Symposium Proceedings

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June 11-12, 2007
Corvallis, OR - USA

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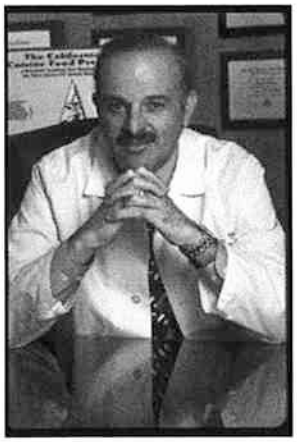
2007 SYMPOSIUM SPEAKERS

Battino, Maurizio
Università Politecnica delle Marche, Italy

Joseph, James
USDA Human Nutrition Research Center on Aging, Tufts University

Crozier, Alan
University of Glasgow, Scotland

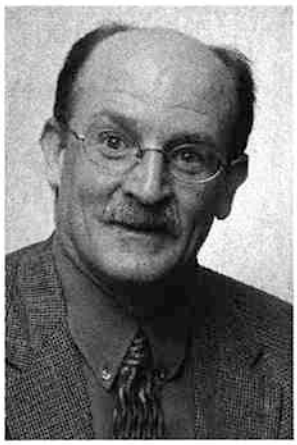
Kalt, Wilhelmina
Agriculture and Agri-Food Canada



Heber, David
Director of the UCLA Center for Human Nutrition at the University of California, Los Angeles

Keynote Speaker

Koli, Raika
Biomarker Laboratory, National Public Health Institute (KTL), Finland



Howard, Luke
University of Arkansas

Kresty, Laura
Ohio State University

2007 SYMPOSIUM SPEAKERS

NO PHOTO AVAILABLE

Lefevre, Michael
Pennington Biomedical Research Center

Puupponen-Pimiä, Riitta
VTT Biotechnology, Finland

Lila, Mary Ann
University of Illinois

Prior, Ronald
USDA-ARS, Arkansas Children's Nutrition Center, University of Arkansas

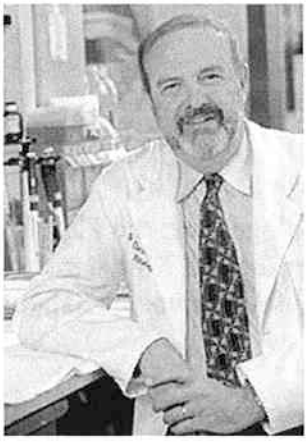
Mazza, Guiseppe
Agriculture and Agri-Food Canada

Reed, Jess
University of Wisconsin, Madison

McGhie, Tony
The Horticulture and Food Research Institute of New Zealand

Seeram, Navindra
UCLA

2007 SYMPOSIUM SPEAKERS



Stoner, Gary
Ohio State University

Tsuda, Takanori
Chubu University,
Japan

SPEAKER BIOGRAPHY

Battino Maurizio

He was born in Verona (Italy) on July 12th, 1961.
He earned his Degree in Biological Sciences (1984) at the University of Bologna and the PhD in Comparative Biochemistry (1990) discussing the Doctorate Thesis at the Italian Minister for University in Rome after 3 years at the University of Catania supported by fellowships of Italian Minister for University.

On September 1991 he won by trial the only place for Assistant & Research Professor in Chemistry and Biochemistry for the Faculty of Medicine available at the University of Ancona.

PRIZES AND FELLOWSHIPS

He was awarded the prize "Vittorio Emanuele II" (1985) at the University of Bologna, obtained an "Ayuda a las Investigaciones de la Junta de Andalucia" fellowship (1991) for delivering investigations at the University of Granada (Spain), he won a fellowship from the Italian Society of Biochemistry (1992).

Post-Doctoral Fellow on 1993 and 1994 at the University of Granada - Spain with a fellowship from the Spanish "Ministerio de Educacion y Ciencia".

On May 2001 The University of Ancona awarded him with the prize "Best Researcher of the Year" for the high quality of his scientific production.

On June 2004 the President of Italian Republic awarded him as Knight of the Order of the Italian Republic.

SCIENTIFIC ACTIVITY AND PUBLICATIONS

Over 120 papers published mostly (85) in international indexed journals (check *PubMed* for most of them).

He delivered over 40 invited lectures at international or national Congresses, Meetings and Symposia and has been **Chairman** of Sessions at several Intl Symposium on "The Biomedical and Clinical Aspects of Coenzyme Q" and at the 1st Public Health Nutrition Congress in Barcelona (Spain).

Co-Editor of (1) Highlights in Ubiquinone Research, Taylor & Francis Ltd., London, 1990, ISBN 0-85066-848-4; (2) Molecular Aspects of Medicine, Pergamon, vol. 15S, 1994, ISSN 0098-2997; (3) Molecular Aspects of Medicine, Pergamon, vol 18S, 1997, ISSN 0098-2997; (4) Guest Editor of a special BioFactors, IOS Press, vol 23(4), 2005, ISSN 0951-6433, issue devoted to the role of berry bioactive compounds in health.

He organized as Scientific Secretary 4 International Symposia.

SPEAKER BIOGRAPHY

Referee of PPP Foundation (London, UK), Society for Free Radical Biology & Medicine and of the following peer-reviewed international scientific journals:

- Aging Clinical and Experimental Research
- Americal Journal of Clinical Nutrition
- Archives of Oral Biology
- Biochimica et Biophysica Acta
- BioFactors
- Botanical Studies
- British Journal of Nutrition
- European Journal of Oral Sciences
- Food & Chemical Toxicology
- Free Radical Research
- Hormone and Metabolic Research
- HortScience
- Infection
- Lipids
- Journal of Chromatography B
- Journal of Lipid Research
- Journal of Psychosomatic Research
- Molecular Nutrition & Food Research
- Nutrition
- Nutritional Genomics & Functional Foods
- Pharmacological Research
- Skin Pharmacology and Physiology

SPEAKER BIOGRAPHY

Alan Crozier

"Absorption and metabolism of dietary phenolics"

Alan Crozier, Professor of Plant Biochemistry and Human Nutrition, Institute of Biomedical and Life Sciences, University of Glasgow, UK

Alan Crozier obtained his PhD at the University of London and carried out research on plant hormones at the University of Calgary in Alberta before taking up a lectureship at the University of Canterbury in New Zealand. After two years he moved to a faculty position at the University of Glasgow from where his research group has published more than 100 papers on plant hormone biochemistry. In 1978-9 he spent a very enjoyable sabbatical at the OSU Forestry School in Corvallis. He has also studied the production of purine alkaloids in tea and coffee and more recently developed an interest in flavonols and other dietary flavonoids in fruits, vegetables and beverages. Alan is currently Professor of Plant Biochemistry and Human Nutrition at the University of Glasgow and his research group is using a number of approaches to investigate the bioavailability of dietary phenolics, principally those occurring in onions, tomatoes, berries, red wine, tea and coffee.

SPEAKER BIOGRAPHY

David Heber, MD, PhD, FACP, FACN

David Heber is the Director of the UCLA Center for Human Nutrition at the University of California, Los Angeles. After graduating from UCLA Magna Cum Laude in Chemistry in 1969 and from Harvard Medical School in 1973, he completed his internship at Beth Israel Hospital and his residency and fellowship training at Harbor General Hospital in Torrance, California. He completed his Ph.D. in Physiology at UCLA in 1978. Dr. Heber has been on the faculty of the UCLA School of Medicine since 1978 and is currently Professor of Medicine and Public Health and the founding Chief of the Division of Clinical Nutrition in the Department of Medicine and the Founding Director of the UCLA Center for Human Nutrition at UCLA.

Dr. Heber is board certified in Internal Medicine and Endocrinology and Metabolism by the American Board of Internal Medicine, and in Clinical Nutrition by the American Board of Nutrition. He directs the NCI-funded Clinical Nutrition Research Unit and the NIH Nutrition and Obesity Training Grants at UCLA. Dr. Heber is a Director of the American Board of Nutrition and past chair of the Education Committee of the American Society of Clinical Nutrition. He was recently elected Chair of the Medical Nutrition Council of the American Society for Nutrition. He has written over 150 peer-reviewed scientific articles and over 50 book chapters, and two professional texts: Dietary Fat, Lipids, Hormones and Tumorigenesis. ; and Nutritional Oncology, a 49 chapter text published by Academic Press in 1999 with a second edition in 2006.

Dr. Heber is included in the 2000, 2001, and 2002 listings of *The Best Doctors in America*, based on a survey of over 35,000 doctors throughout the nation, and was listed in Who's Who in America as of 2001. He has written four books for the public: "Natural Remedies for a Healthy Heart" by Avery Publishing Group in 1998, "The Resolution Diet," by Avery Publishing Group in 1999 ,"What Color is Your Diet?" published by Harper Collins/Regan Books in 2001, and the "L.A. Shape Diet" published by Harper Collins/Regan Books in 2004. His main research interests are obesity treatment and nutrition for cancer prevention and treatment.

SPEAKER BIOGRAPHY

Luke R. Howard

Dr. Howard received his B.S. degree in Horticulture from Purdue University, and his M.S. and Ph.D. degrees in Food Science from the University of Arkansas. He worked as an Analytical Chemist at the Dole Packaged Foods Research and Development Center for two years, and was an Assistant Professor in the Horticultural Sciences Department at Texas A&M University for five years. He has served on the faculty in the Department of Food Science at the University of Arkansas since 1997. His research program is focused on the identification and quantification of bioactive compounds in fresh and processed fruits and vegetables. Dr. Howard has published over 55 scientific articles and book chapters and is a Professional Member of the Institute of Food Technologists.

SPEAKER BIOGRAPHY

James A. Joseph

Lead Scientist ~ USDA/ Chief Neuroscience Laboratory

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Fairmont State College, Fairmont, WV	BS	1963-66	Biology
Virginia University, Morgantown, WV	MA	1967-69	Biopsychology
University of South Carolina, Columbia, SC	Ph.D.	1970-76	Behav. Neuroscience

AWARDS:

1985 Elected to Chair Gordon Conference on Biology of Aging.
1989 Sandoz Prize in Gerontology
2000 Alex Wetherbee Award for Blueberry Res. in Brain Aging

2002 Glenn/AFAR Award for Res. in Aging

2004 Denham Harman Award for Res. in Aging
2005 International Award for Modern Nutrition

PROFESSIONAL EXPERIENCE:

6/93-present--Lead Scientist, Lab Chief, Laboratory of Neuroscience USDA-ARS Human Nutrition Research Center, Boston, MA. (GS 15)

4/88-6/93 --Research Pharmacologist, Sr. Staff Scientist, Lab. of Cellular and Molecular Biology Gerontology Research Center, Baltimore, MD. (GM-14)

6/85 - 4/88--Research Pharmacologist, Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD. (GM-13).

6/82 - 6/85-- Senior Research Biologist, Department of CNS Research, Medical Research Division of American Cyanamid Company, Lederle Laboratories, Pearl River, NY.

3/76 - 6/82--Instructor in Behavioral Biology, Johns Hopkins University Medical School, Baltimore, MD.

3/76 - 6/82--NIH Senior Staff Fellow, Gerontology Research Center, NIA, NIH, Baltimore, MD.

SPEAKER BIOGRAPHY

Wilhelmina Kalt

Research Scientist, Food Chemistry, Agriculture and Agri-Food Canada. Atlantic Food and Horticulture Research Centre, 32 Main St. Kentville Nova Scotia B4N 1J5.
Email: kaltw@agr.gc.ca

Dr. Kalt obtain her Ph.D. degree from North Carolina State University and is currently employed with the Canadian federal agriculture department, Agriculture and Agri-Food Canada.

Dr. Kalt's research on the health benefits of berries has focused on the phenolic components of blueberry species. Through several studies, she has characterized the effects of production and production factors on the level of antioxidant phenolics in blueberries. She has also worked extensively on the separation of berry flavonoid mixtures for *in vitro* studies. Dr. Kalt is currently conducting animal and human studies to assess the bioavailability and functional bioactivity of blueberry flavonoids.

SPEAKER BIOGRAPHY

Raika Koli

Researcher, Doctoral Student
Biomarker Laboratory
National Public Health Institute
Helsinki, Finland

Raika Koli received her M. Sc. in Nutrition from the University of Helsinki (Department of Applied Chemistry and Microbiology). She also worked at the department for several years together with Prof. Marja Mutanen and Adj. Prof. Riitta Freese in projects focusing on the health effects of rapeseed oil and oat betaglucan.

Since 2005 she has been working as a researcher at the Biomarker Laboratory at the National Public Health Institute (KTL) in Helsinki together with Dr. Iris Erlund and Adj. Prof. Georg Alfthan. Her work focuses on the health effects of berries and the bioavailability of polyphenols. This work will be included in her doctoral thesis.

SPEAKER BIOGRAPHY

Laura A. Kresty

Assistant Professor
Cancer Chemoprevention Program
Division of Hematology & Oncology
Department of Internal Medicine
302B CCC Building
410 W 12TH AVE
Columbus, OH 43210
Office Phone: 614-688-7787
Cell Phone: 614-202-0521
FAX: 614-293-4072
E-mail: kresty.1@osu.edu

Research Interests:

The focus of my research is cancer chemoprevention of aerodigestive tract lesions. It has been estimated that 35% of cancer deaths could be prevented through dietary modifications. This in turn has sparked interest in assessing the role of both specific foods and food-based bioactive components as cancer chemopreventive agents. My interests are in identifying and evaluating potential inhibitors utilizing *in vitro* assays, employing clinically relevant *in vivo* models to study carcinogenesis, and translating the evaluation of promising agents into phase I and phase II trials in high risk human cohorts. Currently we are evaluating both natural and some synthetically produced agents including: freeze-dried black raspberries in Barrett's esophagus patients; cranberry proanthocyanidins, strawberry and black raspberry extracts, a licorice extract, isothiocyanates, and a number of histone deacetylase inhibitors utilizing *in vitro assays*; and we are working in a hamster respiratory tract model to evaluate the chemopreventive potential of indole 3-carbinol, anethole dithiolethione, and the polyamine α-difluoromethylornithine. We are also working on novel imaging technologies to detect early epithelial and sub-epithelial changes for more rapid evaluation of chemopreventive agents. Lastly, we are interested in investigating the link between obesity and cancer, specifically esophageal adenocarcinoma.

SPEAKER BIOGRAPHY

Mary Ann Lila

Mary Ann Lila is a Professor in the Department of Natural Resources & Environmental Sciences, and Interim Associate Dean for Research in the College of ACES, University of Illinois. Dr. Lila's research team is internationally recognized for isolation, bioassay, and characterization of bioactive, health-protective components from fruits, medicinal herbs and their *in vitro* cell cultures. Her work is featured in over 130 peer-reviewed publications. Dr. Lila has large ongoing research projects in Egypt, Central Asia, Oceania, Mexico and subSaharan Africa, and she is an Associate Director of the Global Institute for BioExploration (GIBEX). Dr. Lila's team has extensive expertise in the structural chemical characterization of natural products, with full access to one of the world's most advanced NMR and LC-MS facilities in the School of Chemical Sciences.

Dr. Lila was elected to serve as the US correspondent for the International Association of Plant Tissue Culture and Biotechnology (1994-1998), as President of the Society for In Vitro Biology (2000-2002), and as a Fellow of the Society for In Vitro Biology (2003). She was previously Associate Director of the nationally acclaimed Functional Foods for Health Program (1997-2000). Dr. Lila has been honored with the Paul A. Funk Scholarship Recognition Award (the premier research award in her college), the Spitze Professorial Career Excellence Award, the Faculty Award for Excellence in Research, the University Scholar Award, the Amoco Award for Excellence in Undergraduate Instruction, and the Lilly Endowment Teaching Fellowship. In 1999, Dr. Lila won a Fulbright Senior Scholarship to conduct research and outreach in New Zealand, and returns to Australasia at least once/year.

SPEAKER BIOGRAPHY

Dr. G. (Joe) Mazza

- Principal Food and Bioscience Research Scientist at the Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia.
- Adjunct Professor in the Food, Nutrition and Health Program at the University of British Columbia, Vancouver; Food Science Department, University of Manitoba, Winnipeg, and Department of Human Biology and Nutritional Sciences, University of Guelph.
- B.Sc. and M.Sc. degrees in Food Science from the University of Manitoba, and his Ph.D. from the University of Alberta, Edmonton, Canada. Post Doc at the Institute of Chemistry, Université Louis Pasteur, Strasbourg.
- Extensive research experence in the areas of phytochemistry and engineering/processing of functional foods and nutraceuticals, including extraction, separation, characterization, standardization and efficacy of bioactives, especially flavonoids/anthocyanins as antioxidants and anti-inflammatory agents, essential oils for natural flavours/fragrances and as antimicrobials; and proteins and polysaccharides as nutraceuticals and food ingredients.
- Authored/co-authored over 150 research papers, several critical reviews, over 25 book chapters, and four books.
- Founder and editor for the CRC Press Functional Foods and Nutraceuricals Book Series; the editor and a co-author of the 1998 CRC Press book *Functional Foods: Biochemical and Processing Aspects;* a contributor and co-editor of the book *Herbs, Botanicals and Teas as Functional Foods and Nutraceuticals* published by CRC Press in 2000, and a co-editor of the book *Functional Foods: Biochemical and Processing Aspects. Volume 2* published by CRC Press in 2002. The Functional Foods and Nutraceuticals Book Series aims at serving all those involved with and interested in foods and/or food components that provide health benefits beyond those attributable to basic nutritional functions. It offers a comprehensive treatment of the emerging science and technology of functional foods and nutraceuticals which are shown to play a role in preventing or delaying the onset of diseases, especially chronic diseases. He is also a co-author of the 1993 CRC Press book *Anthocyanins in Fruits, Vegetables and Grains.*
- Patented several products/processes in Canada and United States, authored/-co-authored over 165 technology transfer articles/presentations, and contributed significantly to the development and commercialization of several technologies dealing with value-added processing of plant products.
- Received the 1992 Public Service of Canada Merit Award for his outstanding research, and was the 1994 recipient of the W. J. Eva Award of the Canadian Institute of Food Science and Technology (CIFST) for his contributions to food science in Canada through outstanding research and service. In 2002, Dr. Mazza received the Queen's Jubilee Medal for his work in the area of functional foods and nutraceuticals.
- In 2005, he was named by the Information Sciences Institute (ISI) as a world's most cited and influential researcher in Agricultural Sciences, and in 2006 he was elected Fellow of the International Academy of Food Science and Technology (FIAFoST).

SPEAKER BIOGRAPHY

Tony K. McGhie, Ph.D.

Dr McGhie received his B.Sc. from the University of Auckland and M.Sc. from the University of Waikato. He then completed his Ph.D. at The University of Queensland in molecular plant pathology in 1997. Currently, Dr McGhie leads the Healthful Fruit Team of the Horticulture and Food Research Institute of New Zealand. The Team is focused on investigations to better understand the health benefits of fruit consumption. Previously, Dr McGhie has held several research positions in New Zealand and Australia including Marine Research, CSIRO, Hobart; the Bureau of Sugar Experiment Stations, Brisbane; the Veterinary Research Institute, the Department of Agricultural & Rural Affairs, Melbourne; and MAFTech New Zealand.

SPEAKER BIOGRAPHY

Riitta Puupponen-Pimiä

Born	7.4.1956 Helsinki, Finland
Marital status	Married, four children
Languages spoken	Finnish, English, Swedish (basic)
Present position	VTT Technical Research Centre of Finland, Espoo, Finland • Senior Research Scientist • Vice Group Manager

Education
University of Technology, Helsinki, Finland

- M.Sc. (chem. eng., biochemistry), 1983
- Lic. Tech. (biochemistry), 1988
- PhD(Tech) (biochemistry and microbiology), 1995
  (Differentiation and development in plant cell cultures)

Scientific career
Teaching Assistant of Biochemistry, University of Technology

- Plant cell and tissue culture of medicinal plants 1983-1988

Research Scientist, Academy of Finland

- Plant cell and tissue culture 1988-1991
- Plant molecular biology

Research Scientist, VTT Biotechnology (Plant Technology)

- Plant molecular biology 1991-1998

Group Manager, VTT Biotechnology (Plant Biotechnology Group)
1.11.1997-28.2.1998
1.8.2002-31.12.2002

Senior Research Scientist, VTT Biotechnology (Plant Technology)
Vice Group Manager, VTT Biotechnology (Plant Biotechnology Group)

- Bioactive compounds and bioactivity of berries 1998-
- *In vitro* bioactivity assays
- Functional foods
- Food processing

SPEAKER BIOGRAPHY

Other scientific activities

- Supervision of several M.Sc. thesis at the University of Technology, Helsinki and University of Helsinki and diploma works at the University of Applied Sciences Wädenswil, Switzerland
- Scientific secretary of VTT Future Foods Research Program 1997-2000
- Projects manager and coordinator of several VTT and Tekes (Finnish Funding Agency for Technology and Innovation) projects concerning bioactive berry compounds and berry phenolics
- Coordinator of Tekes BERRYDRUG ("Therapeutically active berry compounds") project, 2005-2008
- Member of Management Committee of COST 926 action - Impact of new technologies on the health benefits and safety of bioactive plant compounds, 2004-2007

SPEAKER BIOGRAPHY

Ronald L. Prior, Ph.D.

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 has been with the Agricultural Research Service of the USDA for more than 25 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 provides leadership for their expanding phytochemical and health research program. Dr. Prior has published more than 180 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 most recent 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. Specific research thrusts include studying the anthocyanins in fruits and obtaining data on the proanthocyanidin content of fruits and vegetables as part of efforts to develop a flavonoid database for foods for the USDA food nutrient database. Dr. Prior has developed a food antioxidant capacity database and this information continues to drive some of the specific foods that are studied relative to identifying bioactive components in foods and studying their absorption and metabolism.

Dr. Prior has served as reviewer for the Journal of Nutrition, British Journal of Nutrition, Journal of Agriculture and Food Chemistry, International Journal of Chemical Kinetics, Nutrition and Cancer, Clinical Chemistry, Free Radical Biology and Medicine, Journal of Nutritional Biochemistry, and others. Dr. Prior is a member of the American Society for Nutritional Sciences and the American Chemical Societies Food Chemistry Division.

SPEAKER BIOGRAPHY

Jess D. Reed, Professor

PROFESSIONAL PREPARATION

Oregon State University, Corvallis, Oregon	Animal Science	B.Sc., 1975
Cornell University, Ithaca, New York	Animal Science	M.Sc., 1981
Cornell University, Ithaca, New York	Animal Science	Ph.D., 1983

APPOINTMENTS

Professor, Dept. of Animal Sciences, University of Wisconsin – Madison 7/1/1999 to present

Associate Professor, Dept. of Animal Sciences, University of Wisconsin – Madison 7/1/1995

Assistant Professor, Dept. of Animal Sciences, University of Wisconsin – Madison 1/1/1989

Nutritionist, International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia. 3/3/1983

RESEARCH

Jess Reed is Professor of Animal Nutrition at the University of Wisconsin-Madison with a BSc (1975) from Oregon State and MSc (1980) and PhD (1983) from Cornell. He researches the effects of phytochemicals on animal and human nutrition and health. His early research during his thesis studies and while at the International Livestock Centre for Africa focused on potentially toxic and anti-nutritional phytochmeicals in tropical forage legumes. Highlights of this research included studies on the interactions between proanthocyanidins, protein and fiber in forages in relationship to methods of estimating nutritional value. Reed's research interest has recently shifted to studies on the chemistry of proanthocyanidins in relationship to putative effects on human health and nutrition. This research includes development phytochemical methods for characterizing proanthocyanidin structure coupled with research on the relationship between structure and biological activity in models of atherosclerosis and urinary tract infections.

SPEAKER BIOGRAPHY

Navindra P. Seeram

Navindra P. Seeram, Ph.D., is Assistant Director of UCLA Center for Human Nutrition and Assistant Professor at the David Geffen School of Medicine at UCLA. His doctorate, in natural product chemistry, was obtained from the University of the West Indies and he conducted postdoctoral research at Michigan State University. His research has been widely reported in peer-reviewed journals, book chapters, trade magazines and at scientific conferences. The focus of his research is on the evaluation of bioactive agents from foods, spices, traditional herbal medicines and botanical extracts, using laboratory, animal and human studies, for the prevention and treatment of chronic human illnesses such as cancer and heart disease.

SPEAKER BIOGRAPHY

Gary D. Stoner, Ph.D.

Gary Stoner is a Professor Emeritus in the Department of Internal Medicine at The Ohio State University. He also has joint appointments in the Departments of Pathology, Oral Biology, and Human Nutrition.

Dr. Stoner has conducted studies in chemical carcinogenesis and cancer chemoprevention for more than 30 years and is an expert in the area of aerodigestive tract cancers. Currently, his laboratories are conducting studies of the ability of various chemopreventive agents to inhibit cancer in the esophagus and colon. The results of his research are documented in more than 300 peer-reviewed publications and book chapters. Dr. Stoner has received several awards including the NIH MERIT Award. He has served on multiple NIH Study Sections and as Chair of the Carcinogenesis, Environment and Nutrition Advisory Panel for the American Cancer Society. Currently, he serves as Chair of the newly formed NIH/NCI Chemo/Dietary Prevention (CDP) Study Section. He is past President of the Carcinogenesis and Molecular Biology Specialty Sections of the Society of Toxicology, and of the Ohio Valley Society of Toxicology.

SPEAKER BIOGRAPHY

Takanori Tsuda

College of Bioscience and Biotechnology, Chubu University
1200 Matsumoto-cho, Kasugai, Aichi, 487-8501 Japan

Education; Affiliation and Research Carrier:

1988	M. Agr. Graduate School of Agriculture, Nagoya University
1988-1995	Research Associate, Food Research Institute, Aichi Prefectural Government
1995-2003	Lecturer, Associate Professor, Tokai Gakuen University
1999	Ph.D., Nagoya University
1999	Encouragement Award of Japanese Society for Food Science and Technology
2003	Encouragement Award of the Japanese Society of Nutrition and Food Science
2003-2006	Associate Professor, Research Center for Biomarkers of Preventive Medicine, Doshisha University
2006-	Associate Professor, College of Bioscience and Biotechnology, Chubu University

Research Field:
Functional Food Science, Nutritional Biochemistry

ORAL PRESENTATIONS

Characterization of biologically active compounds and commercial fruit quality in different strawberry genotypes: a study on antioxidant capacity of bioactive compounds and their role *in vitro* and *in vivo*.

Sara Tulipani, Franco Capocasa*, Bruno Mezzetti* and Maurizio Battino
Institute of Biochemistry- Faculty of Medicine and *Dept. of Environmental and Crop Sciences (SAPROV), Università Politecnica delle Marche, Ancona, Italy.

Email: m.a.battino@univpm.it

Many epidemiological studies confirm that an high consumption of fruit is correlated with a lower incidence of several chronic diseases, mainly for the protective role against cardiovascular, degenerative and proliferative diseases. There is convincing evidence that the considerable health benefits of fruit are due to its specific chemical composition, particularly to compounds of nutritional relevance. For these reasons, the nutritional quality of fruit today is becoming an attribute as important as the organoleptic-sensorial quality, and breeding and biotechnological approaches are currently used to increase the content of specific bioactive compounds in fruits.

Strawberry fruit contains high levels of micronutrients, such as essential minerals and vitamins, and phytochemical compounds belonging to the huge class of phenolics. Most of these compounds exhibit antioxidant properties, and their bioactivity seems to be directly or indirectly linked to their relevant role in protecting essential biomolecules from free radicals and reactive oxigen species induced damages. In order to describe the role played by genetic background on attributes that are essential for the nutritional qualities of the fruit, we compared a number of phytochemical parameters of a selected number of genotypes from the Italian strawberry breeding program. In particular, the total flavonoid, anthocyanin, vitamin C and folate contents were measured, and cultivar effects on the total antioxidant capacities (TAC) of strawberries were also tested. In addition, the individual contribution of the main antioxidant compounds was assessed by HPLC separation coupled to an on-line postcolumn antioxidant detection system. The results showed the important role played by genetic background on the chemical and antioxidant profile of strawberry fruits. Significant differences were found between genotypes for the total antioxidant capacity, and for all tested classes of compounds. In particular, very high differences among cultivars and selections were observed on the folate content. The HPLC analyses allowed to resolve four main regions containing the most antioxidant capacity, and a qualitative and quantitative variability in the antioxidant profiles was observed. Furthermore, the relevant contribution of vitamin C and anthocyanins on the antioxidant profile of fruit was confirmed, and a good correlation was found between the total anthocyanin content measured using the pH differential method and the anthocyanin concentrations obtained according to the HPLC data.

These studies show that differences exist between cultivars to be applicable in dietary studies in human subjects. These findings are important since higher levels of micronutrients and phytochemicals may be an important tool to support a higher intake of health-promoting compounds, even in case of low consumption of fruit.

ORAL PRESENTATIONS

- Beekwilder J., Jonker H., Meesters P., Hall R.D., van der Meer I.M., Ric de Vos C.H. Antioxidants in raspberry: on-line analysis links antioxidant activity to a diversity of individual metabolites. J Agric Food Chem. 2005;53:3313-3320.
- Bompadre S., Leone L., Politi A., Battino M. Improved FIA-ABTS Method for antioxidant capacity determination in different biological samples. Free Rad Res 2004;0:1-8.
- Koponen J.M., Happonen A.M., Mattila P.H., Törrönen A.R. Contents of Anthocyanins and Ellagitannins in Selected Foods Consumed in Finland. J Agric Food Chem. 2007;65:1612-1619
- Määtta K. R., Kamal-Eldin A., Törrönen A. R. Identification and quantification of phenolic compounds in berries of Fragaria and Rubus species (Family Rosaceae). J Agric Food Chem. 2004, 52:6178-6187.
- Scalzo J, Politi A, Pellegrini N, Mezzetti B, Battino M. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition. 2005;21:207-13.

Key words: Total antioxidant capacity, strawberry genotype, nutritional quality, phenols, folate

ORAL PRESENTATIONS

Berry Phenolics and Their Fate within the Body

Alan Crozier, University of Glasgow, UK

The diverse spectrum of anthocyanins and phenolic compounds detected in raspberries, blueberries, blackcurrant, redcurrant, cranberries and strawberries determined by HPLC-PDA-MS^2 and HPLC with an on-line antioxidant detector system will be discussed. Research on the fate of anthocyanins within the body following ingestion will also be discussed and illustrated by describing the results of two studies. The first involves monitoring anthocyanins in rat tissues and fluids after acute supplementation with raspberry juice in quantities equivalent to a 70 kg human drinking 500 mL of juice per day. In the second investigation, 200 g of strawberries, with and without 100 mL of double cream, were consumed by human volunteers after which the anthocyanin content of plasma and urine were monitored for a 24 h period. Finally, an investigation will be described in which use was made of hamsters which are a valuable model for the study of atherosclerosis as the development of the disease is similar to its onset in humans. Male hamsters received an atherogenic diet for 12 weeks and at the same time, were fed either raspberry, bilberry or strawberry juice a daily dose of 7.14 ml/kg. After 12 weeks, there was 79-96% reduction in aortic lipid deposition as well as significant reductions in the activity of hepatic antioxidant enzymes in animals that had received a juice supplement. This suggest that, because of their phenolic content, moderate consumption of berries and berry juices can help prevent the development of early atherosclerosis.

Key words: berries, anthocyanins, bioavailability, reduced atherosclerosis

ORAL PRESENTATIONS

What Color Is Your Berry?

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Key Words: berries, phytochemicals, cancer prevention, anti-inflammation, antioxidation

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Berries contain a diverse range of phytochemicals with biological properties. They have antioxidant, anticancer, anti-neurodegenerative, and anti-inflammatory activities. We have studied the phenolics of six popularly consumed berries--blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry derived from the whole fruit using high performance liquid chromatography with ultraviolet (HPLC-UV) and electrospray ionization mass spectrometry (LC-ESI-MS) detection. The major classes of berry phenolics are anthocyanins, flavonols, flavanols, ellagitannins, gallotannins, proanthocyanidins, and phenolic acids. Berry phenolics inhibit the growth of human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), and prostate (LNCaP) tumor cell lines at concentrations ranging from 25 to 200 micro μg/mL. Black raspberry and strawberry extracts showed the most significant pro-apoptotic effects against the colon cancer cell line, HT-29, which produces COX-2. Recently, we have investigated the metabolic fate of pomegranate ellagitannins and have found that they are hydrolyzed to ellagic acid which is then absorbed over a six hour period. By 12 to 24 hours urinary urolithins begin to appear as the result of gut bacterial biotransformation and conjugation in the liver. These urolithins also inhibit prostate cancer cell growth. The in vitro and metabolic studies set the stage for clinical trials in appropriate individuals of the cancer preventive properties of berries in the diet.

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Lay Summary

What Color is Your Berry?

Colors represent families of substances made by plants called phytochemicals, which plants make to protect them from oxidation and to carry out other functions for the plant such as growth regulation and fighting off pests. Mankind evolved on plant foods and our bodies have developed mechanisms to process

these phytochemicals using the same specialized proteins we use to break down drugs that we take, such as aspirin. There are over 25,000 phytochemicals and color provides an easy way to introduce diversity into the diet while reinforcing mindful eating. Americans eat too few colorful fruits, vegetables, and berries. Berries are in the red-purple group and there are many different berries with similar phytochemical profiles. We have tested many of these in the laboratory and they have the ability to inhibit cancer cell growth. Much more research must be done to demonstrate that they have similar effects in humans, but berries are a delicious and healthy way to get the antioxidant and anti-inflammatory compounds that scientists are currently studying for their many health benefits.

ORAL PRESENTATIONS

Processing Effects on Berry Polyphenolics

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Berry fruit are a rich source of polyphenolics including anthocyanins, ellagitannins and flavonols. The polyphenolics found in berries have high antioxidant activity and are reported to possess numerous health-promoting properties. As a result of seasonal production and limited shelf-life, berries are processed into various shelf-stable products (juices, purees, canned). Additionally, berries are commonly preserved by freezing, with frozen fruit serving as the raw material for further processed products. Although significant quantities of berries are consumed in a processed state, little information is available on how different processing methods and storage of processed products influence the polyphenolic content and antioxidant capacity of berries. In this study, frozen blackberries, blueberries and black raspberries were processed into juices (non-clarified and clarified), canned products (water and syrup) and purees. The effect of freezing (IQF) and subsequent storage at -20°C was also evaluated. Processed products were stored for 1 day, 1, 3, and 6 months at ambient temperature and evaluated for polyphenolics by HPLC, polymeric color using a colorimetric assay, and antioxidant capacity using the oxygen radical absorbing capacity (ORAC) assay. Freezing and subsequent frozen storage had a minimal effect on polyphenolics and antioxidant capacity, but significant losses occurred in all thermally treated products in response to processing and during storage. Major losses of anthocyanins and ellagitannins occurred during juice processing, with appreciable levels being retained in the presscake. Anthocyanins were more susceptible to thermal degradation during pasteurization of juices, purees and canned products and more prone to leaching into the liquid medium in canned fruit than flavonols or ellagitannins. Major losses of monomeric anthocyanins occurred during storage of all thermally processed products. These losses were accompanied by significant increases in polymeric color values indicating that anthocyanins are extensively polymerized during storage. The ORAC values remained stable during storage suggesting that polymeric anthocyanins possess potent radical scavenging capacity that offsets the loss of monomeric anthocyanins. Flavonols and ellagitannins showed greater stability than anthocyanins during storage. Results from this study indicate that frozen berries retain high levels of polyphenolics and antioxidant capacity, but extensive losses of polyphenolics, especially anthocyanins occur during processing and storage. Mitigation strategies are needed to prevent losses of polyphenolics during processing and storage.

Keywords: Blackberries, black raspberries, blueberries, processing, storage, polyphenolics, antioxidant capacity

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ORAL PRESENTATIONS

The beneficial properties of berryfruit polyphenolics in brain aging involves reductions in oxidative and inflammatory signalling cascades.

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Numerous epidemiological studies have indicated that individuals who consume a diet containing high amounts of fruits and vegetables exhibit fewer age-related diseases such as Alzheimer Disease (AD). A recent report has indicated that individuals who consumed a diet containing 2.5 servings of fruit and vegetables/day were 40% less likely to develop AD. Research from our laboratory has suggested that dietary supplementation with fruit or vegetable extracts high in antioxidants (e.g., blueberry, BB) can decrease the enhanced vulnerability to oxidative stress (OS) that occurs in aging and these reductions are expressed as improvements in behavior. In addition to their antioxidant and anti-inflammatory activities, there appear to be additional multiple mechanisms involved in the beneficial effects observed from these supplementations. These mechanisms include enhancement of neuronal communication via alterations in neuronal signaling. Previous work, for example, shows that BB supplementation from 4 to 12 months of age in APP/PS1 mice can offset the putative deleterious consequences of amyloid beta deposition on behavior in APP/PS-1 mice by increasing extracellular signal regulated kinase (ERK) and protein kinase C (PKC), two important signaling factors in learning and memory. These same MAP kinases also appeared to be activated in learning in both aged and young rats, are enhanced by blueberry supplementation, and are correlated with behavioral performance. It also appears that polyphenolic compounds, such as those found in BB may exert their beneficial effects by enhancing the endogenous antioxidant and neuronal signaling capabilities of the organism. One of the most striking effects of BB supplementation may involve increases in neurogenesis that were associated with increases in ERK and insulin growth factor-1 (IGF-1). More recent findings suggest that both strawberry (SB) and BB can increase neurogenesis and subsequent cognitive performance in the aged rodents. Taken together, these findings, along with those showing increases in MAP kinase and IGF-1 signaling associated with enhancements in cognitive function in the BB-supplemented animals, suggest that antioxidant-rich fruits such as BBs may improve behavior by enhancing neuronal signaling and ultimately, neuronal communication. However, there appears to be important additional factors involved in the effects of the BB polyphenols. Recent research using BV-2 mouse microglial cells has also indicated that the antioxidant properties of BB and possibly other berryfruits (SB), may be derived at least in part for their abilities to inhibit stress signals (e.g., nuclear factor kappa B, NFκB, and cyclic AMP response element binding protein, CREB, and cytokines) induced by oxidative/inflammatory stressors. In addition it also appears that BB can reduce stress signaling by decreasing the stress induced activation of p38MAPK, CREB, and PKCγ. Similar findings have been found using BB treated primary hippocampal cells that were exposed to oxidative stressors. Thus, in addition to their well known free radical scavenging effects that are seen with assessments of oxygen radical absorbance capacity (ORAC), It appears that BB and possibly other berryfruit (e.g., strawberries) and concord grapes can directly reduce stress signaling. Clearly, however, the antioxidant/anti-inflammatory effects of the berryfruit polyphenols may only represent a small aspect of their beneficial properties in aging.

ORAL PRESENTATION

Distribution of anthocyanins in pig tissues after long-term blueberry feeding

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The effects of anthocyanin (ACN) on physiological processes have been demonstrated in a variety of studies (1). Of particular interest are studies that report ACN effects in *in vitro* and *in vivo* models that may be relevant to human health. In animal studies comparing the effects of berry vs. vegetable consumption on neurological function, berry crops with a high ACN concentration gave rise to the greatest effects (2). While ACN continue to be studied in neuroscience (3), cardiovascular physiology (4), and in primary metabolism (5) information on how anthocyanins are absorbed, metabolized and distributed in the body, is limited.

Most published reports that examine ACN bioavailability only examine the ACN concentration in plasma and urine, are typically done over a short time frame, and employ very high ACN doses. However, to elucidate the potential physiological significance of ACN, it is important to know how ACN are absorbed, metabolized, and distributed to sites in the body where they may be physiologically important (e.g. brain, eyes). Equally critical is an understanding of ACN bioavailability at normal dietary levels rather than at pharmacological levels. In addition, it is important to understand the effects of a complex dietary food matrix and long term intake on bioavailability. Research on ACN bioavailability constitutes a difficult analytical challenge primarily because ACN absorption into animal and human tissues is extremely low (10^{-8} - 10^{-10} per gram of fresh weight of tissue). In contrast, other classes of flavonoids can be found in the body in concentrations up to 100 times higher. Studying ACN bioavailability is made more difficult when food sources have complex (e.g. blueberry, (BB)) rather that simple (e.g. elderberry) ACN profiles (6).

We have undertaken studies to examine ACN bioavailability using a pig model. Pigs are suitable study subjects for understanding human nutrition, since their gastrointestinal absorption is similar to humans (7). Pigs are omnivores and will eat essentially all foods common in the human diet, and they have similar body weights to humans. The usefulness of the pig model in cardiovascular studies is also well-documented (8).

Two separate BB feeding trials using pigs were conducted. Results of the first trial, which examined ACN bioavailability as a function of BB feeding duration and BB dose, will be the focus of this report. The second feeding trial examined the effect of basal diet on ACN bioavailability. In the first trial, pigs were fed diets containing a variety of plant-based feeds. Soy, oats and barley (4:2:1 w/w) constituted 70% (w/w) of the diet. Pig diets were supplemented with whole freeze-dried, powdered blueberries, *Vaccinium corymbosum* L. 'Jersey'. BB were added at 0, 1, 2 and 4% (w/w) of the diet, which was based on ACN concentration and daily feed intake. These feeding levels corresponded roughly to consumption of 0, 1, 2 and 4 cups of BB per day. Pigs (n= 5) remained on experimental diets for 4 and 8 weeks, then fasted for approximately 18 h prior to being euthanized. Selected tissues, organs, and fluids were collected for analysis of their ACN content, including liver, kidney, small and large intestine, various brain regions, eyes, muscle, feces, blood and urine.

Concentrations of native ACN were examined in the first round of analysis. Currently,

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Key words:
Inflammation, aging, oxidative stress, behavior, stress signaling

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ACN conjugates are being examined using similar methods. Results to date showed that native ACN were found in all tissues where they were sought. There appeared to be greater allocation of ACN to specific sites in the body (e.g. kidney > liver). Also the relative proportion of some ACN (e.g. malvidins) was greater in tissues than their proportion in BB fruit. The tissue concentration of the individual native ACNs examined so far ranged from 0.23 to 0.6 ng/g FW tissue. Interestingly, no ACN was detected in plasma and this is likely due to the 18 h fast prior to euthanasia.

Several improvements were made in the methods employed for the study. Tissue extraction methods were modified from those reported by Tsuda et al. (9). Study of the relative stability of ACN in tissue and tissue extracts during long-term-80° storage revealed that ACN were more stable in fresh frozen, compared to freeze dried tissue. With the goal of attaining greater sensitivity during HPLC analysis, Coularray detection was explored as an alternative to DAD monitoring, but was subsequently abandoned. The extremely low concentration (10^{-9} g/ sample) of ACN in resolubilized tissue extracts was found to be stable over a 20 h period before LC-MS analysis. Eluant diversion after HPLC separation was used to minimize fouling of the ion source during MS detection. MS analysis was conducted using an electrospray interface, operating in the positive ion mode, and using single ion monitoring.

It is hoped that the results of this study will contribute to our understanding of the potential significance of ACN at physiologically relevant sites (e.g. brain, eye), when ACN-containing foods are consumed in normal doses over a long period of time.

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ORAL PRESENTATIONS

"The Health Effects of Berry Consumption in Subjects at Risk for Cardiovascular Disease"

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Berries are a rich source of various polyphenols. Experimental studies (mainly *in vitro* studies) indicate that the compounds exhibit biological activities such as anti-inflammatory, anti-aggregatory and antioxidative activities. However, few studies have investigated the long-term effects of polyphenols in humans, particularly using berries as a dietary source.

We investigated the effects of berry consumption on haemostatic function and the bioavailability of polyphenols in a randomized parallel setting. In this study, middle-aged non-medicated subjects (n=72) with cardiovascular risk factors consumed moderate amounts of berry or control products for eight weeks. The subjects in the berry group (n=36) consumed whole bilberries, as well as berry nectar, juice and puree, prepared mainly from lingonberries, blackcurrants and chokeberries. The amount consumed daily was approximately 100 g of berries and 2 dl of nectar or juice. The subjects in the control group (n=36) consumed sugar water, marmalade caramels, and puddings prepared from semolina and rice.

The data is currently being analyzed. At the moment it is clear that various polyphenols were bioavailable from the berries. Significant changes in some indicators of haemostatic function have also been detected. More detailed results will be presented and discussed at the symposium.

Keywords: berries, polyphenols, flavonoids, bioavailability, health effects, haemostatic function, human.

Modulation of apoptosis, cell cycle and MAPK pathways in human SEG-1 esophageal cells by a cranberry extract

Key Words: Chemoprevention, cranberry extract, esophageal cancer, Barrett's, SEG-1

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Abstract:

Over the past 20 years, esophageal adenocarcinoma (EAC) has increased dramatically in the western world and is currently the seventh most common cause of cancer mortality among US males. Diagnosis often occurs late in the course of EAC pathogenesis contributing to the poor overall five-year survival rate of 15%. Esophageal cancer is the third most deadly malignancy supporting the crucial need for improved preventative and treatment strategies. The basis for the increase in EAC remains to be fully elucidated, but has been linked to gastroesophageal reflux disease (GERD), a high fat diet, obesity, and a limited number of additional dietary factors. Conversely, plant based diets have consistently lowered esophageal cancer risk. Recently, cranberry extracts have been shown have chemopreventive potential when evaluated in breast, colon and prostate cancer cell lines. In addition, preliminary work in our lab showed that a cranberry extract inhibited cell proliferation and induced G1 cell cycle arrest in EAC cells.

The purpose of this study was to investigate the potential mechanisms associated with chemoprevention by cranberry proanthocyanidins (PAC) in SEG-1 esophageal adenocarcinoma cells. SEG-1 cells were treated either with 50 μg/ml of PAC or vehicle (veh) and global gene expression arrays were employed to explore potential mechanisms of chemoprotection. RNA was isolated from PAC or veh treated SEG-1 cells six hours post-treatment. Total RNA was extracted, cDNA generated, transcribed into cRNA, and hybridized to Affymetrix human U133 2.0 plus oligonucleotide microarrays to detect PAC-induced changes in gene expression. WEDGE++ analysis was conducted to determine significant differentially expressed genes by treatment. Next, EASE analysis was conducted to identify key biological themes altered following PAC treatment. Microarray findings and real-time PCR validation supported that PAC treatment upregulated the apoptotic genes BAK1, BID, BCL2L1, DFFA, TNFRSF10A, TP73, TRADD and PAC down regulated APAF1, BAG4, FAS, and TRAF2. PAC treatment of SEG-1 cells predominately down regulated cell cycle pathway markers including PCNA, Ki67, CHEK1, CDCs, and multiple Cyclins. In addition, PAC treatment significantly down regulated genes involved in MAPK signaling, a pathway recently reported to be activated by acid and bile exposure, as occurs with gastroesophageal reflux disease. Since aberrant signaling has been implicated in carcinogenesis, other cellular pathways modulated by PAC are being investigated, including WNT, TGF-Beta, and Insulin signaling. Our results support that cranberry PAC's significantly alter multiple processes associated with esophageal cancer progression and further studies are warranted to assess the cancer inhibitory potential of this extract *in vivo*.

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ORAL PRESENTATIONS

Dietary Berries & Performance Enhancement

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Both epidemiological evidence, and an expanding portfolio of scientific research studies have linked the consumption of berry fruits to specific inhibition of human diseases, through antioxidant and/or other mechanisms of action. In addition to their capacity to specifically prevent disease onset, or therapeutically inhibit disease progression, berry fruits also are capable of providing non-specific adaptogenic benefits that enhance human healthspan and longevity. As adaptogens, dietary berries can help to bolster the human body's resistance to a range of physico-chemical stresses, and provide a balancing, normalizing influence on human metabolism. In preliminary tests, mice administered blueberry flavonoids at a 50 mg/kg dosage for two weeks were able to survive oxygen deprivation in a hypoxia chamber for significantly longer than untreated animals. Similarly, treated mice demonstrated enhanced endurance in forced weighted swim trials, in particular after repeated challenges. Parallel treatments in a rat model demonstrated some similar trends, but did not result in significantly enhanced performance. Treatment with mixed flavonoid extracts and proanthocyanidin-rich fractions from freeze-dried blueberries also resulted in significantly elevated intracellular ATP concentration in a rat glial cell line, which has implications for greater cellular efficiency and possible elevated metabolic rate. Upcoming experiments will determine if berry components are stimulating cells to burn more glucose or fatty acids, which may contribute to faster overall metabolism.

Keywords: balance, endurance, metabolic enhancement, immune response, stress resistance

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ORAL PRESENTATIONS

Anthocyanins and Heart Health

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Abstract
Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are responsible for most of the red, blue, and purple colors of fruits, vegetables, flowers, and other plant tissues or products. They are particularly abundant in berries and other fruits with red, blue, or purple color, and in red wines. Approximately 400 individual anthocyanins have been identified. The daily intake of anthocyanins in humans has been estimated at 180–215 mg/d in the United States. This value is considerably higher than the intake of other flavonoids such as flavones and flavonols in the Dutch diet (23 mg/d, measured as aglycones). Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. Servings of 100 g of berries can provide up to 500 mg of anthocyanins.
In recent years, numerous studies have shown that anthocyanins display a wide range of biological activities including antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic activities, improvement of vision, induction of apoptosis, and neuroprotective effects. In addition, anthocyanins display a variety of effects on blood vessels and platelets that may reduce the risk of coronary heart disease. The antioxidant activity (scavenging free radicals, metal chelation, protein binding) of anthocyanins including the protection of LDL against oxidation, has been demonstrated in a number of different in vitro systems. Recently, we found that pelargonidin, cyanidin, delphinidin, peonidin, malvidin, malvidin 3-glucoside, and malvidin 3,5-diglucosides have strong inhibitory effects on NO production in LPS/IFN-γ-activated RAW 264.7 macrophage. At the range of 16-500 μM, these compounds inhibited NO production by >50% without showing any cytotoxicity. Their inhibitory effects were comparable to that of quercetin, which has been extensively studied and shown to exert anti-inflammatory and antioxidant effects. Anthocyanin-rich berry extracts also showed considerable inhibitory effects on NO production, and their inhibitory effects were significantly correlated with the content of total anthocyanins.
The association between grape phenolics and coronary heart disease has been ascribed in part to the presence of anthocyanins in red wine. In addition, several epidemiological studies have shown that coronary heart disease mortality can be decreased by moderate consumption of red wine. The primary mechanisms believed to be responsible for this reduced risk factor include reduced platelet coagulability, and higher circulatory high-density lipoprotein cholesterol (HDL). Other mechanisms such as inhibition of lipoprotein oxidation, free-radical scavenging and modulation of eicosanoid metabolism are also thought to play a role in the reduction of atherosclerosis.
Biological activities of anthocyanins are closely linked to their absorption and metabolism. Recent evidence from several laboratory indicates that absorption of anthocyanins from food is limited and the concentrations found in plasma are in the nM to low μM range. Most other studies have also reported low relative urinary excretion, ranging from 0.004% to 0.1% of the intake. Thus, it is evident that anthocyanins have diverse effects in vitro which suggest potential health benefits in general and reduction of coronary heart disease in particular. However, until the absorption and metabolic fate of anthocyanins in vivo is unravelled, it would be unwise to conclude that a high consumption of anthocyanis will reduce the risk of chronic disease, including heart disease. Definite proof can only be obtained by large, long-

term intervention trials. Such trials need to be seriously considered, and if initiated must be properly designed. In the meantime the evidence for the benefits connected with consumption of anthocyanin-rich products should include a more complete knowledge of the identity of anthocyanin metabolites and their tissue distribution using molecular, cell biology, animal and epidemiological studies. Future in vitro investigations on identifying the physiological effects of anthocyanins/flavonoids should be conducted with chemical structures that exist in the circulation (i.e. parent compound and metabolites) and at similar concentrations.

Key words
Coronary heart disease, anthocyanins, pelargonidin, cyanidin, delphinidin, peonidin, malvidin, flavonoids, antioxidant activity, LDL oxidation, atherosclerosis, anti-inflammatory, apoptosis, NO production, neuroprotective, blueberries, raspberries, strawberries, cranberries, black currants, cherries.

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ORAL PRESENTATIONS

The *In vitro* and *In vivo* Antioxidant Properties of Berry Fruit

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The term 'antioxidant' has become well known over the last decade and the consumption of antioxidants is widely regarded as being beneficial to health and well-being (*1*). This intense interest has lead to a large increase in the number of foods that are promoted as high in antioxidants. Berry fruit are naturally high in antioxidant capacity because of their inherent content of phytochemicals such as flavonoids, phenolic acids and vitamins (*2,3*).

The interest in antioxidants rises from the convergence of two lines of investigation. Firstly, humans as aerobic organisms utilise oxygen for the production of energy and we are therefore continuously exposed to potentially damaging free radicals that may produce oxidative damage. Although humans, and in fact all aerobic organisms, utilise built-in mechanisms for protection from oxidative damage, these mechanisms are not absolute and their reducing efficacy is believed to be responsible for ageing and increased risk for many degenerative diseases. There is accumulating evidence that oxidative damage is associated with many, if not all diseases. Secondly, many population-based (epidemiological) studies have shown that the consumption of fruits and vegetables is beneficial to health and may provide protection against conditions such cardiovascular disease, some cancers, neurodegenerative diseases, and others. Although there is uncertainty about which components of fruits and vegetables are responsible for this effect, a popular hypothesis is that antioxidants are at least partly responsible, and therefore it is desirable to consume more antioxidants. Consequently there is a view that consumption of foods (or supplements) with high concentrations of antioxidants will provide protection from the inevitable oxidative damage, with concomitant protection from degenerative diseases. This paper will discuss the antioxidant capacity of berry fruit and evidence that berry fruit preparations may have biological antioxidant capacity and protect against oxidative damage *in vivo*.

The term 'antioxidant' has a specific chemical meaning and the potency of the antioxidant capacity for any given compound or sample can be measured by a battery of assays. Many different assays exist, and the relative methodology and mechanisms have recently been reviewed (*4*). It is well established that the antioxidant capacities of berry fruit are high, and usually higher than those of most other fruits and vegetables. In our research with 250 genotypes of blackcurrant we have found that antioxidant capacities vary ($ORAC_{FL}$: 71-194 μmol TE/g FW, and FRAP 25-86 μmol TE/g FW). Anthocyanins are the main contributor to antioxidant capacity in blackcurrant ($ORAC_{FL}$ R=0.8655; FRAP R=0.7197) and concentrations of anthocyanins range from 180 to 732 mg/100 g FW in these same genotypes of blackcurrant. Even though the genotypes studied contain substantial vitamin C concentrations (range 68-274 mg/100 g FW), the correlation between vitamin C concentrations and antioxidant capacity was not high ($ORAC_{FL}$ R=0.0743; FRAP R=0.2551).

These results show that blackcurrants, like other berry fruit, have high concentrations of compounds with high antioxidant capacity and that there is potential for increasing the antioxidant capacity of commercial blackcurrant products even further through the breeding of new commercial cultivars. However, it is critically important to determine if the high *in vitro* antioxidant capacities of berry fruit results in an antioxidant effect *in vivo* and a health benefit through the reduction of oxidative damage. The measurement of total antioxidant

capacity (TAC) in blood (plasma or serum) has been considered a good indicator of overall oxidative status (*5*) and a number of human studies have shown increases in TAC following consumption of berry fruit preparations (*6,7*). However, these results have recently been questioned (*8*). The bioavailability of polyphenolic compounds in berry fruit is low and the concentrations resulting in plasma following consumption appear to be insufficient to account for the observed increase in plasma TAC. Furthermore is appears likely that the increases in plasma TAC are a result of increased urate concentrations in plasma that result from the fructose consumed in berry fruit preparations. We recently confirmed this view in a study where no increase in plasma TAC ($ORAC_{FL}$) was observed after a anthocyanin-enriched extract of blackcurrant was administered to pigs despite there being a significant increase in plasma anthocyanin concentrations (*9*).

Although these observations argue against an *in vivo* or biological antioxidant effect for berry fruit, studies with cell cultures have demonstrated an antioxidant protective effect for blackcurrant and Boysenberry polyphenolics when cells are exposed to concentrations (0.13-1 μM) that might be expected to occur in plasma following consumption of berry fruit (*10*).

We have also conducted animal and human studies to investigate the biological antioxidant effect of berry fruit. Oxidative damage was assessed by measuring biomarkers of oxidative damage to protein (carbonyls), lipids (malondialdehyde), and DNA (8-oxo-2'deoxyguanosine), and plasma antioxidant status (plasma TAC ($ORAC_{FL}$) and vitamin E) in rats that had been acclimatised to different base diets and fed a Boysenberry extract with substantial *in vitro* antioxidant (*11*). This study found that after two weeks, the Boysenberry extract showed biological antioxidant activity and raised the TAC of plasma while decreasing some biomarkers of oxidative damage, but the effect was highly modified by the base diet. The base diets (chow (CD), synthetic/soybean oil (SD), or synthetic/fish oil (FD)) had significant effects on the biomarkers of oxidative damage and antioxidant status, with rats fed FD having the lowest levels of oxidative damage and the highest antioxidant status. For example, plasma malondialdehyde (MDA) was 45 ng/mL for the FD-fed rats and significantly higher with 182 ng/mL for the SO-fed rats. When Boysenberry extract was added to the diet, there was little change in 8-oxo-2'deoxyguanosine excretion in urine, protein carbonyls decreased, and plasma MDA either increased or decreased depending on the base diet. For example, the mean protein carbonyl concentration for the CD-fed rats was 0.21 nmol/mg protein for the control rats and was significantly lower at 0.07 nmol/mg protein when 10% Boysenberry extract was added to the diet. Interestingly, MDA concentrations decreased to 36% of the control for the SD rats, increased by 256% for the FD, and remain unchanged for the CD-fed rats when 10% Boysenberry extract was added to the diet.

To determine if daily supplementation with either blackcurrant or Boysenberry juice drinks could improve measures of oxidative damage in humans, a study was undertaken with an elderly population with below-average memory abilities (*12*). The study design was a fully blinded parallel intervention with a placebo control (synthetic colour, flavour, sugar). The study involved 51 participants and the intervention period was for 12 weeks, with an extension period to 24 weeks for 30 participants. Plasma TAC significantly increased for both the Boysenberry and blackcurrant treatments compared with the placebo. Plasma MDA decreased in both the Boysenberry and blackcurrant treatments but the decrease was not statistically significant.

These studies show that berry fruit (blackcurrant and Boysenberry) that have substantial *in vitro* antioxidant activity also demonstrate *in vivo* biological antioxidant activity in cell, animal, and human studies. Our experience shows that biological antioxidant activity is readily demonstrated in cell and animal studies, but biological antioxidant activity (reduction

of oxidative damage) responses are much more difficult to measure in free-living humans. Possible reasons for this include: i) extreme variations in measures of oxidative damage; ii) lack of relevant and specific measures of oxidative damage with sufficient sensitivity; and iii) variation in individual responses because of underlying genetics or health status.

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ORAL PRESENTATIONS

Therapeutically Active Berry Compounds – *In Vitro* and *In Vivo* Effects on Human Health

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Wild berries are traditionally an important part of the daily diet for many people in Nordic countries, where almost 40 edible berry species are grown. In Finland the annual yield of bilberry, which is one of the most important wild berries, has been estimated to be as large as 200 million kg. In addition of being healthy in general, berries have a long history in folk medicine in the Nordic area. Bilberry, for example, has been used as a medicinal herb for treatment of diarrhea and to improve night vision, and cranberry for urinary tract infections. Many health effects of berries are associated to phenolic compounds, which the berries are rich in. Flavonoids, phenolic acids, lignans and polymeric tannins are typical for berries. Many berries, such as bilberry and black currant, are rich sources of anthocyanins, which give the dark red or blue colour to the berry fruit. Some berries, such as cloudberry, raspberry and strawberry contain substantial amounts of polymeric ellagitannins. These compounds are not found in any other common food, so these berries remain their sole source in our diet. Phenolic compounds are potent antioxidants, and exhibit various other physiological activities including anti-inflammatory, antimicrobial, antiallergic, anticarcinogenic and antihypertensive activities in *in vitro* assays or in cell or animal models. Epidemiological studies indicate that diet rich in phenolic compounds correlates with lower risk of cancer and cardiovascular disease (Knekt *et al.* 1997; Vanharanta *et al.* 1999). However, there is still lack of clinical studies verifying these beneficial effects.

Antimicrobially active berry compounds and their mechanisms of action have been intensively studied in our laboratory over the past ten years in several research projects (Puupponen-Pimiä *et al.* 2001, 2005a,b, Nohynek *et al.* 2006). In the *in vitro* studies phenolic berry extracts of common Nordic berries selectively inhibited the growth of harmful bacteria and human intestinal pathogens, without affecting the growth of beneficial lactic acid bacteria. *Salmonella* and *Staphylococcus* strains were the most sensitive bacteria, and cloudberry and raspberry the most efficient berries (Puupponen-Pimiä *et al.* 2001, 2005b). *Campylobacter jejuni* and *Candida albicans* were inhibited with phenolic extracts of cloudberry, raspberry, and strawberry, which all were rich in ellagitannins (Nohynek *et al.* 2006). Several mechanisms of action in the growth inhibition of bacteria seem to be involved, such as destabilization of cytoplasmic membrane and permeabilization of plasma membrane. Antimicrobial activity of berries may also be related to antiadherence of bacteria to epithelial cells. As a consequence of antimicrobial activity, diet rich in berries may affect the composition of intestinal microbiota. So far there is very few *in vivo* data available of the effects of berry phenolics on human gastrointestinal microbiota. Therefore we decided to carry out a large human clinical trial on volunteers with metabolic syndrome, and who have consumed a berry rich diet over a certain period of time. The analyses of this trial focuses on intestinal microbiota and subsequent microbial conversions, on phenolic metabolites of berries and on blood lipids. Bioinformatics are used to evaluate the data collected in the clinical trial in the context of systems biology approach. Clinical trial has just been completed and analyses of faeces, urine and blood samples are in progress. Preliminany results of the

clinical trial will be presented. In this research project selected berry extracts are also screened especially against human pathogens causing gastrointestinal and urinary tract infections, and against inflammatory diseases using various *in vitro* models. *In vitro* and *in vivo* data obtained will be utilised in berry-based functional food and in drug development.

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ORAL PRESENTATIONS

Berries and Berry Anthocyanins: Interactions with Dietary Fat Levels in a Mouse Model of Obesity

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Anthocyanins are the components in berries that give them their dark red or blue colors. They occur primarily as glycosides of their respective aglycone anthocyanidin-chromophores. There are about seventeen anthocyanidins found in nature, whereas only six of them, cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Pn), pelargonidin (Pg), and malvidin (Mv), are ubiquitously distributed. Anthocyanins have an important function in plant physiology. Because of their intense color, anthocyanins are also regarded as potential candidates for natural colorants in the food industry. However, recently, increased attention has been focused on their possible health effects. Anthocyanins have been shown to be strong antioxidants, and may exert a wide range of health benefits through antioxidant and/or other mechanisms. Recent studies (*1, 2*) with anthocyanin extracts from purple corn and cherries have demonstrated a protection against the development of obesity in a mouse model of obesity. The "mix" of anthocyanins in different foods varies, but cyanidin-3-glucoside is a common and predominant anthocyanin in purple corn, cherries and most berries. No studies have been completed on anthocyanins in berries and possible effects on obesity. In our first study, we fed freeze dried powders of whole strawberry and lowbush blueberry to C57BL/6 mice, an obesity mouse model as previously used. Mice received purified diets containing either 10% of calories from fat (Low Fat, LF) or a high fat diet [either 45% of calories from fat (HF45) or 60% of calories from fat (HF60)]. Freeze dried berry powders from whole blueberries (BB) or strawberries (SB) were included at 10% of the diet in both the LF and HF45 diets. Anthocyanin intakes (mg/d) in the first study were from blueberries (3.3, 3.7) and strawberries (0.52, 0.58) for low fat and high fat diets, respectively. In this first study, the LF diet was fed as control and the HF45 diet was used as the obesogenic diet. In the second study, we also provided an extract of the anthocyanins from blueberry and strawberry in the drinking water. Body composition was determined utilizing Echo MRI. Berries added to the LF diet did not alter weight gain, final body weights, body fat or protein (% BW), or diet or energy intake. However, in HF45 mice, weight gain, final body weights, body fat (%) and epididymal fat weights increased and body protein decreased ($p<0.01$) compared to LF mice. In mice fed the HF45 diet plus BB but not strawberry, body weight gains, body fat (% of BW) and epididymal fat weights were significantly greater than in the HF45 fed controls. SB or BB feeding did not alter glucose tolerance although glucose tolerance decreased with age and in HF45 vs LF mice. Baseline plasma glucose was lower in SB vs HF45 fed mice. After 8 weeks of feeding in the second study, mice fed the HF60 diet plus an extract of anthocyanins from BB in the water, had lower body weight gains and body fat than the HF60 fed controls. Berry anthocyanins when fed as the whole berry, did not prevent, and to the contrary, may have increased obesity in a high fat but not in a normal low fat diet. Extracted blueberry, but not strawberry anthocyanins when fed in a high fat diet decreased weight gains and fat deposition. The results suggest an interaction of components in blueberry with level of fat in the diet and a differing response with extracted anthocyanins compared to anthocyanins in the whole berry.

ORAL PRESENTATIONS

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KEYWORDS: Anthocyanin, Anthocyanidin, Flavonoids, Antioxidant, Food Colorant, Cyanidin, Antioxidant, Obesity

ORAL PRESENTATIONS

Cranberry proanthocyanidins and cardiovascular health

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Keywords: cranberries, flavonoids, proanthocyanidins, oxidation, atherosclerosis, cardiovascular disease, inflammation, endothelium-dependent vasodilation, cyclooxygenase-2.

Cranberries contain many polyphenolic compounds that have anti-inflammatory, bacterial anti-adherence and anti-oxidant activity in cell culture, animal models and clinical research. These properties suggest that cranberry consumption may reduce the risk of cancer, cardiovascular disease, and infections of the mouth, stomach and urinary tract. Cranberries contain at least 10 hydroxycinnamic acids, more than 20 flavonols, 6 anthocyanins, and a complex mixture of oligomeric flavonoids known as proanthocyanidins that may exceed one thousand individual compounds. We research the structure of proanthocyanidins in relationship to effects on cardiovascular health.

The earliest form of atherosclerosis is a fatty streak, formed when intimal macrophages have accumulated oxidized LDL in an unregulated manner. Oxidized LDL is recognized by scavenger receptors of macrophages and engulfed. Our research showed that cranberry proanthocyanidins specifically associate with LDL after addition to serum and remain associated with LDL throughout the isolation and preparation procedure. The cranberry proanthocyanidins increase the lag time of Cu^{+2} induced LDL oxidation. Therefore, cranberry proanthocyainidns are effective anti-oxidants when associated with LDL and if absorbed may protect against oxidation of LDL. Proanthocyandins with higher degree of polymerization (DP) may have greater affinity for LDL than oligomers of lower DP and provide more antioxidant protection.

Cyclooxygenase-2 (COX-2) is a regulatory enzyme in the conversion of arachidonic acid to prostaglandins and thromboxanes is associated with production of pro-inflammatory prostaglandins. Inflammation is associated with atherosclerosis and other cardiovascular diseases, several cancers (including colorectal, prostate and skin), arthritis, and Alzheimer's disease. The role of COX-2 in the production of pro-inflammatory prostaglandins and their association with pain and fever suggest that COX-2 has a role in the etiology of these diseases. Evidence linking inhibition of COX-2 to decreased risk of inflammatory diseases indicates that NSAID that are widely used for pain, arthritis and cardiovascular disease may decrease risk of colon cancer and Alzheimer's disease. Our results show that cranberry proanthocyanidins are anti-inflammatory because they inhibit *in vitro* and *in vivo* COX-2 expression.

Feeding experiments were designed to determine if feeding cranberry juice powder (CJP) to familial hypercholesterolemic (FH) swine affect total and LDL cholesterol. In the first trial, eight sows, 4 FH and 4 normal lipidemic (N), were fed a 20% fat baseline diet for 14 days followed by CJP supplement for 28 days. FH sows had 5 to 6 times more total cholesterol than N sows. Comparison of total cholesterol and LDL cholesterol during the feeding of CJP to the baseline indicated that feeding CJP significantly lowered total cholesterol and LDL cholesterol for FH sows, starting at week 1, but had no effect on normal sows. In a subsequent experiment, 6 FH swine littermates were assigned to a control baseline diet for 4 weeks, CJP treatment for six weeks, and a control washout diet for 4 weeks. There was a significant decrease in TC and LDLC in the first week of feeding CJP and the effect of treatment on total cholesterol and LDL cholesterol was significant. Total cholesterol and LDLC continued to decline throughout the 6 week CJP feeding period. There was a significant increase in TC and LDLC during the first week of the washout period although TC and LDLC did not return to baseline levels during the 4

week washout period. Our results suggest that high levels of cranberry intake may be beneficial in reducing cholesterol in hypercholesterolemic humans. More research is required to determine the dietary intake required and the mechanism by which cranberry intake influences cholesterol homeostasis.

Endothelium-dependent vasodilation, mediated through nitric oxide (NO), is impaired in atherosclerosis. Therefore, we hypothesized that cranberry-derived flavonoids would improve vascular function in vessels from pigs with naturally occurring high cholesterol and atherosclerosis. Iliac arteries were obtained at slaughter from adult normal pigs and familial hypercholesterolemic (FH pigs) that had been fed a basal diet or a diet including 15% cranberry juice powder (CJP). Artery segments (4-6 mm axial length) were stretched to 5 g tension suspended in tissue baths and vascular relaxation responses determined in response to bradykinin or S-nitroso-N-acetylpenicillamine. The dose-response to bradykinin induced relaxation was significantly impaired in FH pigs at 6 months of age ($p=0.031$), with a maximum relaxation of $29.2\pm7.7\%$ at 10^{-7} M bradykinin. FH pigs fed CJP for 6 months had relaxation responses that were improved ($56.8\pm10.2\%$ at 10^{-7} M bradykinin) over FH controls, although the difference was not significant ($p=0.068$). In FH pigs that were 12 months of age there was minimal relaxation to bradykinin at all doses, maximum of $12.2\pm3\%$ at 10^{-6} M bradykinin, implying a worsening of vascular function with age. Feeding FH pigs CJP significantly improved the relaxation response curve to bradykinin ($p=0.031$) with maximal relaxation of $34.08\pm8.4\%$ at 10^{-7} M bradykinin. The relaxation curve remained below that observed in normal pigs, although the difference was not quite significant ($p=0.057$). Feeding FH pigs cranberry juice powder improves *in vitro* vascular relaxation to near that of normal pigs. It remains to be determined what specific components of CJP are responsible for this effect.

ORAL PRESENTATIONS

Bioavailablility and Bioactivity of Strawberry Phytochemicals in Animals and Human Subjects

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Studies suggest that consumption of a phytochemical rich diet, which includes fruits and vegetables, contribute towards reducing the risk of some chronic human diseases such as certain cancers and heart disease. Berry fruits such as blackberries, black raspberries, blueberries, cranberries, raspberries and strawberries, are popularly consumed in our diet. We have recently shown that extracts of these commonly consumed berry fruits inhibit the growth and stimulate apoptosis of several human prostate, breast, colon and oral cancer cell lines *in vitro* (Seeram NP et al, J. Agric. Food. Chemistry, 2006, 54, 9329-39)

Strawberries (*Fragaria x ananassa*) contain a wide diversity and high levels of phytochemicals that have potent *in vitro* antioxidant, anti-inflammatory and anti-neurodegenerative properties. However, although the biological effects of these phytonutrients have been demonstrated *in vitro* (i.e. in laboratory tests), there is limited data on these effects exerted *in vivo* (i.e. in the living system). In addition, there are no published studies showing the bioavailability and bioactivities of strawberry phytonutrients or their metabolites in human circulation. We have recently used sensitive analytical liquid chromatography mass spectroscopy (LC-MS) methods to identify the individual phenolic phytochemicals present in strawberries (Seeram NP et al, Food Chemistry, 2006, 97, 1-11). Strawberries were found to contain a wide variety of polyphenols such as ellagic acid, ellagic acid glycosides, ellagitannins, gallotannins, flavonols (quercetin and kaempferol glucuronides and glycosides), anthocyanins (pelargonidin and cyanidin glycosides), flavanols and coumaroyl glycosides.

In the current study we investigated the bioavailablity of strawberry phytonutrients in wild type mice (C57BL6), which were orally gavaged with a standardized strawberry extract. Metabolites of strawberry phytochemicals such as quercetin glucuronide and anthocyanins were found in mouse plasma using LC-MS methods. We also collected plasma and urine from 20 normal healthy female subjects before and after they consumed 250 g frozen strawberries per day for 3 weeks. Several metabolites of strawberry phytochemicals were detected in urine samples using LC-MS methods. We also investigated the *ex vivo* biological effects resulting from strawberry consumption in human plasma and urine including: 1) Oxidative damage: via low density lipoprotein (LDL) oxidation, lymphocyte 8-hydroxydeoxyguanosine and comet assay as well as urinary isoprostanes; 2) Inflammation: via highly sensitive C-reactive protein (hsCRP), and 3) Induction of Phase II enzymes: via serum GST-α, UDP-glucuronosyltransferase and β-glucuronidase activity. This presentation will include an overview of the results obtained from our ongoing human study with strawberries.

ORAL PRESENTATIONS

Prevention of Gastrointestinal Tract Cancers with Berries

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For several years, our laboratory has been evaluating the ability of lyophilized black raspberries to inhibit carcinogen-induced cancer in the rodent esophagus and colon. Some of the known chemopreventive agents in black raspberries include vitamins A, C, E and folic acid; calcium and selenium; ß-carotene, α-carotene and lutein; polyphenols such as ellagic acid, ferulic acid, *p*-coumaric acid, quercetin and several anthocyanins; and, phytosterols such as ß-sitosterol, stigmasterol and kaempferol. In initial studies, lyophilized black raspberry powder was mixed into AIN-76A synthetic diet at concentrations of 5% and 10% and fed to Fischer 344 rats before, during and after treatment with the esophageal carcinogen, *N*-nitrosomethylbenzylamine (NMBA) (1). At 25 weeks of the bioassay, the berries were found to inhibit the number of esophageal tumors (papillomas) in NMBA-treated animals by 40-60% relative to NMBA controls. This inhibition correlated with reductions in the formation of the NMBA-induced O^6-methylguanine adduct in esophageal DNA. Ongoing studies indicate that berries influence NMBA metabolism and DNA adduct formation by inducing Phase I and II enzymes in the esophagus and liver (2). Black raspberries were also tested in a post-initiation scheme and were found to inhibit NMBA-induced esophageal tumorigenesis by 30-40% when administered after treatment of the animals with NMBA (1). Mechanistic studies indicate that they reduce the growth rate of premalignant esophageal cells, in part, through down-regulation of cyclooxygenase-2 (COX-2) leading to reduced prostaglandin production and, c-Jun, one of the activator protein-1 (AP-1) family of transcription factors (3). Berries also inhibit the expression of other genes associated with tumor development in the rat esophagus such as inducible nitric oxide synthase (iNOS) and vascular endothelial growth factor (VEGF) (3, 4).

Black raspberries have also been evaluated for chemopreventive effects in the rodent colon. When administered in the diet at concentrations of 2.5, 5 and 10% after treatment of F-344 rats with azoxymethane, the berries prevented development of all colon tumors by up-to 60% and of adenocarcinomas up-to 80% (5). The berries markedly reduced oxidative DNA damage in azoxymethane-treated animals, and lowered blood cholesterol levels by 10-15%. Similarly, at 10% of the diet, freeze-dried black raspberries caused a 50% inhibition of intestinal tumor development in the *Min* mouse model of familial adenomatous polyposis. The mechanism(s) by which berries prevent colon cancer in rodents are under investigation.

Using biodirected fractionation techniques, studies are being conducted to identify the active inhibitory components in berries. Both organo- and water-soluble extracts of berries were shown to selectively inhibit the growth and stimulate apoptosis of tumorigenic rat esophagus cells *in vitro* and to down-regulate the transcription activator proteins, AP-1 and nuclear factor-kappa B (NF-κB), and their associated kinases in JB-6 mouse epidermal cells (6). They also down-regulate VEGF via inhibition of the P13K/Akt pathway (7). Preliminary studies suggest that the anthocyanins in berries are amongst the most active inhibitory components (8). Recently, we have shown that an ethanol extract of berries is very effective in preventing UV-induced skin cancer in mice.

Based upon the above-described preclinical data, we have initiated prevention trials in humans to determine if berries might exhibit chemopreventive effects in the esophagus and

colon. In an initial Phase I clinical trial involving 10 normal subjects, freeze-dried black raspberries were administered orally for 7 days at a dose of 45 grams per day. This is equivalent to rodents consuming a diet containing approximately 5% black raspberries. The berries were well tolerated with minimal side effects. Ellagic acid and the anthocyanins; cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-xylosylrutinoside and cyanidin 3 –rutinoside, were all absorbed into the blood with peak plasma levels occurring within 2-4 hours of oral berry consumption (9). The absorption of these polyphenols however, was minimal and represented less than 1% of the administered dose. Several Phase IIa clinical trials of lyophilized black raspberries are underway in subjects with Barrett's esophagus and colonic polyps to determine if orally administered berries will modulate various histological and molecular biomarkers of esophageal and colon tumor development. Current progress in these trials will be discussed. Supported by the USDA, and NCI grants CA103180 and CA96130.

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ORAL PRESENTATIONS

Regulation of Adipocyte Function by Anthocyanins; Possibility of Preventing the Metabolic Syndrome

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Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are widely distributed in the human diet through crops, beans, fruits (berry), vegetables and red wine, suggesting that we ingest significant amounts of anthocyanins from plant-based daily diets. In general, anthocyanin pigments are stable under acidic conditions, but are unstable and rapidly broken down under neutral conditions. Therefore, anthocyanins have not been recognized as a physiological functional food factor. However, we demonstrated that cyanidin 3-*O*-□-D-glucoside (C3G), which is a typical anthocyanin, had antioxidative and anti-inflammatory activities based on in vitro and in vivo studies. These findings suggest that C3G has more beneficial effects beyond its antioxidant activity.

Adipocyte is the primary site of energy storage and accumulates triacylglycerol during nutritional excess. Recent studies have revealed that obesity is one of the central causal components in the metabolic syndrome and adipocyte dysfunction plays an important role in the development of this syndrome. Adipocyte synthesizes and secretes biologically active molecules called adipocytokines. Dysregulation of the adipocytokine production is strongly associated with the metabolic syndrome, and amelioration of the adipocyte dysfunction including the adipocytokine expression is one of the important targets for the prevention and treatment the metabolic syndrome.

Our prior study have demonstrated that dietary anthocyanins significantly suppressed the development of obesity, normalized hypertrophy of the adipocytes in the epididymal white adipose tissues and ameliorated hyperglycemia induced by the high-fat diet feeding of C57BL/6 mice (1). Also, we clearly demonstrated that anthocyanins enhance the expression of adipocytokine (adiponectin and leptin), PPAR□ and the adipocyte specific genes in isolated rat adipocytes without the stimulation of the PPAR□ ligand activity (2). However, there must be other anthocyanin responsive genes we did not find out in these studies and they can contribute to understanding the biological basis of anthocyanins and intensively utilize them as physiologically functional food factors. Based on these results, we examined that gene expression profile of human adipocytes treated with anthocyanis using DNA microarray analysis (3). Our observations have demonstrated the significant changes of adipocytokine expression. These data have provided an overview of the gene expression profiles in adipocytes treated with anthocyanins that merit further investigation.

Recently, we have demonstrated that dietary C3G ameliorates hyperglycemia and insulin sensitivity in type2 diabetic mice. The results would be presented in my talk.

Anthocyanins, which are some of the most popular plant pigments as food factors have a unique therapeutic advantage responsible for the regulation of the adipocyte function. Our findings provide a biochemical basis for the use of anthocyanins, which can also have important implications for preventing obesity and diabetes.

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Key Words; cyanidin 3-glucoside, anthocyanins, adipocyte, adipocytokine, obesity, diabetes,

POSTER PRESENTATIONS

Isolation identification and biological activities of phenolic compounds from strawberries

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Oxidative damage is thought to be one of the main mechanisms involved in nearly all chronic diseases. Phenolic compounds, which have strong antioxidant properties, also show anticancer, antiatherosclerotic and anti-neurodegenerative activities. Isolation and identification of food phenolic compounds is necessary since their nature, size, solubility, degree and position of glycosylation and conjugation influence their absorption, distribution, metabolism and excretion in humans. Freeze-dried whole strawberry fruit powder and strawberry fruits were extracted with methanol and further fractionated with different solvents. The fractions were then subjected to column chromatographic methods of separation and pure compounds were isolated and identified using high performance liquid chromatography mass (HPLC-MS) and nuclear magnetic resonance (NMR) spectrometric methods as cyanidin-3-glucoside, cyanidin-3-(6'-coumaroyl)-glucoside, coumaroyl-glucoside, kaempferol-3-(6'-coumaroyl)-glucoside. The extracts and pure compounds were evaluated for antioxidant activities using the trolox equivalent antioxidant capacity (TEAC) assay and ability to inhibit the proliferation of human oral (KB, CAL-27), prostate (LNCaP), breast (MCF-7), and colon (HT-29, HCT116) tumor cell lines at 25-200 μg/mL concentrations. The isolation and identification of strawberry phenolic compounds is necessary to generate standardized materials for in vitro and in vivo studies and for the authentication of strawberry-based food products

POSTER PRESENTATIONS

The Physiological Properties of Blackcurrant Anthocyanins: Improvement of Visual Function and Bioavailability

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Abstract

Blackcurrant contains four anthocyanins, in the proportions of 45% delphinidin-3-rutinoside (D3R), 15% delphinidin-3-glucoside (D3G), 35% cyanidin-3-rutinoside (C3R), and 5% cyanidin-3-glucoside (C3G). In a recent study, we showed that oral intake of 50mg blackcurrant anthocyanins (BCAs) prevented transient refractive alterations induced by working on video display terminals. This preventive effect of BCA was attributed to D3R and C3R causing relaxation of ciliary muscles during contraction induced by ET-1. It is possible D3R stimulates ET_B receptors localized in the ciliary epithelium to release NO, resulting in relaxation of the ciliary muscles. The critical concentration of this relaxing action of D3R and C3R that was significantly different from controls was 10^{-7}M.

Using LC-ESI-MS-MS we also determined the small amount of anthocyanin in plasma after oral intake of 50mg BCA. BCAs were absorbed directly and distributed as intact forms in the blood at a concentration of 10^{-9}M. At this concentration, BCAs may cause ciliary muscle relaxation *in vivo*. However, there is a discrepancy between this concentration and the critical concentration measured in our *in vitro* study.

POSTER PRESENTATIONS

Phenolic composition in Chilean commercial blueberries, strawberries, raspberries and blackberries

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These last five years, the Chilean berry production has increased considerably, focusing it production to North Hemisphere markets such as the United States of America and European Community countries during the winter season in that these countries are in the off season of their own production.

Although the low molecular weigh phenolic composition of commercial berries fruits (blackberry, blueberry, strawberry and raspberry) have been reported in different regions outside Chile (1-3), no satisfactory reports of Chilean berries anthocyanin and low molecular weight phenolic compositions are available.

The present study provides novel data on the anthocyanin and low molecular weight phenolic composition analyzed by HPLC-DAD (4) of commercial blackberry, blueberry, strawberry and raspberry fruits from different regions of Chile.

O'Neil, Duke, Bluecrop, Brigitta and Elliot blueberries cultivars were studied. In the case of raspberry, freeze Heritage and Meeker and fresh Heritage samples were studied. For strawberry and blackberry, Camarosa cultivar and Cherokee, Navaho and Loch Ness cultivars samples, respectively, were analyzed.

For all the cases, similar or higher concentrations of the compounds identified, than those described previously by other authors in these species were found for the samples studied.

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POSTER PRESENTATIONS

Differential brain regional specificity to blueberry and strawberry polyphenols in improved motor and cognitive function in aged rats

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Berryfruits contain a wide diversity of polyphenols belonging to different chemical structural classes; e.g., blueberries (BB) predominate in proanthocyanidins, whereas strawberries (SB) predominate in ellagitannins, although both berries contain anthocyanins. The types and relative amounts of polyphenols in different berries vary, possibly accounting for their relative differences in efficacy in behavioral models. Previously, we have shown that whole, crude berry extracts are able to reverse several parameters of brain aging as well as age-related motor and cognitive deficits when fed to rats from 19-21 months of age. These effects may be the result of direct effects on brain signaling or indirect effects through antioxidant and anti-inflammatory properties of the polyphenols. Thus, the present study examined two different berryfruit diets to determine whether the effects observed are indeed the result of differential effects of the polyphenols on the brain. Old (19 mo) F344 rats were fed a control, 2% BB, or 2% SB diet for 8 weeks prior to motor and cognitive testing. Results showed that SB-fed rats had improved performance compared to the BB-fed rats on the large plank, BB rats were better than SB rats on rod walking, while both diets improved motor function on the rotarod compared to control. Both berryfruit diets enhanced working memory in the Morris water maze. The number of proliferating precursor cells in the dentate gyrus of hippocampus showed a trend for the SB group to be higher than the control group, with the BB group in between these two groups. However, only the behavior of the BB group was significantly correlated with the number of proliferating cells in the dentate gyrus, even though overall the SB group had the greatest number of proliferating cells compared to any other group. We are currently assessing regional localization of the BB and SB polyphenols and their putative differential effects on signaling parameters in these rats, with a view toward determining selective bioavailability and mechanism(s) of action.

POSTER PRESENTATIONS

Challenges and strategies in breeding black raspberries (*R. occidentalis* L.) for improved nutraceutical value

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Black raspberry consumption has increased in recent years due in part to studies showing them to be particularly high in anthocyanins and other polyphenolics indicative of high antioxidant capacity. This has resulted in renewed interest in black raspberry (*Rubus occidentalis* L.) breeding. Present cultivars are ill adapted to the biotic and abiotic stresses of the Pacific Northwest, where the commercial black raspberry industry is centered, and fields must be replanted after 3-5 seasons. An incomplete, partial diallel, consisting of 26 sibling families from 10 parents, was constructed for the study of variation and inheritance of fruit chemistry, vegetative, and reproductive traits in black raspberry. Sibling families of one to eight plants were established at the Oregon State University Lewis Brown Farm in Corvallis, Oregon, and were arranged as a randomized complete block design. Fruit was collected from each plant, and pooled by family within blocks, to study variation in fruit chemistry properties including individual anthocyanin profiles, total anthocyanins, total phenolics, and others. Although there were many similarities, strong trends in phenotype based on pedigree were observed for most traits indicating a strong genetic component. For all of these traits, additive genetic effects were significant and more important than dominance effects. Estimated narrow-sense heritability for total anthocyanins was low (0.30) while heritability of the individual anthocyanins was generally moderate (range = 0.39-0.76). In addition, variation in the proportions of individual anthocyanins was observed in progenies of the wild parent which was not present in named cultivars. The results indicate potential for progress breeding for these traits from careful breeding and selection within the population of plants studied.

POSTER PRESENTATIONS

Effect of the geographical origin on the phenolic composition of the endemic Chilean berry "Maqui" [*Aristotelia chilensis* (Mol.) Stuntz]

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The endemic Chilean berry Maqui (*Aristotelia chilensis*), an edible black-colored fruit produced by a bush with many branches, which grows in dense thickets and that Chilean traditional medicine attributes healing properties, has been described as a rich source of, indole alkaloids, phenolic compounds such as anthocyanins (1,2,3). These compounds confer Maqui fruits and its derivatives important antioxidant properties such as inhibition of LDL oxidation *in vitro* and protection of human endothelial cells against oxidative stress (2).

Considering that phenolic composition depends on environmental (eg. light, temperature) and genetic (eg. specie, ecotype, cultivar) factors, among others, the aim of this work was to know the differences on total phenolic, tannin and anthocyanin concentration in this Chilean native berry from 15 different geographical origins, in order to have better information at the moment of plan pharmacological uses for Maqui fruits. Besides, by using HPLC-DAD (4) the anthocyanin concentration and for the first time the low weight molecular phenolic composition were determined.

The total phenolic content varied from 165.5± 0.3 to 300.4± 0.7 GAE Kg^{-1} of fresh fruit; the total tannin content from 13.3± 0.1 to 41.8± 0.4 g Kg^{-1} of fresh fruit; and the total anthocyanin content varied from 131.4 ± 0.4 to 274.9± 0.6 mg/100 g of fresh fruit. The relative high anthocyanin content and the important presence of flavonol derivatives makes the fruits of *A. chilensis* an interesting source of phenolic extracts for food and pharmaceutical uses, but the effect of the geographical origin of the berries must to be under consideration because its effect on the fruit phenolic concentration.

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POSTER PRESENTATIONS

Low molecular weight phenolic and anthocyanin composition of the "Murta" (Ugni Molinae Turcz.), a Chilean native berry

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Ugni Molinae (Turcz.), known locally as "Murta" or "Murtilla", is a native plant of the Central South region of Chile, used by the Chilean aborigines (Mapuches or Araucanos) of that region since pre-Hispanic times due to its medicinal and nutritive properties.

The murta fruits, small pink to red berries when are fully ripe and the size of a blueberry, were consumed fresh by the aboriginal population as a part of their diet. In the same way, these fruits were used for preparing a psychostimulant drink (murta liquor). Besides, leaves were employed as an analgesic, antiinflammatory, stomachic, antiemetic, antihemoptisic, cicatrizant, antiulcerogenic, vulnerary, and antidiarrhetic agent. Some of these medicinal properties such as scar healing, vulnerary, analgesic, antiinflammatory, intestinal astringent, hemostatic, and psychostimulant (anxiogenic nature) properties have been proven experimentally with *in vitro* pharmacological techniques (1,2).

The aim of this work was, by using spectrophotometrical technics and HPLC-DAD-MS (3), study the total phenolic, low molecular weight phenolic and anthocyanin composition of murta berries from different areas of the South of Chile, in order to know the levels and distribution of these compounds. The ranges of concentration for total phenolics varies from 42.5 to 149.6 GAE Kg^{-1}, for total anthocyanins from 0.7 to 7.0 mg Kg^{-1} and for total tannins from 2.1 to 17.0 mg Kg^{-1}, depending mainly of the area and the ecotype of plant. In the case of the analyses carried out by using HPLC-DAD-MS, the main low molecular phenolic compounds were some phenolic acids and derivatives from flavonol glycosides. Only two anthocyanins were identified in the samples studied.

Under our knowledge, this is the first report of the phenolic composition of Murta and by using this information it could possible establish new potential uses for this Chilean native berry.

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POSTER PRESENTATIONS

Dose-Response and Metabolism of Pelargonidin-3-Glucoside from Strawberries

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A clinical study was conducted to investigate the dose response and metabolism of strawberry anthocyanins. In a crossover study design, twelve healthy adults consumed each of three strawberry treatments. The treatments were 100 g, 200g, and 400 g of pureed strawberries, delivering 15.2 mol, 30.4 mol, and 60.7 mol anthocyanin, respectively. Urine samples were collected for 24 hours after each dose, and samples were analyzed by HPLC with DAD and ion trap mass spectrometry. Pelargonidin-3-glucoside (P3G) was the major anthocyanin form in the treatments, and P3G and three metabolites of P3G (detected as monoglucuronides) were observed in urine after the strawberry ingestion. One predominant monoglucuronide form was detected in urine in masses 10-fold higher than the other two monoglucuronide forms. Increasing dose resulted in increasing appearance of anthocyanins in urine, and mass of each pelargonidin monoglucuronide increased in urine with increasing dose. These results suggest that pelargonidin-3-glucoside absorption and metabolism are not saturated at masses less than or equal to 60 mol, thus showing that more strawberry anthocyanin can be absorbed with increasing dose. This work was supported by the U.S. Department of Agriculture.

POSTER PRESENTATIONS

Study of phenolic composition of leaves from five ecotypes of the native Chilean Strawberry (*Fragaria chilonesis* L. Duch)

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The Chilean wild strawberry (*Fragaria chiloensis* L. Duch.) is a native species of Chile that was domesticated before the arrival of the Spanish conquers. This species is one of the progenitors of the commercial strawberry (*Fragaria* x *ananassa*), and is widely distributed throughout the country in variable agroecological systems (1). Apparently, these diverse habitats have forced this species to develop a high variability of morphological and agronomic traits.

Until now, the majority of the studies of the antioxidant properties of the commercial and native strawerry have been focused in the frbuits (2).

By using biochemical and molecular markers in wild accessions that are a part of the Chilean Strawberry germoplasm collection of the Universidad de Chile, we have differentiated 19 ecotypes. From this point and as a part of a research project, we studied the phenolic composition of the leaves of the five ecotypes selected. Total phenolic concentrations range in the leaves samples from 21,7 ± 2,9 to 54,5 ± 1,1 GAE Kg^{-1} f.w. and the total tannins from 9,5 ± 0,3 to 33,4 ± 0,2 procyanidin equivalents per Kg of f.w. By using HPLC-DAD (3) we have determined the low molecular weight phenolic composition of the samples, in that the ellagic acid and its derivatives and flavonols (aglycones and glycosides) were the most important compounds identified and quantified in the native strawberries leaves. By using these results and as a second part of the research project, leaves extracts will be used in the formulation of some nutraceuticals products.

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