood testosterone or serum CRP levels. In addition lower total PSA level were detected during the course of the trial. In comparison, the controlled group did not demonstrate any significant change for any of the parameters studied throughout the study.

**Conclusion:** This study provides the first evidence that whole cranberry powder may ameliorate LUTS, independent of benign prostatic hyperplasia or C-reactive protein level.

# Strawberry extract protects against oxidative stress and improves mitochondrial functionality in human dermal fibroblasts exposed to hydrogen peroxide

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**Background:** Skin, as the outermost barrier of the body, is directly exposed to a variety of environmental pollutants that can catalyze directly or indirectly the formation of ROS/RSN. After ROS/RNS exposure, both protein and lipid modifications occur, altering the redox status of the intracellular milieu. Exogenous antioxidants from diet fill a beneficial role in improving the endogenous antioxidant defenses of the human body against the development of chronic diseases. Strawberry (*Fragaria × ananassa* Duch.) is an important source of phytochemicals, most of which are natural antioxidants and play a role in preventing human diseases related to oxidative stress<sup>1,2</sup>.

The present work intended to evaluate the *in vitro* effects of the anthocyanin-rich extract of strawberry fruits in cytoprotection against oxidative stress induced by H<sub>2</sub>O<sub>2</sub> in human dermal fibroblasts.

**Material and Methods:** Extracts of strawberry fruits were obtained from frozen strawberry flesh of selected Sveva cultivars. Total antioxidant capacity of fruits was performed by TEAC and ORAC assay, while EPR spectroscopy, in combination with the spin-trapping technique, was used to investigate the strawberry HO<sup>•</sup> scavenging activities. Moreover, identification and quantification of anthocyanins was carried out by HPLC-DAD-MS analyses. Human dermal fibroblasts were treated with three different concentrations of strawberry extracts (0.05, 0.25, 0.50 mg/ml) and subsequently incubated with the oxidant added to the culture medium. Cells were analyzed for viability by MTT assay, intracellular ROS production through fluorescent probe DCF, membrane lipid peroxidation with C11- BODIPY and DNA damage by Comet assay. Moreover, mitochondrial functionality was determined by Seahorse XF-24 Extracellular Flux Analyzer after the addition of the oxidant.

**Results and conclusion:** Strawberry extracts presented high concentration of anthocyanins and showed relevant antioxidant capacity. Fibroblasts incubated with strawberry extracts and stressed with H<sub>2</sub>O<sub>2</sub> presented a smaller intracellular amount of ROS, which results effectively in an increase in cell viability and in a reduction of oxidative damage against membrane lipid. Strawberry extracts were also able to ameliorate the mitochondrial functionality after exposure to pro-oxidant stimuli.

In conclusion, strawberry extract prevented oxidative damage in human dermal fibroblasts. The protective effect could be due to the antioxidant activity of strawberry constituents, especially anthocyanins, that have been shown to exhibit a range of biological effects including; antioxidant activity, anticarcinogenesis, induction of apoptosis, and prevention of DNA damage.

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# Design and selection of black raspberry functional food products and their scale-up production intended for a prostate cancer clinical trial

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Black raspberries (BRB) (*Rubus occidentalis*) have shown great promise as a chemopreventive agent in oral, esophageal, and colon cancers. BRB are rich in anthocyanins, ellagitannins, ellagic acid,  $\beta$ -sitosterol, ferulic acid, bioflavonoids, vitamins, minerals and fiber. When designing a food vehicle for a human clinical trial, the food matrix plays a critical role in the absorption and excretion of the bioactive compounds. We therefore hypothesized that different BRB containing food delivery vehicles designed for human clinical trials can be optimized for palatability and textural consistency while delivering significant doses of bioactive compounds. Our objective was to produce highly palatable BRB containing beverage and confection with different bioactive delivery properties and also to adjust formulation to scale up for human clinical trials.

Three forms of confections (ranging from glassy to rubbery) and nectars (0.4% - 1.2% of pectin as thickening agent) containing freeze-dried whole BRB powder were developed. Dissolution kinetics, rheological properties and storage stability of food products were evaluated, from which final products for clinical trial were selected. Bioactive compounds in food products were identified and quantified with HPLC-photodiode array detection.

Pectin based confections with 2.0 g of BRB per piece (10 g) was selected as the final confection to be used in the clinical trial. This type of confection has intermediate *in vitro* dissolution time (180 min) and also high final anthocyanin delivery (74.7% of total release) compared to hard candy (75 min, 43.2%) and starch based confection (540 min, 47.7%). Moreover, rheological analysis of this confection showed no distinct difference after two months of storage in 4 °C. One type of nectar product with 0.5% of pectin containing either 10 g or 20 g of BRB in 8 oz was selected to deliver intrinsic bioactive compounds systemically for a prostate cancer human clinical trial due to their high palatability and appropriate viscosity. Scale up production of the pectin confections for the clinical trial resulted in slightly weaker gels than lab scale due to greater water retention. Although 10 g nectar was easily scaled up in the commercial setting (UHT processing system with 75 °C for 10 min), the 20 g nectar was too viscous and required batch heating at 95 °C for 15 min and hot filling into sterilized bottles at 70 °C. Final 10 g and 20 g of BRB nectar had  $0.150 \pm 0.0182$  Pa.s and  $0.715 \pm 0.133$  Pa.s of viscosity at  $1.00 \text{ s}^{-1}$  of shear rate. HPLC analysis showed that  $29.0 \pm 0.234$  mg/g of anthocyanins and  $0.918 \pm 0.0247$  mg/g of ellagitannins were in freeze-dried BRB powder. After processing, pectin based confections lost 5.70% of anthocyanins and 9.81% of ellagitannins. Nectar with 10 g and 20 g BRB dosage lost 13.9% and 34.6% of anthocyanins, respectively. For ellagitannins, 33.6% and 13.7 % were lost in 10 g and 20 g dosage nectar. Decrease in anthocyanins after processing may be due to the high temperature heating.

Two BRB food products (confection and nectar) with different physicochemical properties were developed and selected. In addition, scale-up production for the clinical trial was achieved consistently with high retention of bioactive compounds. The modulating effects of food matrix on absorption, distribution and metabolism of bioactive compounds from BRB in systemically delivery will be tested and evaluated in prostate cancer clinical trial.

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## **Geographic and genetic distribution of plants and anthocyanines of Chilean superberry Maqui (*Aristotelia chilensis* (Mol.) Stuntz)**

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The Maqui Berry (*Aristotelia chilensis*) commonly known as “maqui” in Chile, is a common wild, endemic, edible berry in Central and Southern Chile. Maqui is a deeply purple berry loaded with anthocyanins, a potent group of antioxidants which give the berry its coloration and provide many documented health benefits, like protection to inflammatory diseases or metabolic disorders. The Maqui has the highest concentration of antioxidants of any known super berry source, due to the high content of anthocyanins, especially delphinidins, which provides a great capability to trap free radicals (ORAC), with values between 4 to 30 times higher than other berries. It is also packed with other nutrients that give it many powerful and desirable health-promoting qualities. Despite that Maqui is highly distributed geographically in Chile, there is no study of genetic diversity. Currently, Maqui shows a great adaptability to different environments, but there is no knowledge associating anthocyanin and delphinidin concentrations and genetic variability between the plants. In this study, we present the results of genetic diversity between 95 selected Maqui clones from different regions of Chile analyzed by 8 Simple Sequence Repeats (SSR), associated to their geographic distribution and anthocyanin content, determined by HPLC (using an Analytical Method developed by Universidad Austral de Chile). Intra-varietal differences between the Maqui clones were detected by SSR and it was established a relationship between anthocyanin and delphinidin concentration in selected clones. As a result of this knowledge, we are cloning the plants with the highest anthocyanin and delphinidin content in a germplasm bank in order to initiate a breeding genetic program to get the first registered varieties for commercial plantations.

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## **Potential health protective effects of fruit juice enriched with natural polyphenols from black currant press-residue analysed using in vitro model systems**

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Studies have shown that consumption of fruit and berries provides a preventive effect against cancer, cardiovascular disease, and diabetes. One of the reasons for the protective effect is related to the polyphenols found in fruits and berries. Juice is a good source for intake of fruit and berries, however during juice production a large amount of polyphenols end up in the press-residue instead of the juice. We have found a method to extract valuable polyphenols from black currant press-residue. However, pure press-residue extract is not suitable to drink due to bitter and strong taste. The aim of the present study was to investigate sensory and potential biological effects of a fruit juice enriched with natural polyphenols from black currants press-residue. We studied the effect on ACE- inhibition, cell proliferation, and NF-κB in vitro. Based on our results, the enriched fruit juice has potential health effect.

# Composition of Phenolic Compounds in Organically Grown Blackberries and Their Stability during Storage

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Organically grown ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries were used to analyze anthocyanin and phenolic profiles and their stability during storage. Freshly harvested berries were sorted into shiny black (SB) and dull black (DB) and stored at 1°C for 15 days or at 1°C for 13 days followed by 2 days at 20°C. Freeze dried berries were extracted with acidified methanol, and anthocyanins and phenolic compounds were analyzed using high performance liquid chromatography equipped with photodiode array detector and Synergi 4 $\mu$  Hydro-RP 80A column (250 X 4.6 mm) with methanol-formic acid gradient system. Four anthocyanins, cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, and pelargonidin 3-glucoside were detected. Cyanidin 3-glucoside was the predominant anthocyanin in blackberries, representing 87-96% of the total anthocyanin content. Cyanidin 3-glucoside and total anthocyanin contents were generally higher in ‘Natchez’ than in ‘Ouachita’ and tended to increase during storage. However, cyanidin 3-xyloside content in ‘Natchez’ (<0.2%) was lower than in ‘Ouachita’ or ‘Navaho’ (4-7%). Gallic acid and quercetin 3-galactoside or quercetin 3-glucoside contents were generally lower in ‘Ouachita’, but vanillic acid content was the lowest in ‘Natchez’ (2-6% in ‘Natchez’ and 15-30% in ‘Ouachita’ and ‘Navaho’). Total phenolic content was generally higher in ‘Natchez’ and ‘Navaho’, and tended to increase during storage. The results indicate that organically grown ‘Natchez’, ‘Ouachita’, and ‘Navaho’ blackberries have levels of phenolic compounds that range of 17-35 mg g<sup>-1</sup> DW and 220-770 mg 100 g<sup>-1</sup> DW for anthocyanins and phenolic compounds, respectively, and three cultivars used in this study had different phenolic profile. Further, anthocyanins and phenolic compounds were stable and slightly increased during storage.

## Strawberry extract suppressed lipopolysaccharide induced inflammation through inhibiting nuclear factor $\kappa$ B and mitogen-activated protein kinase signaling in Raw264.7 mouse macrophages.

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Phenolic compounds known to present in strawberry (*Fragaria ananassa* Duch.) have been suggested to exert many physiological effects including anti-inflammatory activity. However, the roles of strawberry extracts on immunomodulation are scarcely reported. In this study, we employed multi-step solvent extraction methods using ethyl acetate and acid methanol and separated strawberry (Seolhyang) cultivated in South Korea into 6 fractions (F1~F6) according to their properties. Among those fractions, fraction 4 (F4) showed highest activity in inhibiting lipopolysaccharide (LPS)-induced iNOS expression in a dose dependent manner without affecting the COX2 expression. In addition, F4 strongly recovered the degradation of inhibitory  $\kappa$ B (I $\kappa$ B) by LPS and suppressed the phosphorylation of MAPK such as ERK and JNK, indicating that F4 contain the active constituents regulating the inflammation. To determine the potent molecules responsible for the activity, we performed metabolome analysis between fractions and found that ellagic acid in F4 is the molecule significantly different from other active fractions, F3 and F6. In addition, we confirmed that ellagic acid itself inhibited the expression of LPS-induced iNOS protein. In conclusion, the strawberry extract with acid methanol possess anti-inflammatory activity via regulating NF- $\kappa$ B and MAPK signaling and metabolomic approach suggest that ellagic acid may be one of key metabolites regulating inflammation in strawberry, Seolhyang.

# **Effect of processing on ascorbic acid and total monomeric anthocyanins in blackcurrant (*Rubus nigrum*) juice**

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Blackcurrant (*Rubus nigrum*) berries are rich in ascorbic acid and anthocyanins (pigments), and a lot of people drink their blackcurrant juice because they believe it is healthy. But how much is left after the berries are processed into juice and stored prior to consumption? And which effect do various process parameters have?

After harvesting the berries (varieties; Narve Viking and Ben Tron (50/50)) were rinsed and frozen at -20 °C. The berries were thawed at 2 °C for four days to -1 °C at processing. Prior to juice pressing by a 40 liter hydro press, the berries were subjected to one of three pre-treatments: 1, no treatment, 2, homogenizing by hand; and 3, homogenizing by hand, heating to 90 °C followed by cooling to 60 °C.

After pressing, the juice was heated up to and kept for 1 minute at 78 °C before bottling. In addition, juice from treatment 2 was heated to different stages; 62 °C, 72 °C, 78 °C, 85 °C, 90 °C and 100 °C for 1 minute, 100 °C kept in ten minutes, 100 °C kept in twenty minutes and 100 °C kept in thirty minutes before bottling. Juice was also made on a household steam-cooker (treatment 4), and after the processing time (1.5 hours) the juice was bottled immediately from the steam-cooker. All bottles were kept cold until next day, when samples were analyzed for ascorbic acid and total monomeric anthocyanins (TMA). The rest of the bottles were stored in dark at +4 °C and +20 °C, for analyzes after three and six months.

Different treatments of the berries prior to pressing had some effect on the ascorbic acid content, and huge effect on the amount of TMA. The heating step prior to pressing gave 30% less ascorbic acid and four times more anthocyanins compared to juice from untreated berries. The juice made in a household steam-cooker had only half the contents of ascorbic acid and TMA compared to the juices produced on the hydro press. Ascorbic acid was surprisingly stable during heat treatments with different temperatures, while TMA decreased 15%, 25% and 38% during heat treatment up to 78 °C, 100 °C and 100 °C in 30 minutes respectively. The results after storage will be presented on the poster and answer the question about content of ascorbic acid and TMA in different blackcurrant juices as consumed.

## **Carotenoid and Tocopherol Content of Raspberries and Blackberries**

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Raspberry and blackberry color is from the water soluble anthocyanins, present at 200 to 1000 mg/kg fresh weight. In contrast, carotenoids are one tenth of the anthocyanin content yet contribute much of the flavor volatiles to caneberries. Additionally, tocopherols in caneberries contribute a small amount of vitamin E precursor. Nine raspberry and nine blackberry cultivars were harvested from North Carolina plantings for relative comparison of carotenoid and tocopherol profiles. Freeze dried material free of seeds was extracted with hexane:ethanol:acetone and analyzed using a Hitachi HPLC equipped with photodiode array and a YMC C30 4.6 x 250 um column. Peaks at 470 nm were quantified using standards for alpha and beta carotene and lutein. Peaks at 290 were quantified using alpha, delta, and gamma tocopherol. No beta tocopherol was detected. Fruit tissue contained high amounts of alpha and delta tocopherol while pyrenes contained high amounts of gamma tocopherol. A number of peaks were found to be esters of alpha and beta carotene, and of beta cryptoxanthin in raspberry. The lutein content of raspberries was 0.1 (Nova) to 0.9 (Caroline) ug g dry weight. Alpha carotene ranged from 0.01 (Nova) to 0.5 (Caroline) and beta carotene was 0.01 (Nova) to 0.2 (Caroline). In contrast, blackberry had no detectable alpha carotene and beta carotene was 10 fold higher than in raspberry (1.4 ug g dry weight). Alpha and beta carotene are direct precursors to alpha and beta ionone which impart floral and fruity notes in caneberries.

# Berry flavonoid epicatechin extends lifespan in aging mice via improving physical activity, inflammation and antioxidants

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## Abstract

**Purpose** Epicatechin, a flavonoid present in berries, cocoa and tea, may have beneficial effects on human health. In the present study, we investigated the hypothesis that dietary epicatechin intake increases survival rate in aging mice through promoting physical activity and improving inflammatory status and Antioxidants.

**Methods** Two-year old male mice (C57BL/6J) were fed with/out epicatechin by drinking at 0.25% (0.25 gram epicatechin in 100 mL water) for 8 weeks. The mortality of the mice was monitored daily, and body weight, food intake, water intake were assessed weekly. Physical activities were measured using computer controlled automatic rotarod and behavioral core chamber at the beginning and the end of the experiment. A young control group (1 year-old male C57BL/6J) was used in the physical activity experiments and endpoint sample collections. Inflammatory markers from blood were assessed by ELISA kits. Antioxidant glutathione concentration and total superoxide dismutase activity from liver were also measured.

**Results** After 8 weeks, only 46.2% of mice survived in the old control group (OC), whereas the survival rate was 84.6% in the epicatechin group (OE). The duration time on the rotarod was  $121 \pm 20$ ,  $78 \pm 15$  and  $127 \pm 23$  seconds in the young control (YC), OC and OE groups, respectively. Similarly, both of the vertical counts and the distance traveled in the behavioral core chamber were reduced in the OC group compared to the YC group, however epicatechin intake reversed these reduced behavioral measurements. Moreover, serum level of monocyte chemoattractant protein-1 (MCP-1), one of the key chemokines that regulate migration and infiltration of monocytes/macrophages, was radically increased in the OC group (617.74 pg/mL) compared to the YC group (75.13 pg/mL), but epicatechin intake significantly reduced the MCP-1 level in the OE group (341.28 pg/mL). Consistently, levels of antioxidants glutathione and superoxide dismutase in the livers of the OE group were significantly greater than the OC group, whereas these antioxidants were not altered in the OC group as compared to the YC group.

**Conclusion** Epicatechin may be an anti-aging agent by improving physical activity, inflammation and antioxidants.

# **The effect of acute blueberry anthocyanin interventions on executive function performance in 7 – 9 year old children**

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Anthocyanins, a group of flavonoids which are found in high concentrations in various foods and drinks such as blueberries, are postulated to promote healthy brain function in adults. Similar effects in a school-aged population, it could be argued, may enhance learning and thus influence academic attainment. Indeed, initial research from our laboratory has shown improvements in word recall, word recognition, and executive function (EF) in 7–9 year old children supplemented with either fresh or freeze dried blueberries. When tested in local schools on an EF response interference flanker task, though no benefit for reaction time was found, children showed significantly improved accuracy performance ( $p < .05$ ) on the more cognitively demanding incongruent trials. This effect was particularly evident 3 hours after supplementation with a 253mg anthocyanin dose. In order to further investigate these actions on EF, we assessed the effects of 253mg anthocyanins in a more controlled lab environment using an extended EF task battery. We included (1) an attentional network task which targets the response interference, alerting and orienting aspects of EF, (2) a switching task which taps cognitive switch costs and response interference, and (3) a stop-go task to investigate response inhibition. Testing at 3 hours post-supplementation, we found trends related to response interference. However, in this instance, the intervention resulted in faster reaction times for the less cognitively demanding congruent trials in the attentional network task ( $p = .053$ ) and shape response trials of the switching task ( $p = .076$ ). No additional effects were found for incongruent trial accuracy or for our measures of alerting, cueing, switching costs or inhibition. Given that our initial improvement in EF results were gathered in a distracting school environment, our findings may indicate that the positive benefits of blueberry supplementation subtly differ depending on the level of external distraction at the point of testing. Work is continuing to further investigate these effects.

## **Changes in Anthocyanin Content and Polymeric Color during Processing and Storage of Chokeberry Juice**

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Anthocyanin-rich chokeberries are commonly consumed as juices and nectars, but limited information is available on how unit operations used in juice processing and storage of juice impacts anthocyanin content of the berries. This study was undertaken to determine the major steps in juice processing where major losses of anthocyanins occur, and to determine the stability of anthocyanins over 6 months storage at 23°C. Samples of frozen berries, blanched berries, enzyme treated mash, presscake, non-pasteurized and pasteurized juice were analyzed for anthocyanin content by HPLC and percent polymeric color using a spectrophotometric assay. Similar measurements were performed monthly on juice samples stored for 6 months at 23°C. Blanching resulted in the greatest losses of cyanidin glycosides (40-55%), followed by pressing (21-31%), and pasteurization (17-22%). Percent polymeric color values fluctuated from frozen berries (16%) to non-pasteurized juice (13%), but increased following pasteurization (29%). Following pasteurization only 14% of the total anthocyanins present in frozen berries used for processing were recovered in the juice, with cyanidin hexosides (15-21%) showing greater retentions than pentosides (6-8%). Cyanidin glycosides declined linearly over 6 months of storage at 23°C and were accompanied by increased polymeric color values. After 6 months, only 2.3% of the original total anthocyanins were present in the juices, with cyanidin hexosides showing greater retentions (2.4-5.9%), than pentosides (1.9-2.3%). Percent polymeric color values increased from 35% to 45% from 1 to 6 months of storage indicating that the small amounts of free anthocyanins remaining were present (almost 50%) as anthocyanin-tannin polymers. Methods are needed to ameliorate the extensive losses of anthocyanins that occur during chokeberry juice processing and storage.

## **Decrease in Health Benefits of Juices with Increased Processing**

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The juice and functional beverage industry is rapidly advancing a plethora of products driven by a consumer awareness of the health benefits of phytonutrients contained in “superfruits.” Shelf-life stability, flavor retention, and off-flavor problems are often associated with these antioxidant-rich beverages. Processing technologies may result in loss, or negatively affect the stability and/or bioavailability, of these compounds. In this work, changes in anthocyanins, volatiles, and sensory were monitored at selected steps as berries were pressed, pasteurized and filtered.

Pasteurized, not-from-concentrate, 100% juices from frozen blueberries were produced in a food-grade commercial-like pilot plant. Commercially frozen ‘Tifblue’ blueberries (*Vaccinium ashei*) were processed into a mash in a 40 quart steam jacket kettle at 95°C then cooled and juiced with pectolytic enzymes and hydraulically pressed on a GoodNature X-1. Filtration occurred with a 200,000 molecular weight cut-off (0.2 µm) XP-201 polyvinylidene fluoride (PVDF) membrane run at ambient (~25°C) through a pilot Aquious Bro/Buf Membrane Filtration unit. The raw juice was divided and pasteurized as a non-clarified and ultrafiltered clarified juice on a MicroThermics Electra UHT/HTST Lab-25EDH at 90 °C for 10 sec. followed by hot-filling at 85 °C, cap twisting, inversion of the glass bottles for 20 sec. and ice water bath chilling (~5 minutes). Samples were then stored at 4 °C for 0, 1, 2 and 4 months in the dark.

There is a marked decrease (~75%) in anthocyanins from whole berries compared to the processed juice. Profiles of volatile compounds change during processing as aldehydes become more dominant and esters decrease. Sensory evaluation showed a significant difference between filtered and unfiltered juice.

### **Impact of interspecific hybridization on anthocyanin accumulation in blueberries (*Vaccinium* spp.)**

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Blueberry, a rich source of anthocyanins with important implications for human health and chronic diseases, has become a major fruit commodity in the USA. This study was designed to assess the impact of genetic introgressions among blueberry species on anthocyanin concentration and profile. Blueberries of commercial cultivars, NC breeding selections/clones, and F<sub>1</sub> populations with varying ploidy levels and degrees of introgression from multiple species were used to address this question. Ripe fruits from blueberry genotypes, grown in Piedmont Research Station at Salisbury, NC were evaluated for anthocyanin concentration and profile in two consecutive years (2010 and 2011). Total anthocyanin concentrations ranged from 160 – 464 mg/100g fresh weight in the commercial cultivars while NC selections ranged from 173 – 408 mg/100 g fresh weight. For the F<sub>1</sub> crosses, population means for total anthocyanins ranged from 227 – 537 mg/100 g fresh weight. The highest anthocyanin accumulation was observed in the NC1223 x Columbus cross, with the wild *virgatum* (*ashei*) background. Ploidy level and degree of introgression among blueberry species were observed to affect total and individual anthocyanin species. However, the expression of the interspecific introgressions in blueberry species was genetic background-dependent.

## **Polyphenol-rich fruit extract attenuates glucose and free fatty acid mediated tube formation in vitro in human umbilical vein endothelial cells (HUVEC)**

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High concentrations of glucose and free fatty acids (FFA) are known to increase oxidative stress resulting in endothelial dysfunction. Our previous study suggested that polyphenolic antioxidant-rich fruit extract modulated endothelial function via a redox-sensitive mechanism. Therefore, we hypothesized that polyphenol-rich fruit extracts of strawberry (SB) and wild blueberry (WB) would attenuate endothelial dysfunction in vitro after stress induced by glucose and FFA.

Human umbilical vein endothelial cells (HUVEC) were exposed to three combinations of glucose and/or FFA concentrations. Glucose (5-15 mM), FFA (0.5-2 mM), glucose + FFA combinations (5-15 mM for glucose and 0.5-2 mM FFA) aimed to mimic physiological concentrations ranging from normal to diabetic conditions. HUVEC were treated with 0.2 mg/ml SB or WB for 2 h in 2% fetal bovine serum (FBS) containing media followed by nutrient combinations overnight. The cells were then washed twice with phosphate buffered saline (PBS) and then moved to a Matrigel and 4% FBS medium for assessment of capillary-like tube formation, i.e., angiogenic activity, an indicator of endothelial function. Observations were made 0-10 h post overnight treatment.

Our previous study showed that SB and WB increase angiogenic capacity compared to the PBS control. [1] In this study, we were interested in determining the effects of SB and WB on HUVEC under nutrient-induced stress. Consistently, higher concentrations of glucose and FFA caused more stress to HUVEC and decreased their tube formation capability compared to HUVEC treated with lower concentrations of glucose and FFA. Moreover, capillary-like tube formation was significantly ( $p < 0.05$ ,  $n = 4$ ) improved when cells were pre-treated with solutions containing 0.2 mg/mL SB and WB extracts compared to HUVEC treated with glucose (10–15 mM) and or FFA (1-2 mM) without pretreatment with fruit extracts. This study provides evidence for the action of SB and WB extracts on prevention of stress-related effects on endothelial cells upon exposure to glucose and FFA.

Reference:

1. Tulio AZ Jr., Chang C, Edirisinghe I, White KD, Jablonski JE, Banaszewski K, Kangath A, Tadapaneni RK, Burton-Freeman B, Jackson LS. Berry fruits modulated endothelial cell migration and angiogenesis via phosphoinositide-3 kinase/protein kinase B pathway in vitro in endothelial cells. *J Agric Food Chem.* 2012 Apr 15.

## **Inhibition of *Streptococcus mutans* amyloid fibril formation by cranberry fractions**

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Biofilm formation is necessary to establish urinary tract infections, dental caries and numerous other infectious conditions. *Streptococcus mutans* is an organism that forms biofilms and was recently shown to be an amyloid-forming organism. Amyloids are insoluble fibrillar protein aggregates that have been associated with protein misfolding and disease states such as Alzheimer's, but the concept of functional amyloid formation as a directed process involved in microbial pathogenesis is recently emerging. A panel of well-characterized cranberry fractions was tested to determine if they inhibited *S. mutans* biofilm formation. The fractions did not affect bacterial growth, but inhibited biofilm formation. As a potential mechanism of biofilm inhibition, inhibition of amyloid fibrillization by *S. mutans* extracellular proteins was assessed using a Thioflavin-T fluorescence assay. Amyloid formation was inhibited by cranberry fractions containing proanthocyanidins. Thus, cranberry bioactive compounds may inhibit biofilm formation by preventing amyloid fibril development of the amyloidogenic proteins produced by *S. mutans*. (Fractions provided by OSC Inc. Support by Univ Schol Prog, UFL, and NIDCR R01DE21789)

## **The Impact of Blueberry Polyphenols on Osteoblast Differentiation and Bone Nodule Formation**

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Dietary polyphenols present in fruits and vegetables may play an important role in bone metabolism through modulation of osteoblasts, the bone-forming cells. Blueberries contain a rich mixture of polyphenolic compounds that exhibit anti-inflammatory and anti-oxidant actions in various tissues. Hence, polyphenols present in blueberries may directly stimulate osteoblasts, and favorably alter bone formation. Utilizing murine MC3T3-E1 (pre-osteoblast) cells, we evaluated whether blueberry polyphenols (BBP) in a dose-dependent manner increase markers of bone formation in presence and absence of an inflammatory agent. Cells were treated for 7 days with various doses (0, 10, 50, 100, 200 ug/mL) of BBP and challenged with tumor necrosis factor-alpha (TNF- $\alpha$ ). BBP dose-dependently increased ( $p < 0.05$ ) alkaline phosphatase, a marker of bone formation and osteoblast differentiation in presence of TNF- $\alpha$ . Using colorimetric In-Cell ELISA, we observed an increase in bone morphogenetic protein-2 (BMP-2) with BBP treatment in absence of TNF- $\alpha$ . Treatment with BBP in presence of TNF- $\alpha$  only showed modest effects on BMP-2. Alizarin red staining of cells treated with BBP resulted in dose dependent increase in staining density, an indicator of bone nodule formation. The highest level of nodule formation was observed with 50 ug/mL BBP. However, no change in nitrite levels, a marker of inflammation was observed with BBP treatment in presence and absence of TNF- $\alpha$ . The influence of BBP on molecular mechanisms involved in osteoblast differentiation and activity is being further investigated using real time PCR.

## **Effect of Blueberry Polyphenols on RANKL-Mediated Osteoclast Differentiation and Activity**

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Bone is a dynamic tissue with osteoblast and osteoclast participating concomitantly in its remodeling process. These cells are derived from different progenitor pools and under different molecular control. Osteoclast maturation requires stimulation by receptor-activated nuclear kappa ligand (RANKL), a downstream product of inflammation and an important regulator of bone resorption. Plant flavonoids such as polyphenols have been shown to have anti-inflammatory action in various tissues. Blueberries are a rich source of polyphenolic compounds. Using mouse macrophage cells (RAW 264.7), we evaluated whether blueberry polyphenols (BBP) dose-dependently inhibit RANKL-induced osteoclast differentiation and activity. Our result demonstrated that BBP in presence of RANKL reduces tartrate resistant acid phosphatase (TRAP) activity and TRAP staining of multinucleated osteoclastic cells. BBP did not significantly influence levels of nitrite, a marker of inflammation. Western blot analysis demonstrated a decrease in protein expression of cyclooxygenase-2 (COX-2) in cells treated with BBP. However, the protein expression for inducible nitric oxide synthase was not affected. Further elucidation of the molecular mechanisms related to the inhibitory action of BBP on osteoprotegerin, an osteoclastogenesis inhibitory factor and nuclear factor of activated T cells (NFATc1), the master transcription factor for osteoclast differentiation induced are currently being evaluated.

# Phytochemical Profiles of Alaskan Berries and their Importance to Human Health

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Wild berries are prized in Alaska. They are delicious fresh, frozen, dried, or turned into any number of tasty creations: jams, muffins, fruit leathers, sauces, and agutuk (otherwise known as Alaskan/Eskimo ice cream, made from seal oil and berries). They are also supercharged with antioxidants; polyphenolic metabolites that can ameliorate metabolic disorders such as obesity, diabetes and metabolic syndrome. In this study, two wild Alaskan berries were investigated; lingonberry (*Vaccinium vitis-idaea*), known as lowbush cranberry, and bog blueberry (*Vaccinium uliginosum*). The two Alaskan berries were investigated in parallel with American cranberry (*Vaccinium macrocarpon*), and lowbush blueberry (*Vaccinium angustifolium*) to rigorously characterize their polyphenolic constituents. Total phenolics (TP), total anthocyanins (ANC) and total proanthocyanidins (PAC) were determined by colorimetric methods; individual ANC, and low oligomeric PAC were evaluated by HPLC. LC-ESI-MS and MS/MS were used to identify compounds according to their accurate mass and fragmentation pattern in both the positive and negative ion modes.

Fresh lingonberry contained 8.48 mg/g TP, 2.57 mg/g ANC, and 3.78 mg/g PAC, which were  $\geq 2$  fold the concentrations in American cranberry. Bog blueberry contained higher levels of TP and ANC (6.55, 2.77 mg/g, respectively) than lowbush blueberry (4.70, 2.40 mg/g, respectively), however the latter was much higher in their PAC content (3.44 versus 1.11 mg/g for bog blueberry).

ANC profile for lingonberry showed two major peaks for cyanidin galactoside and arabinoside with a minor peak for cyanidin glucoside but no detectable levels of peonidin glycosides, contrary to American cranberry which showed balanced levels of both cyanidin and peonidin glycosides. The ANC profile of bog blueberry was very close to lowbush blueberry, except that the former was deficient in acylated anthocyanins.

The PAC profile for lingonberry indicated both A and B-type components with predominantly the B-type, contrary to American cranberry, where PAC A-type predominated. Bog blueberry showed nearly equal levels of type-A and B- dimers and trimer PAC, while lowbush blueberry showed much lower levels of type-A PAC.

HPLC-MS and MS/MS analyses identified chlorogenic acid as a major phenolic acid in lowbush blueberry, but it was not detected in other berries. Quercetin and myricetin flavonol glycosides were present in all berries.

The four studied berries contained distinctly different levels and profiles of polyphenolic components, which are expected to have an impact on their health-relevant properties. These differences in phytochemical profiles prompted us to comparatively investigate the antioxidant and anti-inflammatory activities of extracts from each berry genotype.

# Anti-allergic Potential of Peanut Proteins Complexed with Polyphenols from Berry Fruits

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**Hypothesis:** Berry fruit juices and other plant extracts with diverse polyphenolic profiles and concentrations are capable of binding to peanut proteins, which may condition the relative allergenicity of those proteins.

**Abstract:** Studies from the United States, the United Kingdom and France estimate peanut allergy to affect about 0.4% of children and about 0.5% to 1% of the general population. A primary mechanism for the allergic response in sensitive individuals is immunoglobulin E (IgE) binding to epitopes on various peanut proteins followed by downstream cascades. Phytochemicals such as polyphenols have substantial binding affinity for edible proteins with the potential to form soluble and insoluble protein-polyphenol complexes. Berry fruit juices and other plant extracts were complexed (sorbed) with light roast 12% fat peanut flour (LR12) which was subsequently screened for anti-allergic properties. Alterations in secondary protein structure in all enriched LR12 matrices could be observed using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy. Western Blotting used to test soluble fractions for IgE-binding capacities showed that LR12 complexed with proanthocyanidin-rich sources substantially decreased IgE-binding to peanut proteins. The cranberry-enriched LR12 matrices have reduced the basophil activation capacity in an *in vitro* degranulation assay using whole blood from peanut allergic individuals by approximately 50% in median as compared to un-modified LR12. Serum from C3H/HeJ mice made allergic to peanut proteins and challenged with un-modified LR12 or cranberry 2x dilute-enriched LR12 matrix was assayed for mouse mast cell protease-1 (MMCP-1) as a marker of degranulation. Mice challenged with cranberry 2x dilute-enriched LR12 matrix had substantially lower levels of MMCP-1 in their sera than mice challenged with un-modified LR12. Spleen cells were used to examine effects of cranberry polyphenolics on T cell cytokine secretion. Results indicate a dose-dependent suppression of both IL-13 and IFN- $\gamma$  attributable to cranberry. The evidence suggests that bioactives particularly from proanthocyanidin-rich sources may be capable of modifying peanut epitopes and modulating allergenicity forming a reduced allergenic ingredient with potential use in clinical oral immunotherapy applications.



2013 Berry Health Benefits Symposium  
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June 18-20, 2013  
Concord, North Carolina - USA