CSREES Award Number: 2005-35503-16186
Title: Enzymatic Synthesis of Trans-Free Structured Lipids and Their Food Applications
University: University of Georgia
Project Director and Co-PD: Casimir C. Akoh (PD) and William L. Kerr

ABSTRACT:
Incorporation of stearic acid into canola oil to produce *trans*-free structured lipid (SL) as a healthy alternative to partially hydrogenated fats for margarine formulation was investigated. Response surface methodology was used to study the effects of Lipozyme RM IM from *Rhizomucor miehei* and *Candida rugosa* lipase isoform 1 (LIP1) and two acyl donors, stearic acid and ethyl stearate, on the incorporation. Lipozyme RM IM and ethyl stearate gave the best result. More SLs were synthesized using Lipozyme RM IM and the products compared to SL made by chemical catalysis, and fat from commercial margarines. After short-path distillation, the products were characterized by GC and RPHPLC-MS to obtain fatty acid and triacylglycerol profiles, ^{13}C NMR spectrometry for regiospecific analysis, X-ray diffraction for crystal forms, and DSC for melting profile. Stearic acid was incorporated into canola oil, mainly at the *sn*-1, 3 positions for the lipase reaction and no new *trans* fatty acids formed. Most SL products did not have adequate solid fat content or β' crystal forms for tub margarine, although these may be suitable for light margarine formulation. Ongoing studies include increasing solid fat content and regulating the crystal forming habit of the SLs by using emulsifiers and/or by blending the SLs with palm oil fractions.

Publications/Presentations:
Lumor, S.E., Lee, G.C., Shaw, J.F. and Akoh, C.C. 2006. Enzymatic incorporation of stearic acid into canola oil: effects of lipase and acyl donor types. Presented at the American Chemical Society National Meeting, Atlanta, GA, March 26-30.

Lumor, S.E., Jones, K.C., Ashby, R., Strahan, G., Kim, B.H., Foglia, T.A., and Akoh, C.C. 2007. Characterization of structured lipids synthesized by incorporating stearic acid into canola oil for possible food application. Presented at the 98th AOCS Annual Meeting and Expo, Quebec City, Canada, May 13-16.

Lumor, S.E., Lee, G.C. Chang, S.W., Shaw, J.F., Kays, S.E., and Akoh, C.C. 2007. Effects of lipase and acyl donor types on the incorporation of stearic acid into canola oil to produce *trans* free fat. J. Agric. Food Chem. (in review).

Lumor, S.E., Jones, K.C., Ashby, R., Strahan, G., Kim, B.H., Foglia, T.A., Kays, S.E., and Akoh, C.C. 2007. Characterization of *trans* free structured lipids synthesized by incorporating stearic acid into canola oil for possible food application. J. Agric. Food Chem. (in review).

CSREES Award No. 2005-35503-16168
Project Title: Interactions and consequences of lipid and protein oxidation in muscle foods
Principal Investigator: Hebert O. Hultin
Co-principal Investigator: Ann T. Ballesteros
University of Massachusetts/Amherst

Lipid oxidation in muscle foods causes unpleasant sensory changes. In muscle foods, phospholipids associated with membranes are the primary substrate for lipid oxidation. The objectives of this study were to determine if gallic acid esters (GAE) of varying alkyl chain lengths could be directed into the membranes of washed cod muscle and whether these GAE demonstrate oxidation inhibition against hemoglobin-mediated lipid oxidation.

Washed fish muscle was prepared from minced cod muscle and adjusted to pH 7.2. Oil was added to provide competition between phospholipids and triacylglycerols. GAE were individually prepared in ethanol at equivalent molar concentrations before addition. Cod muscle membranes were precipitated using differential centrifugation and analyzed spectrophotometrically to determine GAE presence. Hemoglobin prepared from haddock frames was added to the samples at 6µmol/kg washed cod for the oxidation studies. Lipid oxidation was monitored over seven days by measuring lipid peroxides and thiobarbituric acid reactive substances (TBARS).

GAE detection in the membranes, with or without added oil, increased as alkyl chain length increased. However, the samples treated without oil showed higher overall GAE uptake values (ppm, lipid basis) than samples with oil. The relationship between GAE membrane concentration and effectiveness at inhibiting lipid oxidation is currently under investigation.

CSREES Award Number: 2005-35503-16147
Title: Hexanal Synthesis in Isolated Soy Proteins
University of Kentucky
Project Director: William L. Boatright

Soybeans are the second largest food crop in the U.S. Soybean proteins are underutilized in human foods because of their undesirable taste. Hexanal is one of the most powerful odorants from aqueous isolated soy proteins (ISP). This investigation examined the mechanism by which hexanal is synthesized in aqueous ISP mixtures.

Solid state electron paramagnetic resonance (EPR) spectroscopy was used to determine the level of free radicals from aqueous ISP mixtures containing either erythrobate or dithiothreitol (DTT). Headspace hexanal levels in aqueous ISP were measured using gas chromatography/mass spectrometry.

Hexanal levels from ISP (that lacked residual lipoxygenase activity) were increased by as much as 13-fold with the addition of sodium erythrobate (2.3 mM) or DTT (6.5 mM). Solid state EPR spectroscopy of ISP combined with 2.3 mM sodium erythrobate and frozen in liquid nitrogen exhibited a 18-fold increase in carbon-centered radical (g = 2.005). The level of carbon-centered radicals in ISP combined with 6.5 mM DTT were lower than in the control. Free iron can react with either DTT or erythrobate to catalyze hexanal synthesis, and these free iron catalyzed reactions were inhibited by EDTA. Hexanal syntheses in ISP, or its fractions, were not inhibited by EDTA. These findings demonstrate that the synthesis of hexanal in ISP with added DTT and erythrobate is a free radical initiated reaction.

Publications:
Boatright, W.L. and G. Lu, Hexanal Synthesis in Isolated Soy Proteins, *American Oil Chemists' Society Annual Meeting Technical Program Book of Abstracts*, Louis, MO, July 2006
Boatright, W.L. and G. Lu, 2007. Hexanal Synthesis in Isolated Soy Proteins, *Journal of the American Oil Chemists' Society*, 84(3):249-257.
Lei, Q. and W.L. Boatright, 2007. Sulfite Radical Anions in Isolated Soy Proteins, *Journal of Food Science,* 72(5):C302-307

CSREES Award Number: 2005-35503-16234
Title: Inverse Gas Chromatographic Measurement of Flavor Interactions with Solid Food Matrices under Controlled Relative Humidity
Institution: University of Illinois
Project Director: Keith R. Cadwallader

Abstract:
Binding of flavor compounds to soy proteins can cause flavor fade in soy products. The interaction between volatiles and soy protein in solution has been the subject of a number of studies; however, very little attention has been given to these interactions in dry and semi-dry food systems. Currently, there is limited information concerning binding of volatile flavor compounds by soy proteins in low-moisture foods. The objective of our study was to develop an inverse gas chromatography (IGC) system for measurement of volatile flavor-ingredient interactions in low-moisture food systems.
We have developed a rapid and sensitive inverse gas chromatographic (IGC) method for the quantitative measurement of interactions between volatile flavor compounds and solid food matrices [*J. Agric. Food Chem.* 52: 6271-6277 (2004)]. The unique aspect of this system is its ability to precisely control relative humidity, thus allowing our group to be the first to study the influence of relative humidity (an environmental parameter that greatly affects binding and one that is critical to the safety and stability of low moisture foods) on the interaction and binding between volatile flavor compounds and soy protein in the solid state.
This novel IGC technique was also applied to study the influence of flavor compound chemical structure (*structure-function relationship*) and environmental relative humidity on flavor-soy protein interactions [*J. Agric. Food Chem.* 54: 1838-1843 (2006)]. Both the chemical and stereo structure of the flavor compound, as well as the relative humidity were found to greatly influence binding potential, especially in the case of polar flavor compounds.
The impact of this research was demonstrated in a third study, where binding of selected butter flavor compounds to wheat versus soy-containing crackers was measured by combining IGC and sensory analysis techniques [*J. Agric. Food Chem.* 54: 5516-5520 (2006)]. Results showed that IGC data could be used to predict sensory impact of flavor binding in a real food system. Our group is now focusing on the use of IGC to study flavor-flavor interactions (i.e. competitive binding) in dry food systems by use of IGC coupled with atmospheric chemical ionization mass spectrometry (APCI-MS).

Publications:
Zhou, Q. and Cadwallader, 2007. Measurement of flavor-soy protein interactions in low moisture solid food systems by inverse gas chromatography. In *Food Flavor: Chemistry, Sensory Evaluation and Biological Activity.* ACS Symposium Series. American Chemical Society, Washington, D.C. (In Press).

Zhou, Q., Lee, S.-Y. and Cadwallader, K.R. 2006. Inverse gas chromatographic evaluation of the influence of soy protein on the binding of selected butter flavor compounds in a wheat soda cracker system. *J. Agric. Food Chem.* 54: 5516-5520.
Zhou, Q. and Cadwallader, K.R. 2006. Effect of flavor compound chemical structure and environmental relative humidity on the binding of volatile flavor compounds to dehydrated Soy protein isolates. *J. Agric. Food Chem.* 54: 1838-1843.

Zhou, Q. and Cadwallader, K.R. 2004. Inverse gas chromatographic method for measurement of interactions between soy protein isolate and selected flavor compounds under controlled relative humidity. *J. Agric. Food Chem.* 52: 6271-6277.

CSREES Award Number: 2005- 35503-16146
Title: "Novel Functional Ingredients Using Milk and Soy Protein Formulations"
University: North Carolina State University (NCSU)
Project Director (PD): Christopher R. Daubert
Co-PDs: Debra A. Clare, Sankar Pichan, Prachuab Kwanyuen, and George L. Catignani

Development of alternative whey/soy formulations, exhibiting enhanced functionality and stability, will impact future design of new ingredient products. The objectives of this research are to devise optimal methods for preparing (a) modified whey/soy mWPC,mSPI) protein-carbohydrate conjugates via the Maillard reaction, (b) transglutaminase (TGase) polymerized mWPC aggregates, and (c) calcium supplemented modified whey protein concentrates (Ca^{++}- mWPC) and then to characterize biochemical/rheological properties of each end product. Color analysis and SDS-PAGE coupled with protein and glycoprotein staining confirmed Maillard reactivity. TGase-mWPC crosslinking reactions were quantified with OPA assays. Mechanical spectra, shear/temperature ramps, and small strain oscillatory testing were accomplished. Emulsion stability in corn oil was assessed. Scanning electron microscopy was performed. The results showed mWPC/mSPI glycoproteins and TGase-mWPC dispersions exhibited superior performance characteristics with respect to emulsion stability. The viscosity of mWPC-lactose/mSPI-dextran solutions was increased 2 fold compared to native constituents. Mechanistic theories were developed to explain mSPI gelling. Gelation temperatures were raised while gel strength and viscosity decreased after TGase polymerization of mWPC solutions. Calcium addition elicited gel formation at lowered mWPC protein concentrations accompanied by a dramatic increase in gel strength (4°C). Scanning electron micrographs illustrated altered protein network structure. The aim of this project is focused on delivering novel valued-added protein/glycoprotein ingredients.

Publications:
Clare, D.A., Lillard, J.S., Ramsey, S.R., Amato, P.M., and Daubert, C.R. (2007) "Calcium Effects on the Functionality of a Modified Whey Protein Ingredient" has been submitted to the *Journal of Agriculture and Food Chemistry*
Cramp, G.L., Kwanyuen, P., and Daubert, C.R. (2007) "Molecular interactions of a modified soy protein isolate" has been submitted to the *Journal of Food Science*

Invention Disclosures:
Clare, D.A. and Daubert, C.R. (2007) Calcium Effects on the Functionality of a Modified Whey Protein Ingredient"

Daubert, C.R., Pichan, S., Lillard, J.S., and Clare, D.A. (2007) "Modified Protein-based, low carbohydrate food ingredient and process for making the same"

CSREES 2004-35503-1520
Title: Novel Ferulyol esterases for ferulic acid extraction from Agro-biomass
University: School of Chemical and Biomolecular Engineering, Georgia Institute of Technology
Projector Director: Rachel R. Chen

Agro-industrial by-products (such as corn bran and corn fiber) are a potential source of value-added phenolic acids, such as ferulic acid. Ferulic acid is a precursor for vanillin and additionally, can be used as an antioxidant in the food and pharmaceutical applications. For enzymatic extraction of ferulic acid from agriculture residues, novel hemicellulases are needed. In particular, feruloyl esterase (FAE) plays a key role in the release of ferulic acid from agro-biomass. Through bioinformatics-assisted discovery, we identified, cloned and overexpressed several hemicellulases useful in the enzymatic extraction of ferulic acid. A hypothetical protein AN1772.2 of Asperigillus nidulans exhibited a 56% identity with a known type-C ferulic acid esterase (FAE) from Talaromyces stipitatus. In addition, it contained a 13-amino acid conserved region flanking the characteristic G-X-S-X-G motif of a serine esterase, suggesting a FAE function for the protein. The putative FAE was successfully cloned from the genomic DNA and expressed in yeast. The recombinant protein exhibited high FAE activities and about 86% of the enzyme activity was found in the growth medium, indicating that the native signal peptide was effective in the yeast expression system. The recombinant FAE is stable over an unusually wide range of pH (4.0-9.5), has a pH optimum of 7.0, and a temperature optimum of 45^{o}C. A substrate specificity profiling reveals that the enzyme is a type-B FAE, despite its strong sequence homology with type-C FAEs. This result has been published in Applied Biotechnology and Microbiology 2007, 73(6), 1323-1330.

CSREES Award Number: 2006-55503-17103
Title: Lipid polymorph impact on food quality: use to enhance Food Science education
University: University of Illinois, Urbana, IL
Project Director: Nicki J. Engeseth, Ph.D.

To maintain global competitiveness, NAS emphasized US enhancement of education in K-12 science and engineering. To increase University placement in scientific disciplines early educational exposure is pertinent. This integrated project creates experiential learning workshops exposing students with basic science interests to food science, through studying chocolate.

Our long term goal is to determine the chemistry of lipids in chocolate as affected by storage conditions and translate this into the impact on human perception of chocolate texture and flavor release. Chocolate quality is evaluated by textural and descriptive sensory analysis. Atomic force microscopy generates topographical images and estimates of grain size and roughness. Flavor is evaluated by gas chromatography and polymorphs characterized using X-ray diffraction and differential scanning calorimetry.

Temperature fluctuations had more influence on texture perception than storage at high temperatures or high relative humidity. Chocolate stored at high temperature transitioned from polymorph V to VI, significantly impacting sensory and instrumental texture and flavor. Cycling temperatures and duration have also been investigated.

Our research will represent some of the first publications on the correlations of sensory and instrumental analysis of chocolate quality, providing a more thorough understanding of the chemistry behind the human perceptual changes with storage of chocolate and a fascinating teaching tool.

Abstracts/Publications:

Andrae, L.M. and Engeseth, N.J. Impact of temperature fluctuations on lipid polymorphism, fat bloom, and surface properties of dark chocolate. To be presented at the Annual Meeting of the Institute of Food Technologists, Chicago, IL July, 2007.

Engeseth, N.J., Andrae-Nightingale, L.M. Impact of storage on flavor and texture perception of chocolate. Proceedings of the 61st Annual Production Conference of the Pennsylvania Manufacturing Confectioners' Association. April, 2007 (Hershey, PA)

Engeseth, N.J. and Nightingale, L.M. 2007. Impact of storage on flavor and texture perception of chocolate. Manuf. Confect. 87(3):50-56.

Andrae-Nightingale, L.M., Lee, S.-Y. and Engeseth, N.J. 200X. Textural changes in chocolate characterized by instrumental and sensory techniques. Submitted.

Andrae-Nightingale, L.M., Lee, S.-Y. and Engeseth, N.J. 200X. Impact of storage on dark chocolate: I. Texture and polymorphic changes. In review prior to submission.

CSREES AWARD NUMBER: 2005-35503-15381
DEVELOPING DESIGN CRITERIA FOR RAPID AND UNIFORM COOLING OF PRODUCE IN CONSUMER PACKAGES
Singh R.P. and Ferrua M.J.
Dept. of Biological and Agricultural Engineering, University of California at Davis.

Reducing the post-harvest losses of fresh horticultural commodities is a topic of major importance around the world. Optimal design and efficiency of the forced-air cooling process are critical factors to enhance the postharvest life of high-value produce, such as strawberries.

The specific goal of this research is to develop a better understanding and modeling of the transport phenomena during the forced-air cooling process of packed products.
A computational fluid dynamic (CFD) model was used to analyze the flow field and temperature history of fresh strawberries, being cooled in a commercial forced-air cooling facility. Navier-Stokes equations were solved within a computational model of the commercial packaging structure. By modeling the rate of moisture evaporation and assuming uncoupled heat and momentum processes, the cooling rate of individual products was predicted.

CFD analysis showed a complex and uneven distribution of the airflow, only 25% of the total airflow enters the packages. The simulated cooling process showed significant differences between and within packages, the $7/8^{th}$ cooling time within individual packages ranged from 62-150 min. The CFD model was validated against experimental flow field and temperature measurements.
CFD modeling provides a powerful design tool to improve the design and efficiency of the forced-air cooling process.

Publication

Ferrua, M.J. and Singh, R.P, 2007. A nonintrusive flow measurement technique to validate the simulated laminar fluid flow in a packed container with vented walls. Paper accepted to be published by the *International Journal of Refrigeration*.

CSREES Award Number: 2005-35503-16303
Authors: R.W. Hartel[1] and L. Yu[2]
[1] Department of Food Science
[2] Department of Pharmacy
Title: Moisture Penetration into Sugar Glasses
Institution: University of Wisconsin
Madison, WI

Long-term stability of amorphous sugar systems to moisture uptake is critical in both food and pharmaceutical applications. Increased water content during storage leads to loss of sensory properties (flavor loss, stickiness, graining) in foods and loss of drug stability in pharmaceuticals. Thus, our objective is to quantitatively map the penetration of water into amorphous sugar systems by using a Raman microspectroscopy technique. Measurement of water diffusion coefficients in and around the glass transition region is needed to understand the nature of moisture penetration into sugar glasses.

Moisture penetration into sugar glasses does not follow classic Fickian (exponential) diffusion. Instead, a sharp moisture front is observed as moisture penetrates into the system. Exterior to the front, a high moisture syrup layer exists where crystallization occurs when insufficient inhibitor is present, whereas interior to the front, the original stable glass is found. Penetration of the front and crystallization are related to relaxation of the glassy matrix, which depend on environmental storage conditions and glass composition.

Such studies not only enhance our understanding of the mechanisms of moisture uptake, they also lead to potential methods of slowing or inhibiting changes associated with water sorption in foods and pharmaceuticals.

CSREES Award Number: 2005-35503-16129
High hydrostatic pressure process parameters impact on soy components extractability and characteristics
Iowa State University
Stephanie Jung

The objective of this work is to determine whether high-pressure processing (HPP) could qualify as an alternative to the conventional thermal treatment of soymilk and tofu. We investigated the effect of pressure alone and combined with a mild thermal treatment on soymilk key enzymes, nutritional factors, protein/functional properties and microbiological attributes.
□-Glucosidase and lipoxygenase were inactivated under pressure at 25 °C but combined temperature was necessary to efficiently destroy trypsin inhibitors. Vitamin B6 content of soymilk was maintained after treatment up to 800 MPa at 25 and 75 °C. When appropriate pH was selected, soymilk color and viscosity were maintained at 400-600 MPa, 25 and 75 °C. Its isoflavone distribution was unchanged at 25 °C but at 75 °C, the isoflavone distribution was modified due to the effect of adiabatic heating. HPP at both temperatures was able to extend shelf life of soymilk to 28 days, two weeks longer than untreated soymilk. Mesophilic microorganisms were injured after HPP treatment and their recovery was observed during 28 days of storage in all conditions tested except in anaerobic storage after a treatment at 500 and 600 MPa, 1-5 min, 75 °C.
This study showed that HPP could destroy antinutritional factors while maintaining soymilk vitamin and isoflavone content. Pressurized soymilk has an extended refrigerated shelf life of 4 weeks and unique emulsifying properties.

Publications:
Ileana Sala. 2006. Effects of high-pressure processing on soymilk enzymes, proteins and isoflavones. Iowa State University Master thesis. May 2006, 146 p.
Lakshmanan, R., de Lamballerie-Anton, M., Jung, S. 2006. Effect of soybean-to-water ratio and pH on pressurized soymilk properties. Journal of Food Science 71:E384-391.

CSREES Award Number: 2004-35503-14839
Title: Conjugation Reactions Between Whey Proteins and Dextran
University: University of Wisconsin-Madison
Project Director and Co-PDs: John Lucey, Dan Zhu, Srinivasan Damodaran

The Maillard reaction can produce protein and polysaccharide conjugates that have been reported to have improved functionality, e.g. emulsification. The reaction is performed by heating powdered mixtures at high temperatures for long periods of time. Our objective was to use aqueous solutions to form whey protein (WP)-dextran conjugates via the initial stage of the Maillard reaction (Schiff base formation). We investigated the impact of temperature, pH, concentrations of the two macromolecules, and time on conjugates formation. Difference UV spectroscopy was used to identify conjugate formation (peak at ~304 nm). Increasing WP and dextran concentrations and lowering pH from 8.5 to 6.5 promoted conjugate formation. The use of high dextran concentrations (30%) may have helped to prevent excessive protein denaturation/aggregation. Covalent attachment of dextran to WP was confirmed by SDS-PAGE with both protein and carbohydrate staining. The Schiff base (conjugate) appeared to be stable and more advanced colored products did not readily form during processing or storage. The conjugate was purified by SEC and affinity chromatography. The emulsifying properties of purified conjugates are being studied. This study indicates that conjugates can be produced in aqueous protein-polysaccharide mixtures and conditions can be selected that avoid excessive protein denaturation or undesirable color formation.

Publications:
Zhu, D. Damodaran, S. and J. A. Lucey. 2007. The formation of WPI-dextran conjugates in aqueous solutions via the Maillard reaction. Oral presentation at 2007 IFT annual meeting. Presentation number 163-05.

CSREES Award Number: 2006 35503 17449
Title: Processing Strategies for the Production of Long-Chain Fatty Acids by Algae
University: West Virginia University
Project Director: Kristen Matak
Co-PD: Jacek Jaczynski

Effect of Different Initial Growth Media on Growth Parameters of *Crypthecodinium cohnii* ATCC 30772

D. L. James, E. R. Parsons, J. Jaczynski, and K. E. Matak, Division of Animal and Veterinary Sciences, West Virginia University, PO Box 6108, Morgantown, WV 26508

Crypthecodinium cohnii, a heterotrophic marine alga, deposits docosahexaenoic acid (DHA) as 30-50% of total fatty acids. The media recommended by ATCC for initial growth of *C. cohnii* is expensive, both in labor and chemicals required. It is not available for purchase; therefore objective (1) was to see if *C. cohnii* growth rate was affected by initial growth medium. Lipid production may be enhanced if *C. cohnii* were grown in continuous rather than batch cultures; therefore, (2) processing parameters for the continuous culture of *C. cohnii* were ascertained.

C. cohnii was initially grown in complex ATCC media 460 and simple media (9g/L glucose, 2g/L yeast, and 27.8g/L salt) then transferred to standard growth media (25g/L glucose, 5.5g/L yeast, and 25g/L salt) for the duration of the study. Growth was measured by optical density (OD) every hour for 60 hours. Conductivity, pH, and dissolved oxygen (DO_2) were also measured.

C. cohnii grew equally in both media ($P<0.05$). There was no difference in pH, conductivity, or DO_2 between the two media ($P>0.05$). The OD indicated a lag phase for ~13 hr, followed by an exponential phase until ~48 hr growth. The pH dropped during the exponential phase. These data indicate optimum retention in a pH-controlled bio-reactor is 48 hr and for high-cell-density continuous-mode process, the ideal flow rate would be 5.2 ml/min for a 15L culture.

CSREES Award Number: 2005-35503-16328
Title: Control of organosulfur transformation in Alliums through processing for retention and enhancement of health-related bioactivities
University: University of Wisconsin-Madison

Project Director and Co-PDs: Kirk L. Parkin, Scott D. Rankin
This project is focused on preventing volatile losses of the objectionable pungent principle propanethial-*S*-oxide (lachrymatory factor, or LF) in fresh-minced onion (*Allium cepa*) preparations, while trying to retain the corresponding *S*-1-propenyl groups as thiosulfinates (TS) within the tissue matrix. Such a result would enhance the retention of health-promoting bioactive organosulfur components in processed *Allium* tissues. The objective is to control the product of alliinase action on 1-propenyl-cysteine sulfoxide (1-PeCSO) upon tissue disruption by exploiting an *S*-thioalk(en)yl-exchange reactions by "trapping" 1-propenyl sulfenic acid (1-PeSOH) as TS species. Control was exerted over alliinase source, pH, levels/profiles of alk(en)yl-CSO, levels and species of pre-formed TS, and the blending of one tissue preparation with another. Results showed that leek alliinase favored the trapping of 1-propenyl group as TS greater than red onion alliinase. Modulation of alk(en)yl-CSO and pre-formed TS levels, combined with limiting the rate of alliinase action, favored trapping of 1-PeSOH as TS. Informal sensory analysis revealed that the tissue preparations with greater retention of 1-propenyl groups as TS possessed milder flavor intensity than native crude onion extracts (rich in LF). These results indicated that simple processing protocols were effective for manipulating TS/LF profiles in freshly minced *Allium* preparations for improved quality.

Scholarly Activity:
Qin Ren (2006). Thiosulfinate manipulation in *Allium* tissues and biological effects of thiosulfinates on cells *in vitro*. Ph.D. Dissertation, University of Wisconsin-Madison.
Xiao, H. and K.L. Parkin (2007). Isolation and identification of potential cancer chemopreventive agents from methanolic extracts of green onion (*Allium cepa*). Phytochemistry 68:1059-1067

CSREES Award Number: 2006-35503-17179

Assessment of Peppers (*Capsicum spp.)* for Capsinoids – A Newly Characterized Class of Natural Antioxidants

Satyavan Singh*, Brian Perkins*, Robert Jarret†, Vincent Russo‡, and Rodney Bushway*
Department of Food Science and Human Nutrition, University of Maine, Orono, ME*
United States Department of Agriculture, Athens, Georgia†
United States Department of Agriculture, Lane, Oklahoma‡

Abstract

Capsicum fruit contain a number of phytocompounds, including the newly characterized capsinoids that have positive effects on human health. Closely related to the pungent capsaicinoids, the non-pungent capsinoids exhibit antioxidant activity and have been shown to promote energy metabolism and reduce body fat in rats. Since little is known about the quantities of these compounds in both sweet and pungent pepper fruit, we have developed an efficient analytical method to quantify the naturally present E-capsiate and dihydrocapsiate from the core accession of the USDA's *Capsicum* germplasm collection. *Capsicum* fruit grown in Athens, GA and Lane, OK were analyzed for the capsiate content using a high performance liquid chromatography method (HPLC). Fresh-frozen and lyophilized fruits were extracted with acetonitrile, filtered, and injected into an HPLC system equipped with a monolithic column, gradient pump, and ultraviolet detector. Of 500 samples analyzed to date, 50 samples contain detectable levels (13-410 ug / g) of E-capsiate at a measured at a wavelength of 280 nm. Ten of the E-capsiate positive samples also had detectable levels of dihydrocapsiate (13-96 ug / g). HPLC-FLD (Em: 280nm; Ex: 3200nm) confirmed the presence of capsiate in the pepper samples. It is hoped the results will benefit both consumers and producers of peppers.

CSREES Award Number: 2005-35503-16153
Interaction of Flavors with Macromolecules: Tannins and Proteins
Pavla Polášková and Susan E. Ebeler
Department of Viticulture and Enology, University of California Davis, Davis, California 95616, USA

The physical-chemical interaction of flavor with nonvolatile food components can dramatically affect the intensity and perception of the flavoring agent. Consumer demand for flavorful, low fat and low salt foods, which often use proteins or carbohydrates as fat replacers have highlighted the need for complete understanding of the effect that all food components have on overall flavor release in the final product.

We used diffusion based NMR techniques, headspace-solid phase microextraction (HS-SPME), and sensory studies to obtain information on flavor-matrix interactions. These studies are giving us a better understanding of the complex relationships between chemical composition and flavor volatility, release, and perception.

According to our experiments, headspace concentration of many volatile compounds significantly decreases if polyphenols and proteins are present in solution. The strength of interactions is strongly dependent on the structure of both volatiles and macromolecules and is most profound for compounds containing extended conjugated π-orbitals. A number of different interactions have been proposed to explain the association of flavor compound with other wine components. This includes reversible Van der Waals interactions and hydrogen bonds, hydrophobic interactions and π-π interactions.

CSREES Award Number: 2005-35503-16134
Title: Role of heme crevice microenvironment in oxidative processes
University of Wisconsin-Madison
Project Director: Mark P. Richards

Color deterioration and lipid oxidation in muscle foods remain major agricultural problems yet the mechanisms involved remain poorly understood. Our objective was to obtain crystallographic structures of heme proteins from aquatic and terrestrial animals that exhibit a wide range of capacities to incur oxidative processes. Comparative analysis of these structures should elucidate mechanisms of the oxidative processes involved. A number of crystallographic observations help explain why perch and trout IV hemoglobin (Hb) autoxidize and lose hemin much more rapidly compared to bovine Hb at post mortem pH. Even though each of these hemoglobins has a histidine residue at site CD3 in the beta chains, only in bovine Hb is the histidine close enough to the heme propionate group to electrostatically stabilize heme in the globin. Mechanisms of enhanced autoxidation and hemin loss due to amino acid differences at four specific sites include: failure to deflect protons from liganded dioxygen, undesirable alignment of the distal histidine, steric forces that facilitate dissociation of species coordinated to the iron atom of the heme group, and increasing access of water to the heme pocket. The mechanisms of oxidation described in this work can be used to better design antioxidant strategies in muscle foods.

CSREES Award Number: 2005-35503-16132
Composite microcapsules for delivery of sensitive food ingredients
Moshe Rosenberg, D.Sc., Dept. Of Food Sci. & Technol., University of California, Davis

The research is aimed at developing highly functional composite microcapsules consisting of zein- or SPI-coated droplets of sensitive core material embedded in wall matrices consisting of carbohydrates. Core-in-wall emulsions (CIWE) consisting PUFA, either of the investigated proteins and maltodextrin have been developed and investigated for their PSD, stability and surface excess properties. A controlled de-amidation process allowed, for the first time ever, utilization of zein as effective encapsulating agent in all-aqueous systems. Results indicated that as little as 2.5% of the investigated proteins enabled the formation and stability of functional CIWE. Results obtained with CIWE were processed and a series of model spray dried microcapsules have been developed and investigated for their microstructural properties, core retention, encapsulation efficiency and oxidative stability. Results indicated that in all cases, core retention > 95% was accomplished with high encapsulation efficiency that was affected by the DE value of the maltodextrin. Accelerated tests at 50°C indicated the oxidative stability of PUFA encapsulated in SPI-coated droplets embedded in MD-based wall system. Microcapsules are currently being investigated for their long-term oxidative stability. Results will enhance our capability to successfully deliver sensitive food ingredients in a way that can enhance the health and well being of consumers.

CSREES Award Number: 2005-35503-15374
Title: Continuous flow high pressure sterilization of low-acid food
University: University of Georgia
Project Director and Co-PDs: Rakesh Singh and Romeo Toledo

Abstract: Not all the useful soybean nutrients are totally incorporated in commercially produced soymilk because the large-sized solids are removed from whole comminuted soybean suspension to obtain a smooth texture. By eliminating filtration or centrifugation steps in soymilk processing, all essential soybean solids will be in soymilk. Our objective was to process soymilk by high pressure homogenization thereby retaining all essential soybean solids and to study its effect on particle size distribution, rheological and ultra-structural properties of soymilk. After dehulling and blanching, whole dehulled soybeans-deionized water mixture (1:3w/w) was ground, comminuted using Megatron/Fitzmill/Stonemill, and high pressure homogenized using continuous flow high pressure throttling (CFHPT)/microfluidizer/Gaulin one-stage homogenizer at different pressure levels. Particle size was reduced significantly with increased homogenization pressure. Ultrastructural results showed fat-protein network disruption with increasing pressure, particle hydration for heated samples, uniform size and dispersion of particles with increasing pressure, and smallest particle size for Megatron-CFHPT samples. Significant difference in apparent viscosity (μ_{app}) was obtained with Megatron-CFHPT samples having highest μ_{app} but same solid content as other treatments. Sterilization of soymilk was achieved in CFHPT as it was homogenized. Soymilk processing method using CFHPT sterilization was developed which provides excellent quality soymilk while retaining all essential soybean solids.

CSREES Award Number: 2002-35503-12546
Title: Evaluation of a Low Phytic Acid Gene in Wheat
University: University of Idaho
Project Director: Edward Souza

ABSTRACT:
The presence of phytic acid in wheat grain contributes to water pollution and increases the cost of animal feed. We conducted research to measure effects of the LPA trait on wheat yield, flour milling or end-use quality and quantify mineral composition of flour fractions. We used three genetic backgrounds to measure the effects of the LPA trait in replicated field testing across two years and two locations. We did not observe consistent differences in grain yield or field performance related to the LPA trait among three genetic backgrounds evaluated in the study. The concentration of inorganic P in LPA flour was 3 times the concentration in WT flour, and Mg concentration in LPA flour was 25% greater than in WT flour. The LPA genotypes were not associated with detrimental effects on flour protein concentration, dough mixing properties, or bread loaf volume. The low phytic acid trait has the potential to improve the nutrition of people in the developing countries who have primarily grain based diets. The increased concentration of magnesium in the flour of low phytic acid wheats may be significant for US consumers. Magnesium deficiency in the US is linked to osteoporosis and juvenile onset of Type II diabetes.

PUBLICATIONS
Guttieri, M. J., K. M. Peterson, and E. J. Souza. 2006. Milling and Baking Quality of Low Phytic Acid Wheat. Crop Sci. 46: 2403-2408.
Guttieri, M. J., K. M. Peterson, and E. J. Souza. 2006. Agronomic Performance of Low Phytic Acid Wheat. Crop Sci. 46: 2623-2629.
Guttieri, M. J., K. M. Peterson, and E. J. Souza. 2006. Mineral Distributions in Milling Fractions of Low Phytic Acid Wheat. Crop Sci. 46: 2692-2698.

CSREES Award Number: 2005-35503-16348
Title: Identification of Molecular Mechanisms of Stress-Resistance in Turkeys to Improve Meat Quality
University: Michigan State University
Project Director: Gale Strasburg

To study the molecular mechanisms that explain how heat stress influences turkey meat quality, we evaluated heat stress effects on thyroid hormone response and meat quality in growth-selected (commercial) and genetic unimproved (RBC2) turkeys. Birds were subjected to heat stress (12 h at 35°C, 12 h at 27°C) for different durations before harvest. The thyroid hormone concentrations and various meat quality traits were analyzed. The thyroid hormone concentrations in commercial birds followed a cyclical pattern during heat stress, but thyroid hormone concentrations in RBC2 birds were stable until birds reached the maximum stress of 120 h. Commercial birds had higher $pH_{15\ min}$, and lightness color, but lower cook loss and marinade uptake than RBC2 birds during the heat stress. The T_3 concentration in birds of both lines was positively correlated with cook loss, but was negatively correlated with marinade uptake. These results reveal that the thyroid hormone response during heat stress was less stable in commercial turkeys than in RBC2 birds and the thyroid hormone response in commercial turkeys in response to heat exposure might influence the consistency of meat quality. The results of this study provide a potential application in selecting turkeys for producing consistent meat quality.

CSREES Award Number: 2005-35503-16213
Hyperspectral Fluorescence Imaging to Detect Black Walnut Shell Fragments
Fischell Department of Bioengineering
University of Maryland, College Park

Differentiation of walnuts' shell and meat has great potential application in harvest walnuts industry. The purpose of this project is to classify the walnuts shell and meats in hyperspectral fluorescence imagery. The black walnuts after harvested were provided by USDA AMS and Growers and were scanned by a hyperspectral imaging system developed by ISL at USDA.

The hyperspectral fluorescence imaging technique was demonstrated to be capable of analyzing the difference of walnut shell and meat. The Principle Component Analysis and Gaussian Mixture Model based Bayesian Classifier was applied to do the discrimination between walnut shell and meat. Meanwhile, a Gaussian-kernel based Support Vector Machine approach was also used to classify the walnuts shells and meat in hyperspectral fluorescence imagery. A total of 5496 samples were studied, and an overall 95.6% recognition rate was achieved. Furthermore, the Independent Component Analysis based optimum wavelength selection approach was proposed for walnut shell and meat classification. An overall 90.6% pixel recognition rate was achieved given 10 optimal wavelengths. With above result, it has potential to achieve 100% object recognition rate. In the next stage, the corresponding real-time processing system will be built, including camera settings, conveyor controller and real time rejection shells from the shell/meat mixture.

U.S. Patent:
Tao, Y., L. Jiang, and B. Zhu. 2006. Methods and Device for Automated Walnut Shell Fragment and Meat Differentiation. *Provisional Patent No. 60/884,501.*
Publications:
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Jiang, L., B. Zhu, H. Jing, X. Chen, X. Rao, and Y. Tao. 2007. Gaussian Mixture Model Based Walnut Shell and Meat Classification in Hyperspectral Fluorescence Imagery. *Trans. of ASABE*. Vol.50(1): 153-160.
Zhu, B., L. Jiang, F. Jin, L. Qin, A. Vogel and Y. Tao. 2007. Walnut Shell and Meat Differentiation Using Fluorescence Hyperspectral Imagery with ICA-kNN Optimal Wavelength Selection. *Sensing and Instrumentation for Food Quality and Safety*. (Accepted).
Jin, F., L. Jiang, B. Zhu, L. Qin, X. Rao, and Y. Tao. 2007. Classification of Walnut Shell and Meat using Gray scale and Rotation Invariant Features and Self-Organizing Map. *Trans of ASAE*. (in review).
Jiang, L., B. Zhu, X. Rao, G. Berney, and Y. Tao. Black Walnut Shell and Meat Discrimination using Hyperspectral Fluorescence Imaging. *2007 ASAE Annual Meeting 073089*.
Jiang, L., B. Zhu, X. Rao, G. Berney, and Y. Tao. ICA Based Band Selection for Black Walnut Shell and Meat Classification in Hyperspectral Fluorescence Imagery. *2007 ASAE Annual Meeting 073088*.

CREES Award Number: 2005-35503-16151
Antifreeze Glycoproteins as Cryopreservation Agents: Structural Requirements for Activity
University of Pennsylvania
Jane Vanderkooi

Ice crystals are a main factor behind food spoilage. There is growing evidence that suggests antifreeze glycoproetins may inhibit recrystallization during freezing, storage, transport, and thawing and thus preserve food texture by reducing cellular damage and minimizing the loss of nutrients. The goals of the grant are to: examine the role of glycosylation in thermal hysteresis, determine the role of the polypeptide backbone in water-protein interaction and to characterize interactions between glycoprotein and water. Infrared spectroscopy and computation were used to study how crystallization can be disrupted. Small flexible molecules such as glycerol disrupt the H-bond network of water, sugar groups additional order water molecules around themselves, and antifreeze
protein inhibit ice recrystallization by interfering with ice surfaces. The water Hbonding network in living yeast cells did not change H-bonding when trehalose synthesis was stimulated, suggesting subtle effects of regulation of water organization in cells.

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(5) Dashnau, J. L., Nucci, N. V., Sharp, K., and Vanderkooi, J. M. (2006) Hydrogen bonding and the cryoprotective properties of glycerol/water mixtures. *J. Phys. Chem. 110*, 13670-13677.
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CSREES Award Number: 2005-35503-16223
Title: Improved Quarantine Treatments for Tropical Fruit Using Thermal Energy
University: Washington State University
Project Director and Co-PDs: Tang, J.; Wang, S.; Mitcham, E.; Armstrong, J.

Abstract
Current tropical fruit treatments are lengthy, may lead to quality losses. The goal of this research was to study postharvest control of pest in tropical fruit with radio frequency (RF) energy. We combined surface heating by hot water with internal RF heating to improve treatment heating uniformity to reduce treatment times. Dielectric properties of four fruit flies and six subtropical and tropical fruits were measured using open-ended coaxial-line probe techniques at 20-60°C. The results showed that dielectric loss factors of the fruit and the insects decreased with increasing frequency and increased linearly with increasing temperature at RF frequencies. Thermal mortality of four targeted fruit flies was determined using the WSU heating block system. The 0.5^{th} order kinetic model had the best fit to the survival ratio at all treatment temperatures for the fruit flies. Treatments at 48 or 50°C for 6 or 2 min are effective to control all life stages of the fruit flies. With above information, RF treatments were developed for persimmons. 48°C+6min provided insect control with acceptable fruit quality. This project addresses quality issues related tropical fruit quality in postharvest pest management, and will enhance long-term sustainability and competitiveness of US tropical fruit industry.

Publications
Birla S.L., Wang S., Tang J., 2007. Computer simulation of radio frequency heating of model fruit immersed in water. *Journal of Food Engineering*, in press.
Wang S., Monzon M., Johnson J.A., Mitcham E.J., Tang J., 2007. Industrial-scale radio frequency treatments for insect control in walnuts: II. Insect mortality and product quality. *Postharvest Biology and Technology*, 45: 247-253.
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Wang S., Birla S.L., Tang J., Hansen J.D., 2006. Postharvest treatment to control codling moth in fresh apples using water assisted radio frequency heating. *Postharvest Biology and Technology*, 40(1): 89-96.
Wang S., Monzon M., Gazit Y., Tang J., Mitcham E.J., Armstrong J.W., 2005. Temperature dependent dielectric properties of selected subtropical and tropical fruits and associated insect pests. *Transactions of the ASABE*, 48(5): 1873-1881.

CSREES Award Number: 2004-35503-14122
Title: Mechanism of oxidation-induced functionality changes of myofibrillar protein
University: University of Kentucky
Project Director: Youling L. Xiong

Muscle proteins are susceptible to oxidation that occurs ubiquitously in meat processing and storage. The objective of this study is to determine the molecular mechanism involved in the alteration of muscle protein functionality under normal ionic, pH, temperature, and three oxidative conditions that are commonly encountered in meat processing and storage. Myofibrillar protein isolates (MPI) are exposed to different oxidizing agents. Physicochemical and biochemical analyses show that MPI, particularly myosin, are very susceptible to iron-catalyzed radical-generating systems as well as to metmyoglobin-oxidizing systems. The MPI are also susceptible to oxidizing linoleic acid. Structural changes in MPI, including myosin conformational stability, damage of amino acid residues, formation of protein carbonyls, and loss of myosin K^+- and Ca^{2+}-ATPase activity, lead to spontaneous cross-linking and aggregation of myosin and other myofibrillar components. Depending on animal species or muscle fiber types, and the specific oxidizing system, the observed protein covalent cross-linking occurs either predominantly on the tail (rod or light meromyosin) or on the head (s1 or heavy meromyosin) portion of myosin. Addition of antioxidative potato protein hydrolysates inhibits some of the MPI changes. The results indicate that oxidant-dependent variation in functional properties of muscle proteins and in textural characteristics of processed muscle foods under oxidative conditions is caused by altered protein-protein interaction pattern and subsequent protein aggregation.

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