CSREES Award Number: 2005-35503-16186

Project 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

Presentation Title for 2008 PD Meeting in New Orleans: Canola Oil-Based Structured Lipids for Formulating *T*rans-Free Margarines

ABSTRACT:

Two types of structured lipids (SLs) for formulating *trans*-free margarines were synthesized by (1) lipase-catalyzed acidolysis of canola oil (CO) with stearic acid (SA) and (2) lipase-catalyzed interesterification of CO with palm stearin (PS) and palm kernel oil (PKO), respectively. CO/SA-based SLs were further blended with palm mid-fraction (PMF) and were treated with emulsifiers, such as sucrose stearate (S-170), distilled monoglycerides (DMG), and sorbitan tristearate (STS). We determined the atherogenicity and physical properties of SLs and the textural properties of margarines made with the SLs. Among a series of blends of CO/SA-based SLs and PMF, the SLs prepared from the 40% blend (SA/CO, w/w%) and PMF blend in the weight ratio of 70:30 (SLs:PMF, w/w) had predominantly β′ polymorphs and had the most desirable solid fat content for margarine formulation. However, the addition of S-170, STS, and DMG to the blend did not further improve its polymorphic properties. Among a series of CO/PS/PKO-based SLs, the SLs prepared from 50:30:20 and 60:25:15 blends (CO:PS:PKO, w/w/w) had lower and similar atherogenic index than the commercial *trans* (CTMF) and *trans*-free margarine fats (CTFMF), respectively and had more β′ than β polymorphs. The margarines made with them had similar textural properties to margarines made with

CTMF and CTFMF, respectively. Therefore, CO/SA-based SLs:PMF blends and CO/PS/PKO-based SLs were suitable for formulating *trans*-free margarines with desirable textural properties or low atherogenicity.

Pressure-Assisted Thermal Processing: Key Engineering Properties and Process Improvement

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The purpose of this research was to develop in-situ methods to measure thermal conductivity, density, compressibility, and electrical conductivity of selected foods as a function of process pressure-temperature. The reaction volume and pH of weak acid buffers under pressure were also determined.

Experiments were conducted using pressure equipment that had provision for in-situ temperature, voltage, DC current and impedance measurements. Thermal and electrical conductivity of food materials under pressure were estimated using a line heat source probe and electrical conductivity sensor, respectively. A variable volume piezometer estimated the compressibility, density, and reaction volume of food materials. The sensors were calibrated using suitable calibration materials, and the experimental data was compared against published literature to establish sensor specific calibration factors.

Thermal conductivity of food materials increased with increase in pressure, moisture and temperature, but decreased with increasing fat content. Density of foods tested increased as a function of pressure at a rate that decreased with increasing pressure. Compressibility of tested foods decreased as a function of concentration. Temperature and pressure had a significant effect on electrical conductivity for all samples; conductivity increased as a function of pressure, peaked between 200 and 500 MPa and decreased above 500 MPa. Phosphoric acid and citric acid showed negative reaction volumes that decreased as a function of pressure. Sulfanilic acid and MES had relatively pressure stable, slightly positive reaction volumes. Equilibrium constants and pH were calculated as a function of pressure; pH changes from 0.1 to 400 MPa at 25˚C were -0.57 for citric acid, -1.24 for phosphoric acid and 0.18 for MES, and to 200 MPa, -0.07 for sulfanilic acid. Results of the study will facilitate the evaluation of the process uniformity during high-pressure pasteurization and sterilization and further development of mathematical models for process optimization.

Improvements in Modified Food Starches, James N. BeMiller, Department of Food Science, Purdue University, West Lafayette, IN

The overall objective of this aspect of my research is to determine any effects of channel constituents on corn starch granule reactivity towards reagents approved for use to make modified food starches. Summarized are accomplishments in meeting the specific objectives of this project, which are –

(1) Preparation of corn starch samples with channel proteins and/or lipids intact and removed.
(2) Determination of the influence of channel constituents on granular reactions.
(3) Determination and characterization of channel and granule proteins and lipids.

To date, the research has resulted in 2 published papers, 2 accepted papers (in press), and 3 papers in preparation. Two research projects are in progress.

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. Human consumption of isolated soy proteins (ISP) has increased gradually in the last decade in part because of perceived health benefits associated with consuming soy proteins. Solid-state electron paramagnetic resonance (EPR) spectroscopy of commercial samples of ISP revealed a symmetrical free-radical signal typical of carbon-centered radicals (g=2.005) ranging from 2.96 x 10^{14} to 6.42 x 10^{14} spins per gram of soy protein. The level of free-radicals in ISP was 14-times greater than similar radicals in sodium caseinate, 29-times greater than egg albumin and about 100-times greater levels than casein. Nine soy protein powdered drink mixes contained similar types of free-radicals up to 4.10 x10^{15} spins per gram of drink mix or up to 6.4-times greater than the highest free-radical content found in commercial ISP. Levels of carbon-centered radicals in newly prepared laboratory ISP samples were very low immediately after preparation and then gradually increased during storage of the “dry” ISP in the dark at 22°C. After 10 to 25 weeks of storage free-radical contents had increased to levels similar to those observed in commercial ISP samples. This demonstrates a previously unreported mechanism for the generation of protein free-radicals in ISP. Storing the ISP samples under nitrogen greatly slowed the increase in free-radicals over time, while storing ISP under oxygen greatly accelerated the formation of radicals.

ISP free-radicals formed during storage can be released when the ISP is hydrated and directly or indirectly catalyze a wide array of reactions including those contributing to undesirable flavor and odor compounds.

New Publications for 2007-2008:

Lei, Q., and W.L. Boatright Sulfite Free-Radicals and One-Electron Oxidation of Methionine in Soy Products, *Institute of Food Technologists Annual Meeting Technical Program Book of Abstracts*, Chicago, IL, July 2007.

Boatright W.L., M.S. Jahan, B.M. Walters, A.F. Miller, D. Cui, E.J. Hustedt and Q. Lei , 2008. Carbon-Centered Radicals in Isolated Soy Proteins, Journal of Food Science, 73(3):C222-226.

Lei, Q., W.L. Boatright, 2008 Lipoxygenase Independent Hexanal Formation in Isolated Soy Proteins Induced by Reducing Agents, Journal of Food Science, in press.

Characterizing Stress Responses of Industrial Strains of Bifidobacteria to Extend Cell Survival in Foods

J. Broadbent[1], T. Oberg[1], A. Abdalla[2], S. Ingham[2] and J. Steele[2]

[1]Department of Nutrition and Food Sciences, Utah State University, Logan, and [2]Department of Food Science, University of Wisconsin–Madison

Clinical studies have shown that consumption of live bifidobacteria has beneficial effects on human health. Unfortunately, these bacteria often do not survive well in food products due to acid, oxygen, and salt stress conditions in the food. As a result, there is a need for technologies that better assure the survival of bifidobacteria at high numbers in bioactive foods. This project is designed to provide a more fundamental understanding of how bifidobacteria survive stress conditions commonly encountered in food systems, and identify potential strategies to enhance long-term survival in foods. To accomplish this, we have investigated intrinsic and inducible stress resistance in commercial strains of *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium longum*. Results have demonstrated that intrinsic resistance to acid, salt, and H_2O_2 is commonly much higher in strains of *B. lactis* versus *B. longum*, but substantial intraspecific differences in stress resistance have also been observed. In addition, experiments to induce and characterize an acid tolerance response (ATR) in *B. lactis* and *B. longum* found this treatment significantly enhanced acid resistance in some, but not all of the strains tested. These results suggest there is pronounced intraspecific variation in both intrinsic and inducible stress resistance among *B. lactis* and *B. longum*, which supports our hypothesis that fundamental knowledge of mechanisms for stress resistance in these cells will reveal new approaches to enhance their survival in probiotic foods.

Effect of various parameters in a continuous lactose crystallization process (CLCP)

1. Justification

Lactose is recovered from whey or permeate concentrate by crystallization. The goal of the crystallization process is to obtain large lactose crystals (100-200μm) with a narrow Crystal Size Distribution (CSD). CSD is dependant on the crystallization kinetics. It can be controlled by various process parameters, including supersaturation, temperature, residence time, agitation rate, etc.

2. Objective

This study aims to investigate the effect of various process parameters in a CLCP. Based on this understanding, the most influential parameters will be identified.

3. Methods

A baffled draft-tube 200ml jacketed cooling crystallizer was used. The crystallization was studied at varying supersaturation level (1.7-2.0), agitation rate (400–1200 rpm), temperature (40-60^{o}C) and residence time (17-20 min). Supersaturated solution at desired temperature was pumped into the crystallizer at a varying mass flow rate to a fixed location near the impeller. The solution was seeded at time t = 0. Lactose slurry was continually removed from the crystallizer at the same mass flow rate as the inlet. Lactose crystals were collected once the process reached steady state, after 8-10 residence times. CSD was analyzed by Malvern Master Sizer 2000.

4. Results

The population balance model was used to obtain growth rate (G) and nucleation rate (J) using CSD data. It was observed that rotational speed (rpm), supersaturation, temperature and residence time had a significant effect on CSD. G, J and suspension density showed a non-linear relationship with most of the parameters.

5. Significance of your research to the food science field.

In the food industry, most of the lactose is manufactured using a batch crystallizer. The lactose obtained by this process has wide CSD. This is a big hurdle to subsequent post processing, where a large portion of the lactose crystal produced is lost. The knowledge developed from the continuous process can be used as a basis for the optimization of batch processes.

USDA-NRI PI meeting, August 2008

Proposal Number: 2005-01261

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

Binding of flavor compounds to soy proteins strongly affects flavor perception of a soy product. Several studies have been conducted to explain the binding of flavor compounds by soy proteins; however, the majority of these studies have been conducted in protein solutions using static or equilibrium techniques. The limited information about binding interactions in low-moisture systems prompted the application of nontraditional techniques such as inverse gas chromatography (IGC). The objective of our study was to develop an IGC system for measurement of volatile flavor-ingredient interactions in low-moisture food systems.

Our group has developed a rapid and sensitive IGC method for the quantitative measurement of volatile-flavor compounds and solid food matrices [*J. Agric. Food Chem.* 52: 6271-6277 (2004)]. The effects of relative humidity (RH) and flavor compound chemical structure were studied for single flavor compounds and soy protein using this method. Both variables (RH and chemical structure) were found to greatly influence binding potential, especially in the case of polar flavor compounds [*J. Agric. Food Chem.* 54: 5516-5520 (2006)]. Further studies showed that IGC data could be even used to predict sensory impact of flavor binding in a real food system [*J. Agric. Food Chem.* 54: 5516-5520 (2006)]. Nevertheless, there was a need to understand more complex systems involving multiple flavor compounds interacting at the same time. A modification of our IGC system with an atmospheric pressure chemical ionization mass spectrometer (APCI-MS) allowed our group to be the first to study competitive binding and sorption parameters between multiple flavor compounds and soy protein. Five different volatile flavor compounds with equal carbon chain lengths but having different chemical structures were used in binary combinations to assess the effect of multiple compounds on the binding forces detected for single compounds. Results show that the presence of saturated aldehydes and hydrocarbons, when introduced as background probes, did not appear to affect the adsorption of alcohols by soy protein isolate at 0 % or 30% RH. Unsaturated aldehydes and ketones significantly affected the adsorption of saturated aldehydes at 0% RH. However, an increase in relative humidity (30%) reduced this effect, due to binding site competition with water molecules. Our group is now focusing on the effect of protein modification on the flavor-flavor interactions with dry soy systems. The impact of this research will be useful to the food industry in order to develop new processes for off-flavor removal in soy products, improve masking agent efficiency and target preferential flavor release.

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 Propsal Number: 2006-00907
Title: Improving Soymilk Quality by UHT Processing.
University: North Dakota State University
Project Director: Sam K. C. Chang and Associates: S. H. Yuan, Z. S. Liu, and B. J. Xu.

Justification: Soymilk has health benefits. High trypsin inhibitor activity (TIA) is undesirable to health. Soy odor is a major factor limiting consumer acceptance. Ultra-high temperature (UHT) processing is relatively new for manufacturing soymilk. Simultaneous elimination of TIA and soy odor by UHT processing has not been studied.

Our **objective** was to determine TIA and odor compounds in soymilk processed by blanching and UHT methods.

Methods: Proto soybean was soaked and blanched at 70-85 C for 30 s to 7.5 min. The blanched beans were made into soymilk, which was heated by indirect and direct UHT methods at 135-150 C for 10-60 s using the Microthermics processor. Eight soy odor compounds were extracted and quantitated using gas chromatography. Commercial soymilk products were analyzed for comparison. Experiments were completed in duplicate and data analyzed by statistical methods.

Results showed that blanching eliminated 100% of hexanal in the blanched soymilk, but 58-70% of the residual TI activities still remained in the blanched soymilk. The blanching conditions of 80 C and 2 min were selected for UHT processing since these conditions produced blanched soymilk without the eight soy odor compounds. Additional TI inactivation was achieved by UHT methods with residual activities ranging from 10-35% of that of the raw soymilk. Hexanal ranging from 10-110 ppb and 170-670 ppb were formed during direct and indirect UHT processing, respectively. As compared to the laboratory processed soymilk, some commercial products contained high residual TI activities (30-60%), indicating that these products had not been processed adequately. Post-blanching oxidation of the unsaturated fatty acids was a significant factor for producing the undesirable soy odor in the end products.

Significance: The study shows that significant TI activities remained both in laboratory and commercial soymilk even though soy odor had been mostly eliminated. The results are important to the soy food industry and consumers.

Publications:

Yuan S. H. and Chang, K. C. 2007. Selected odor compounds in soymilk as affected by soybean materials and direct steam injection. J. Food Sci. 72:S481-486.

Yuan, S. H. and Chang, S. K. C. 2007. Selected beany odors in soymilk as affected by chemical composition, and lipoxygenases in five soybean materials. J. Agric. Food Chem. 55:426-427.

Xu, B. J. Yuan, S. H., and Chang, S. K. C. 2007. Comparative studies on the antioxidant activities of the hydrophilic extracts of selected legumes against copper-induced human low-density lipoprotein oxidation *in vitro*. J. Food Sci. 72:S522-S527.

Xu, B. J., Yuan, S. H. and Chang, S. K. C. 2007. Comparative analyses of phenolic

composition, antioxidant capacity and color of cool season legumes and other selected food legumes. J. Food Sci. 72:S167-177.

Xu, B. J. and Chang, S. K. C. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. J. Food Sci. 72:S159-166.

Yuan, S. H. 2007. Enhancing Soymilk Quality by Heat Processing. MS Thesis. North Dakota State University. Fargo, ND.

CSREES 2004-35503-15220

Title: Enzymatic recovery of ferulic acid from corn fiber and its bioconversion to vanillin

University: School of Chemical and Biomolecular Engineering,
Georgia Institute of Technology

Projector Director: Rachel R. Chen

Agro-industrial by-products, such as corn fiber, are a potential source of value-added phenolic acids. In particular, ferulic acid is of interest as it is a precursor to vanillin, and itself an antioxidant with potential pharmaceutical applications. Feruloyl esterases (FAE) is crucial for the release of phenolic compounds from plant biomass. Therefore, discovery of novel FAEs with desirable properties has been our research focus. From screening microbes that are known to be associated with corn, we discovered a novel feruloyl esterase from *Fusarium proliferatum* strain NRRL 26517 known to be capable of utilizing corn fiber xylan. The enzyme was a 31 kD protein and exhibited a pH optimum at 6.5-7.5. Unlike other feruloyl esterases, it is stable over a broad pH range (5-9). Its unusual insensitivity to pH makes it a potentially useful enzyme in applications that require acidic or alkaline conditions. In a bioinformatics-assisted approach, we identified a hypothetical protein AN1772.2 of *Asperigillus nidulans* showing 56% identity with a known type-C ferulic acid esterase (FAE) from *Talaromyces stipitatus*. The putative FAE was successfully cloned from the genomic DNA and expressed in yeast. 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. Since release of ferulic acid require synergistic action of many hemicellulases and FAE is only one of the enzymes needed, we

investigated another enzyme discovery approach by directly searching ferulic acid releasing activity using authentic substrate, corn fiber. This resulted in a discovery of a filamentous fungus *Neosartorya spinosa* NRRL185 capable of producing a full complement of enzymes to release ferulic acid. A partial characterization of the extracellular proteome of the microbe revealed the presence of at least seven cellulases and hemicellulases activities, including multiple iso-forms of xylanase and ferulic acid esterase. The recovered ferulic acid was bio-converted to vanillin, demonstrating its potential application in natural vanillin synthesis. The enzymatic ferulic acid recovery accompanied a significant release of reducing sugars (76-100%), suggesting much broader applications of the enzymes and enzyme mixtures from this organism.

In conclusion, a combination of traditional screening and bioinformatics-assisted led to the discovery of ferulic acid esterases and hemicellulase-producing microbes. These enzymes and microbes are useful not only in applications originally targeted (ferulic acid recovery) but also in plant biomass utilization for biofuel production. A follow-up grant application has been successful and research in hemicellulase continues in our laboratory.

Publications:

Hyun-Dong Shin and Rachel R. Chen, Production and Characterization of a Type B Feruloyl Esterase from *Fusarium proliferatum* NRRL 26517, *Enzyme Microbial Technol.*, 38(3-4), 478-485 (2006)

Hyun-dong Shin, Shara McClendon, Frank Taylor, and Rachel Chen, A complete enzymatic recovery of ferulic acid from corn residues with extracellular enzymes from *Neosartorya spinosa* NRRL185, *Biotechnol. Bioeng.*, 95(6), 1108-1115 (2006)

Hyun-dong Shin and Rachel Chen, A type-B feruloyl esterase from *Aspergillus nidulan* with unusually wide pH applicability, *Applied Microbiology and Biotechnology*, 73(6), 1323-1330 (2007).

Simulating the Food Engineering (FE) Learning Experience using Computational Fluid Dynamics (CFD): A Case Study on Membrane Filtration

The adoption of CFD into the food industry has been slow but progressive. More food engineers are turning to CFD simulation to model/optimize and design food processes. However, CFD is not generally available in undergraduate FE curriculums. In addition, many students in FE courses tend to find it difficult to understand and apply FE concepts in solving food processing problems. A solution is to use CFD to create innovative FE teaching tools that illustrate FE concepts while training undergraduate food engineers in its effective use. This study aims to explore the incorporation of CFD into the FE learning experience, with a specific emphasis on the development of virtual course material. The proposed learning methodology will be illustrated by a case study on a membrane filtration system. This study consisted of three parts, i.e. simulation, experimentation and analytical verification. In the experiment, a constant pressure membrane filtration system for 1% skim milk solution was setup. A CFD simulation was done as a CFD training exercise. Darcy's law of filtration was used for analytical verification. The power of CFD simulation is illustrated by the good correlation between simulated, experimental and analytical results. A series of virtual course materials were developed by the CFD post-processing tools. These include an animation of the streamlines, movies showing the transient changes in the volume flow rate, velocity and pressure profiles, etc. These virtual materials enabled a better understanding of the correlation between the multiple solution fields. The new learning paradigm will be beneficial to both junior food engineers and instructors. Food engineers will graduate with the knowledge of CFD simulation. All virtual course material will be made available online for FE instructors. Ultimately, the expected result is an overall increase in the awareness and the application of CFD in the food community.

Optimization of hydrolysis conditions and characterization of ice crystal growth inhibition peptides from gelatin hydrolysate

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ABSTRACT

The inhibition of ice crystal growth in ice cream mix by gelatin hydrolysate (GH) produced by papain action was studied. The GH obtained after hydrolyzing bovine hide gelatin (Type 225B40) for 30 min at 37 °C and pH 7.0 at an (crude) enzyme to substrate ratio of 1:10 inhibited ice crystal growth in an ice cream mix. The ice crystal growth was monitored by thermal cycling between -14 to -12 ^{o}C at a rate of one cycle per 3 min. The active peptides in the GH were then fractionated using Sephadex G-50 and SP-Sephadex C-25 chromatography. The MALDI-TOF mass spectroscopic analysis revealed that the molecular weight of ice crystal growth inhibiting peptides was in the range of 700–1000 Da. The possible mechanism of ice crystal growth inhibition by peptides from GH was explored. Molecular modeling of model gelatin peptides revealed that they form an oxygen triad plane at the C-terminus with oxygen-oxygen distances similar to those found in ice nuclei. Binding of this oxygen triad plane to the prism face of ice nuclei via hydrogen bonding appears to be the mechanism by which gelatin hydrolysate might be inhibiting ice crystal growth in ice cream mix.

Transglutaminase Polymerization of Whey and Soy Proteins

Christopher R. Daubert, Debra A. Clare, Prabhasankar Pichan, Prachuab Kwanyuen, and George L. Catignani

ABSTRACT

Transglutaminase promotes protein crosslinking reactions through an acyl transferase mechanism involving protein-bound glutaminyl residues and primary amines including the ε-amino group of lysine residues in soy, myosin, gluten, oat globulin, peanuts, casein and whey. In this study, a modified whey protein concentrate (mWPC) and two distinct soy protein dispersions were prepared in deionized water, pH 8.0, and crosslinked with microbial transglutaminase (TGase) at an enzyme/substrate ratio of ~5 units of activity/g protein. Test fractions, prepared in the presence and absence of 10 mM dithiothreitol (DTT), were then incubated with the enzyme for various time intervals at 40°C. Representative samples were analyzed with respect to the (a) SDS-PAGE banding profile, (b) degree of crosslinking as determined by OPA assays, and (c) impact of covalent modification on various rheological parameters, including apparent viscosity and gelation properties.

SDS-PAGE results showed that polymer formation occurred in all experimental mWPC dispersions treated with TGase, even those devoid of dithiothreitol (DTT); however, quantitative OPA analyses revealed ~30% cross-linking in whey samples containing 10mM DTT after a 3h treatment period compared to 15% polymerization in equivalent solutions prepared under non-reducing conditions. These finding were attributed, in part, to maintenance of the redox status of the active cysteine residue found in the enzymatic catalytic site. Conversely, the inclusion of the reducing agent had a negative impact on the apparent viscosity of soy protein solutions.

Pseudoplasticity was observed in all shear rheological experiments. Furthermore, the apparent viscosity and gel strength of TGase-mWPC dispersions was slightly lower than non-treated controls while the gelling temperature was raised. Again, the properties of soy were different compared to whey in which the apparent viscosity and gel strength of TGase-treated SPI dispersions was greatly enhanced. Likely, these differences can be attributed to the unique role of disulfide bonds in whey versus soy protein dispersions. Ultimately, these approaches may provide novel whey/soy-based food ingredients with unique functional characteristics for expanded application within the world marketplace.

Role of Physical Structures in Food Oils on Lipid Oxidation

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Nutrition recommendations are causing manufacturers to change lipid profiles in their products to contain more polyunsaturated fatty acids and less hydrogenated fats. These trends result in food products that are more susceptible to lipid oxidation. Edible oil contains polar lipids such as diacylglycerols, free fatty acids, phospholipids that are surface active and form association colloids such as reverse micelles and lamellar structures the presence of water. The overall objective of this project is to gain a better understanding of how the properties of water-oil interfaces in bulk oil impact the oxidation of polyunsaturated lipids. Initial Studies utilized a fluorescence probe, 5-dodecanoylaminofluorescein (DAF) to study both the physical and chemical properties of stripped corn oil containing oleic acid and phosphatidylcholine. The fluorescence intensity of DAF increased with increasing water concentration in the edible oil. Addition of oleic acid decreased DAF fluorescence due to the ability of the free fatty acid to decrease the pH of the aqueous phase of the bulk oil. Phosphatidylcholine increased DAF fluorescence due to its ability to increase DAF exposure to the aqueous phase. Oleic acid had no impact on interactions between DAF and water soluble peroxyl radicals while phosphatidylcholine decreased peroxyl radical degradation of DAF. These results suggest that DAF could be a useful analytical tool to study the impact of the aqueous environment of bulk oil on lipid oxidation.

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. This integrated project creates experiential learning workshops exposing students with basic science interests to food science, through studying chocolate.

Our research goal is to determine chemistry of lipids in chocolate affected by storage and translate this into impact on human perception of chocolate texture and flavor release. Cocoa butter crystallizes in six structures, called polymorphs. Triglycerides reorganize to polymorph VI during long-term storage. Rate of transition may be exacerbated by temperature cycling, commonly used in research. Cycling temperatures and durations cited throughout literature are inconsistent and may provide varying results.

Temperature cycling was compared below, at, and above the normal melting point of chocolate to distinguish impact of cycling parameters on chocolate characteristics. Lipid polymorphism was analyzed by X-ray diffraction and differential scanning calorimetry. Atomic force microscopy was used to visualize surface roughness.

Dark chocolate cycled at 30, 32, and 34ºC transitioned to polymorph VI throughout cycling. Fat bloom developed on chocolate cycled at 32 and 34ºC; cycling at 30ºC was not sufficient to form fat bloom. Cycling at 37ºC resulted in recrystallization in polymorph V and formation of 'sugar-cocoa bloom' likely caused by separation of sugar and fat phases. Increasing temperature during cycling significantly impacted microstructural characteristics, such as surface roughness and grain number. Further research is necessary to confirm break in emulsion, lecithin instability, and/or separation of fat and sugar phases in samples stored at temperatures >35ºC.

Interactive workshops are being developed for high school students to study food science through chocolate experimentation. Examples of interactive events and workshop plans will be presented.

Our research will provide a thorough understanding of chemistry behind human perceptual changes with chocolate storage and is creating a fascinating educational tool for introducing students to food science.

Abstract

Cameron Faustman and Mark Richards
University of Connecticut and University of Wisconsin-Madison

The interaction between lipid oxidation and myoglobin (Mb) redox stability leads to undesirable consequences for both of these sensory parameters, and significant quality loss in muscle foods. A mechanistic basis for the interaction between lipid oxidation and Mb redox instability has not been provided. The objectives of our recently funded project are to (1)determine the rate of heme release in bovine and porcine Mbs as a function of alkylation by monounsaturated aldehydes; (2)compare the rate of heme release in native vs. mutant Mbs as a function of Mb alkylation. This will be accomplished using mutant Mbs with altered numbers of histidine and/or other amino acid residues to determine the effect on conformational stability, and the propensity for heme release; (3)map the sites of alkylation as a function of site-directed mutagenesis in mutant Mbs utilizing mass spectrometry (MS) and MS/MS analyses and relate these to heme release measurements obtained #2; and (4)measure the effect of heme release in Mbs on lipid oxidation in muscle foods models, and determine the effect of oxidizing lipids on redox stability of mutant Mbs. Accomplishing these objectives will contribute to our understanding of postmortem muscle biochemistry as it affects meat color stability and lipid oxidation.

Title: Mechanism responsible for astringency of whey proteins at low pH.

E. Allen Foegeding, Bongkosh Vardhanabhuti, Paige Luck and MaryAnne Drake. Department of Food, Bioprocessing, and Nutrition Sciences. North Carolina State University.

Whey proteins are the major ingredients in sports beverages due to their high nutritional quality. These beverages at low pH are clear but highly astringent. In this study, the roles of protein solution pH and protein modification on astringency were investigated. A second experiment determined the effect of added phosphoric acid used to lower protein solution pH by determining the astringency of phosphate buffers. Finally, the binding of whey proteins and mucin was evaluated as an *in-vitro* determination of the astringency mechanism

The effects of pH (3.5 to 5.5, adjusted with phosphoric acid) and protein modification on astringency of beverages containing 4% (w/w) β-lactoglobulin (β-lg) were evaluated by a trained descriptive sensory panel. Modified proteins were made by heating (60°C for 30 min) in the presence of ribose to promote the initial phase of Maillard browning and thereby decreasing the positive charge of β-lg. In the second experiment, the role of buffering capacity was evaluated using 15 or 30 mM phosphate buffer at pH 3, 4 and 6, representing similar amounts of phosphoric acid needed to adjust 4% β-lg to the same pH. The astringency of β-lg beverages increased as pH decreased and Maillard reaction-modification significantly decreased astringency at lower pH where astringency was greatest ($p<0.001$). This suggested that a decrease in positive charge reduced the astringency. The astringency of protein solutions was rated higher than phosphate buffers at all pH values ($p<0.001$); while the effect of phosphate buffer concentration was insignificant. These results indicated that astringency of protein beverages was pH dependent and influenced by the protein molecule rather than the buffering capacity of the protein.

To study the astringency mechanism, β-lg (pH 2-7) was mixed with mucin and turbidity as well as the pH of the mixtures were measured. Turbidity increased when mixing β-lg at low pH with mucin and was at maximum when the pH of the mixtures was near the pI of β-lg (4.4-5.4), suggesting that precipitation of whey proteins alone could be a factor in the astringency mechanism. However, the turbidity of β-lg-mucin mixtures at these pH values was greater than β-lg alone, while mucin at these pH values was not turbid. This suggested an interaction between mucin and β-lg, which was confirmed by the presence of both proteins in the pellet as shown by size exclusion chromatography coupled with multi-angle laser light scattering.

We conclude that the astringency of whey protein beverages was dependent on pH and the amount of positively charged proteins. The astringency mechanism could be contributed from the precipitation of proteins as well as direct interaction between β-lg and salivary mucin, and possibly other saliva proteins. We are currently investigating the interactions between β-lg and other salivary proteins as well as studying the effect of different sugars in reducing the astringency.

Understanding sensory texture of foods based on food structure, oral processing and mechanical properties

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There is a constant demand on food processors to produce low-calorie or low-fat alternatives to traditional foods; or develop new products based on calorie-restricted guidelines. Changing food composition generally changes food quality by altering appearance, texture, flavor and flavor delivery. Therefore, understanding the mechanisms responsible for food flavor and texture is essential when tailoring food to meet nutritional needs such as reduced calories, while at the same time maintaining an acceptable level of quality.

Our work has shown that fracture properties of a model food (agar gels) predict sensory texture terms with a high degree of correlation. In contrast, cheese texture is much less predictable based on fracture properties. We hypothesized that the oral processing of cheese and agar gels would be different and therefore our first experiments concern implementing a standard procedure to evaluate oral processing of foods.

Oral processing measurements were performed using electromyography (EMG) and 3-dimensional jaw tracking (JT-3D). EMG analysis was used to measure muscle activity during chewing by attaching surface electrodes on the left and right side masseter (M), anterior temporalis (AT) and anterior digastric (AD) muscles. Locations of AT and M muscles were determined by palpation when subjects clenched their teeth while AD was located when they opened their mouth. After transforming raw EMG signals into wave forms with the LabView Graphical Programming System, several variables were analyzed for the mastication process. Mandibular movement was also recorded by electromagnetic JT-3D simultaneously. A small magnet was attached to the lower frontal incisors, being half on teeth and half on gingiva, with a nontoxic adhesive. A headgear tracking the position of the magnet was used to measure the movement of jaw in three dimensions (anterior-posterior, vertical, lateral). Using EMG and JT-3D several neuromuscular and kinematic variables such as number of chews, total duration of the chewing sequence, total muscle work, mean muscle work per chew, relative work of elevator muscles (AT and M), relative work of working vs. balancing side, vertical and lateral amplitudes, opening and closing velocities were calculated. Initial experiments have been conducted with different bolus sizes of gums in order to establish a standard method and to evaluate the effect of different bolus sizes on chewing pattern.

The next phase of the research will evaluate model foods (whey protein, agar and alginate gels) with systematic variations in textural properties to establish links among food structure, fracture properties, oral processing and sensory texture.

Moisture Penetration into Sugar Glasses

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Abstract:
Moisture sorption by amorphous low-moisture foods and pharmaceuticals is one of the main causes for degradation of these products. In the present work, moisture uptake by different sugar glass systems (sucrose:corn syrup (50:50 solid basis) and sucrose: other sugar (60:40 solid basis)) was studied at storage temperature of 23°C and desired relative humidity (RH) (65% and/or 75%) using bulk moisture uptake and Raman micro-spectroscopy (RS) techniques (one-dimensional moisture penetration into thin films of sugar glass). In the case of RS, moisture content along one-dimensional locations was accurately measured using microscopic mapping. Sorption penetration profiles of moisture content vs. penetration depth were obtained. Moisture penetration profiles typically showed a fairly sharp boundary between intact glass in the interior and a high moisture content surface layer, which slowly moved into the sample interior over time. In bulk moisture uptake, samples exposed to 75% RH picked up higher moisture compared to 65%. The moisture uptake was highest in sucrose:fructose system, whereas sucrose:lactose system had the lowest (among sucrose:other sugar systems). In the case of sucrose:corn syrup systems samples made with a 42 or 36 DE corn syrup had similar levels of moisture uptake (mg/mm^2) while samples made with 62 DE corn syrup had a greater level of moisture uptake.

Bridging the Gap between Starch Granule Architecture, Molecular Structure, and Reactivity

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Starch represents the second greatest biomass on the planet and a superb source of functional biopolymers. Annual world production of isolated starch for food/industrial markets climbed to 60 million tons in 2004, with 59% isolated in the U.S. The majority of starch utilized in food applications is first modified chemically, while in the native granular state, to improve its physicochemical properties in accordance with the intended function. Though chemical modification is important industrially, starch reactivity is complex (not fully understood), as it is governed by at least two levels of structural organization - granular and molecular structures. A more comprehensive understanding of relationships between reaction conditions, starch reactivity (granular and molecular aspects), and their link to modified starch properties, is key to maximizing starch utilization and development of value-added products.

The proposed research will focus on two botanical sources of starch: 1) corn, (most utilized worldwide/important to U.S. economy), and 2) wheat, (emerging importance) The overarching goal of this project is to further bridge the knowledge gap between starch granule and molecular reaction patterns, as they relate to starch properties, via a multi-faceted approach. The PDs have identified key granule features (channels, surface and granule matrix proteins) important to reactivity, and have developed novel research tools to address their influence in starch reactions (granular and molecular levels). Knowledge resulting from this research is anticipated to foster development of strategies to control site(s) of reactions in starch granules, through modification of reaction conditions, to produce starch products with improved or novel properties.

Project No. IOW05055
Agreement: #2005-35503-16129
High hydrostatic pressure process parameters impact on soy components extractability and characteristics
Iowa State University
Jung, S.
Termination: August 2008

High-pressure processing (HPP) could qualify as an alternative to the conventional thermal treatment of soymilk and tofu. Effect of pressure alone and combined with a mild thermal treatment on soymilk key enzymes, isoflavones, protein/functional properties and microbiological attributes were determined (Sala, 2006; Lakshmanan et al., 2006; Jung et al., 2008; Smith et al., 2008).

Pressurized soymilks (400 and 600 MPa, 25°C, 10 min) were compared to untreated soymilk and heat-treated soymilk (95°C for 30 min) for their use in tofu making (0.3% GDL, 80°C, 30 min). Impact of the soymilk pH (6.0 and 6.5) on the tofu attributes was also determined. At pH 6.5, HPP increased tofu yield to 71% compared to 45% and 60% for untreated and heat-treated soymilk, respectively. At pH 6.0 the attributes, including hardness, strength, and gumminess of tofu from 400 MPa soymilk were higher than that of 600 MPa soymilk. The more pronounced changes in textural attributes due to pH adjustment were observed for tofu from heat-treated soymilk.

Ongoing investigations involve the characterization of changes in the tertiary and quaternary structure and native state of soy proteins, enzymes and trypsin inhibitors determined by circular dichroism and differential scanning calorimetry. These modifications are correlated to inactivation rates of the enzymes and TIs, and textural properties of tofu. Processing parameters (pressure level, dwell time, temperature) to produce tofu under pressure are currently identified. We identified that *Salmonella typhimurium* was very sensitive to pressure. Investigations are currently performed to determine if a cross-protection effect takes place against HPP when *Salmonella typhimurium* is pre-stressed with starvation and acid-adaptation.

Such studies not only enhance our understanding of high-pressure processing, they also lead to the identification of potential new products with better functionality, improved shelf life and enhanced safety.

Publications:

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Title: Molecular-Based Design and Optimization of Sub-Critical Water Processing of Grapes/By-Products

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Award No.: 2006-00903

Project Type: C - NRI Competitive Grant

Effective period: 09/01/2006 – 08/31/2009

Considerable progress has been made towards optimizing the extraction of anthocyanins from dried red grape pomace using a combination of pressurized solvent extraction (PSE) and predictive methods based on the solubility parameter theory. Using an accelerated solvent extraction (ASE) module, laboratory-based experiments were conducted over a range of temperatures (100 – 140^{o}C) using the following solvents: water, acidified water, 70% ethanol, and 70% acidified ethanol. Ethanolic solvents and water extracted higher levels of total anthocyanins than acidified water, with acidified ethanol extracting more than water, but similar amounts as ethanol. The optimal extraction temperature range was 100 – 120^{o}C. An additional ASE study investigated the effect of increasing the amount of ethanol in hydroethanolic solvent mixtures (10-90%) from 100 to 140^{o}C on the recovery of anthocyanins. The optimal extraction conditions were 10% hydroethanolic solvent and a temperature of 100-120^{o}C. The above experimental results correlated well with predictive results obtained by using solubility parameter correlations and a Hansen three-dimensional solubility parameter approach using a Hsp3D graphical software program. For model anthocyanins, miscibility and solubility in water were predicted to occur between 100-150^{o}C, however solubility parameter theory predicted higher solubility of model anthocyanins in subcritical ethanol over a lower temperature range, namely from 25-100^{o}C. Preliminary batch extraction experiments with hot pressurized carbonated water at pressures up to 300 bar showed increasing recovery of anthocyanins at 120^{o}C using very quick extractions (7.5 min) yielding red-colored aqueous extracts indicative of flavylium cation formation under acidic conditions.

Control of lipid oxidation in extruded salmon jerky

Presentation Number: 091-19

Abstract Division: Aquatic Food Products

Presentation Start/End Time: Monday, Jun 30, 2008, 2:00 PM - 5:30 PM

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An extruded jerky-style salmon snack has been developed to appeal to consumers who wish to increase their consumption of omega-3 fatty acids. Deterioration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) due to lipid oxidation is a major concern for this healthy snack. A shelf-life study was conducted to evaluate the effect of antioxidants on oxidative stability of this intermediate-moisture food product. A control sample with no added antioxidants and four samples with antioxidants (rosemary, mixed tocopherols, THBQ and ascorbyl palmitate) were extruded in duplicate in a Coperion ZSK-25 twin screw extruder. All antioxidants were added as 0.02% of the lipid content. Salmon snacks from each formulation were placed in 3 mil barrier pouches, flushed with nitrogen and stored in an Environmental Specialties ES2000 stability chamber at 35°C, 75% relative humidity. Analyses were conducted at 4-week intervals. A modified Folch procedure was used to extract lipids. SafTest kits for malonaldehyde and peroxides were used. AOAC method 991.39 was used for fatty acid analysis. Color and headspace volatiles were also measured. All measures of lipid oxidation increased in all samples over time; omega-3 fatty acids and CIE L*a* b* values decreased. Only the rosemary treatment had lower malonaldehyde and peroxide values ($p \leq 0.05$) than the control by 12 weeks. Formation of headspace propanal was retarded by all antioxidants through week 4only rosemary, tocopherols and TBHQ were lower than the control by week 8. At 12 weeks, all treatments had similar or higher propanal values compared with the control. Ascorbyl palmitate was most effective for retention of EPA and DHA. The control sample lost 35% of its EPA and over 40% of its DHA during the 12-week period. When protection of omega-3 fats in these extruded snacks has been optimized, the product will offer consumers a convenient source of healthful omega-3 lipids.

USDA-CSREES National Research Awardee

Antioxidative Hydrolysates from Alkali-treated Catfish and Tilapia Protein Isolates

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Antioxidants obtained from natural sources are gaining popularity among consumers and food manufacturers. A large amount of byproducts are generated from seafood processing and these byproducts constitute an important source of natural antioxidants, particularly protein hydrolysates. The objective of our research was to prepare hydrolysates from catfish and tilapia white muscle using various enzymes and evaluate their antioxidant activity in aqueous solution and in muscle food model systems.

Catfish and tilapia protein isolates were prepared by a pH-shift alkali process. Tilapia isolate was hydrolyzed using each of these five enzymes: Cryotin-F, Protease-A-Amano, Protease-N-Amano, Flavourzyme and Neutrase to 7.5, 15 and 25 % degree of hydrolysis (DH). Catfish isolate was hydrolyzed using Protamex to 5, 15 and 30% DH. Catfish hydrolysates (CH) were further divided into a soluble supernatant fraction and a precipitated hydrolysate fraction. The hydrolysates were tested for their ability to scavenge DPPH radicals and for their ability to inhibit the formation of TBARS in a washed tilapia model system. Tilapia hydrolysates (TH) were also tested for their ability to chelate ferrous ion and ability to inhibit lipid hydroperoxides in a washed system. CH were tested for ORAC values, reducing power and for their ability to chelate cupric ions.

Results showed that, among TH, antioxidant activity increased with increased degree of enzyme hydrolysis and varied with the type of enzymes used. All TH significantly ($p<0.05$) inhibited the development of lipid hydroperoxides and TBARS. However, the ability of hydrolysates to scavenge DPPH radicals and chelate metal ions was not reflected by the ability to inhibit the formation of TBARS and PV. Hydrolysates prepared using Cryotin were most effective in muscle model system while, hydrolysates prepared using Flavourzyme were more effective DPPH radical scavengers and metal chelators. In general, hydrolysates with low molecular weights were significantly ($p<0.05$) better antioxidants than those of high molecular weight.

Among CH, DPPH radical scavenging ability and reducing power decreased, while ORAC value, metal chelating ability and ability to inhibit TBARS increased with an increase in % DH. Hydrolysate samples showed higher DPPH scavenging ability and ferric reducing ability, while supernatant samples had higher metal chelating ability.

The results from our studies show that catfish and tilapia protein hydrolysates can be used as natural antioxidants. However, the antioxidant activity differs with the type of enzymes used, %DH and the type of food system in which the antioxidants are tested.

CHARACTERIZING CRANBERRY PROANTHOCYANIDINS THAT PROMOTE URINARY TRACT HEALTH

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Abstract:

Two cranberry [*Vaccinium macrocarpon,* Ait.] cultivars, 'HyRed' and 'Stevens', were harvested from a northern Wisconsin site. 'HyRed' cranberries accumulate anthocyanins earlier and more rapidly than 'Stevens'. Both cultivars were analyzed by Matrix Assisted Laser Desorption / Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) and were found to contain proanthocyanidin (PA)-anthocyanin oligomers as previously reported. However, 'HyRed' was also found to contain anthocyanin-anthocyanin oligomers and direct linked anthocyanin-proanthocyanidin oligomers. This is the first report of these compounds in cranberry, although the presence of similar compounds in grape skins has been previously published. Reverse phase (C18) liquid chromatographic separation profiles were developed to separate PA oligomers from anthocyanin-PA oligomers. Preliminary results indicate that anthocyanin-PA oligomers do not inhibited the adherence of uropathogenic bacteria to uroepithelial cells. We are continuing to develop separation technologies and investigate the effects of fruit processing and storage on the presence of PA-anthocyanin oligomers in cranberry fruit and cranberry juice. Condensation reactions between anthocyanins and PA occur at the pH of cranberry juice. Therefore, anti-adherence activity of juice may decrease over time in proportion to the formation of these compounds. Results may be used by the cranberry industry to enhance food quality attributes, by relating pigmented anthocyanin-PA oligomers to color stability, astringency and consumer acceptance of cranberry fruit, juice and other products. This information will be used in future research designed to determine the impact on UTH in relationship to interactions between anthocyanins and PA as a result of fruit development, processing and storage.

Influence of moisture on aggregation of protein during storage

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The nutritional bar market is a growing market with bar types focused on muscle mass , weight loss, endurance and general health. Whey protein isolates (WPI) have been widely used in bars due to their high nutritional value, bland flavor and chewy texture. In addition whey protein hydrolysates (WPH) offer an easy-to-digest protein source, reduces allergenic potential and provides a softer texture. For most bar products, various sweeteners such as high fructose corn syrup are also included to provide a proper taste and texture. Unfortunately whey protein based bars become undesirably hard during storage. Possible mechanisms for this include moisture-redistribution, protein aggregation, and the Maillard reaction between the added sugars and whey. Moisture-redistribution plays an important role in the early stages where the water/sugars/polyols redistribute in the protein particles reducing the overall plasticizing effect. Protein aggregation results from protein-protein interactions through the intermolecular disulfide bonding and/or non-covalent interactions. This reaction as also been used to explain the loss of effectiveness of dry protein inhalation drugs, such as insulin which undergo aggregation during storage. This disulfide bond formation leads to an aggregated macromolecular network and increased hardening in protein bars. In addition, reducing sugars like HFCS cause the Maillard reaction, resulting in a darker color, loss of nutritional value 80% loss in 4 days at 45°C and hardening. Polyols are often added to lower the water activity for micro spoilage control and softening. Glycerol lowers the water activity best and gives the softest texture compared to xylitol, sorbitol or maltitol. Polyols also delay insoluble protein aggregation, except for propylene glycol, which gives the most hardening due to protein interactions. Partially replacing WPI with WPH provides a softer texture, mostly due to a lower glass transition temperature than WPI. However, WPH increases the Maillard browning, because of the increases in the amount and accessibility of free amine groups on a weight basis. Thus, the amount and type of whey proteins and amino groups, sweet humectants and water content of protein nutritional bars significantly affects sensory and nutritional quality.

Protein-Protein Interactions during High Moisture Extrusion of Fibrous Meat Analogs

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Abstract

Soy protein, mixed with gluten and starch, was extruded into fibrous meat analogs under high moisture and high temperature conditions. The protein solubility of samples collected at different extruder zones and extrudates made with different moistures was determined by 11 extraction solutions consisting of 6 selective reagents and their combinations: phosphate salts, urea, DTT, thiourea, Triton X-100, and CHAPS. Protein solubility by most extractants showed decreasing patterns as the material passed through the extruder, but the solution containing all 6 reagents, known as isoelectric focus (IEF) buffer, solubilized the highest levels and equal amounts of proteins in all samples, indicating that there are no other covalent bonds involved beside disulfide bonds. Regarding relative importance between disulfide bonds and non-covalent interactions, different conclusions could be made from protein solubility patterns, depending on the type of extracting systems and a baseline used for comparison. The observation points out pitfalls and limitation of current protein solubility methodology and explained why controversy exists in the literature. Using the IEF buffer system with omission of one or more selective reagents is considered as the right methodology to conduct protein solubility study, and thus recommended. Results obtained with this system indicate that disulfide bonding plays a more important role than non-covalent bonds in not only holding the rigid structure of extrudates but also forming fibrous texture. The sharpest changes in protein solubility occurred when the mix passed through the intermediate section of the extruder barrel, indicating formation of new disulfide bonds during the stage of dramatic increase in both temperature and moisture. After this stage, although the physical form of the product might undergo change and fiber formation might occur as it passed through the cooling die, the chemical nature of the product did not change significantly.

Formation of an intramolecular disulfide bond in porcine µ-calpain inhibits activation and activity of porcine µ-calpain.

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Examination of mechanisms that regulate calcium dependent protein degradation in muscle continues to be a significant area of inquiry in muscle biology. The active site cysteine of calpain not only facilitates effective catalysis, it also makes the enzyme sensitive to oxidation as characterized by its decreased activity in the present of an oxidant. It is hypothesized that oxidation of the active site cysteine residue of µ-calpain is responsible for the reversible inactivation of calpain that has been observed. Oxidation of cysteine is very complex and more than 10 different sulfur oxidation states are found in vivo. Porcine µ-calpain contains 11 cysteines, which are all likely to be subject to oxidation. This makes it clear that sulfur oxidation state may contribute to regulation of calpain proteolytic activity. These experiments utilized LC-MS/MS analysis to determine the specific consequence of the oxidation of µ-calpain by H_2O_2. The effect of different oxidative and reducing conditions on µ-calpain activity was examined by incubating µ-calpain with either with 200 µM hydrogen peroxide (H_2O_2), 0.03%, 0.1%, or 0.2% ß-mercpatoethanol (MCE) or without H_2O_2 and MCE. Activity was determined in the presence (0.2%) or absence (0%) of MCE. Pre-incubation with H_2O_2 resulted in an inhibition of autolysis, activation and activity. However, when activity was evaluated under reducing conditions, the effects of incubation with H_2O_2 were reversed. This illustrates that oxidation of µ-calpain by 200 µM H_2O_2 is reversible. Pre-incubation with H_2O_2 results in the inability of N-ethylmaleimide (NEM) to irreversibly µ-calpain. This indicates that the pre-exposure to H2O2 likely oxidizes the active site cysteine, making it unavailable for blocking by NEM. The LC-MS/MS analysis of the oxidized µ-calpain revealed a peak at m/z 1032.5 that was not present in the control. The MS/MS data revealed that the peptide 105-133 contains a disulfide bond between Cys(108) and Cys(115). The disulfide bond was confirmed by reduction of the peptide. The finding that the active site cysteine in µ-calpain is able to form a disulfide bound has to our knowledge not been reported before. It is hypothesized that this is part of a unique mechanism for regulation of µ-calpain.

Key Words: calpain, oxidation, LC-MS/MS

Molecular Mobility and Oxygen Permeability in Amorphous Solid Foods

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The stability and quality of amorphous solid foods are largely determined by molecular mobility: matrix mobility modulates the rates of molecular diffusion, and diffusion rates modulate the rates of chemical reaction and physical change. This project investigates how molecular mobility and molecular interactions modulate the permeability and diffusion of O_2 in amorphous model proteins and in real foods.

We use measurements of phosphorescence lifetime/intensity and emission spectra from intrinsic (tryptophan) and extrinsic (erythrosin and vanillin) spectroscopic probes to investigate local molecular mobility in amorphous protein films, either pure or doped with plasticizers or other small molecules, and in real foods. Since oxygen is a contact quencher of phosphorescence, comparison of the phosphorescence lifetime in the presence and absence of oxygen (air) provides a measure of the oxygen quenching constant, a parameter proportional to the rate of oxygen permeability through the matrix. We report here studies of the following model systems and foods: β-lactoglobulin films doped with a variety of small molecules including sugars, sugar alcohols, polyols, and fatty acids; pure gelatin films; pure α-lactalbumin films; pure legumin films; collagen films; and meat-stuffed collagen casings (sausages).

Small molecules modulate the molecular mobility and oxygen permeability of β-lactoglobulin films in a dose-dependent manner. In general, sugars and sugar alcohols decreased mobility and oxygen permeability, polyols increased both mobility and permeability, and fatty acids decreased mobility with no effect on permeability. The fibrous protein gelatin was both less mobile and less permeable to oxygen than globular proteins such as β-lactoglobulin, bovine serum albumin, and α-lactalbumin. The flavor molecule vanillin provided a novel and sensitive indicator of molecular mobility in amorphous solids. Oxygen permeability was directly related to local matrix molecular mobility in a wide range of small globular, fibrous, and large multimeric proteins, although the proportionality varied with molecular structure. And studies of both collagen films and sausage casings indicated that the phosphorescence technique is versatile enough to provide information about local mobility and oxygen permeability in real foods as well as model systems.

Docosahexaenoic Acid Production By The Marine Alga *Crypthecodinium cohnii* In A Continuous Mode Process

D. Inan*, S. Beamer, J. Jaczynski, K. Matak

ABSTRACT

Docosahexaenoic acid (DHA;22:6 n-3), an omega-3 fatty acid found in fish like sardine and salmon, has significant health benefits. Unfortunately, fish consumption may increase exposure to environmental pollutants, such as methyl mercury. The marine alga *Crypthecodinium cohnii* is an important source of DHA because it can accumulate lipid >20% of its biomass with a large fraction of DHA (30-50%). Commercially, DHA production by *C. cohnii* is conducted in bioreactors using a batch-mode process. **The purpose of this study was to investigate DHA production by *C. cohnii* using a novel, continuous-mode process.** Cultivation of *C. cohnii* was conducted in two 15 L computer controlled bioreactor vessels. Temperature of both vessels was maintained at 27˚C during the growth mode of the study and standard media (25g/L glucose, 5.5g/L yeast, and 25 g/L salt) was administered to both bioreactors. After 40 h, the system was switched to "continuous" mode where one vessel was maintained as a growth vessel at 27˚C, and the other as a lipid accumulation vessel at 17˚C. In continuous mode, standard media was administered to the growth vessel and a 25% glucose solution was administered to the lipid accumulation vessel. Results showed that *C. cohnii* growth was maintained in continuous production. Maximum biomass, fat and DHA concentrations achieved were 3.9 g/L, 0.54 g/L and 0.151 g/L respectively. DHA production rate reached homeostasis by h 88, and maximum DHA production rate was 43.27 mg/h (at h 112). This level of production was maintained for the remainder of the continuous cultivation (140 h). More research is needed to optimize processing parameters in continuous mode to produce a viable alternative to batch mode processes. Future research will look at the effect of different temperatures and carbon sources on *C. cohnii* growth and DHA accumulation in a continuous mode process.

Utilization of Interfacial Engineering to Improve Food Emulsion Properties
David Julian McClements and Eric Andrew Decker

The overall objective of this project was to develop new strategies for improving food emulsion stability and performance based on interfacial engineering. During the project we have shown that lipid droplets can be coated by multilayer biopolymer layers that have much improved stability to environmental stresses, such as pH extremes, high mineral contents, thermal processing, freeze-thaw cycling, drying and mechanical agitation. We have also shown that the improved stability of these multilayer emulsions can be related to the physicochemical properties of the biopolymer coatings, *e.g.*, composition, thickness, charge and environmental responsiveness. This knowledge can be used to rationally design emulsion systems with improved functional performance. As part of the work we developed a mathematical model to predict the impact of interfacial properties on multilayer emulsion stability. This model can be used to theoretically predict the optimum conditions for multilayer formation and to determine how specific interfacial properties impact the stability of multilayer emulsions. The knowledge gained from this project has been disseminated through scientific publications, reports in trade journals, presentations at scientific meetings and consulting with the food industry. We have had great interest from the pharmaceutical, food and cosmetic industries in utilizing this technology in their products. The project will also improve the competitiveness of the US food industry by leading to the development of novel encapsulation technologies for use in functional food applications.

Syneresis sensor technology development for curd moisture content control

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The cheese making industry is a very important segment of the US agriculture and produces approximately 30% of world cheese production. Syneresis is a major process in cheese making and involves the dewatering of curd particles. The flow of whey must be controlled to obtain the desired cheese moisture content. Better curd moisture control is needed to decrease the production of under-grade cheese and improve cheese quality. A promising optical sensor technology has been developed that predicts curd moisture content during syneresis with a SEP of 1.72% over a range 50-90%. This new technology consists of a unique sensor that measures light backscatter at a wavelength of 980 nm and yields a response which, with data processing, yields the kinetics of syneresis, and regression models which predict cutting time, whey fat losses, cheese yield and curd moisture content as a function of time. This technology offers the potential for improved process control for both high and low moisture cheese manufacture. Two patent applications have been filled at the US patent office on that technology.

ABSTRACT

Title: Determination of Capsinoids in *Capsicum spp.* obtained from USDA's germplasm collection.

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Some peppers (*Capsicum spp.*) contain a recently characterized class of non-pungent phytochemicals called capsinoids. These compounds were discovered by thin-layer chromatography in 1998. They are structurally similar to the extensively studied pungent capsaicinoids, with the exception that an ester group is present in the capsinoids instead of an amide moiety, which is found in the capsaicinoids. The major capsinoids include E-capsiate, dihydrocapsiate, and nordihydrocapsiate. These compounds exhibit antioxidant activity, enhancement of adrenal catecholamine secretion, promotion of energy metabolism, and suppression of body fat accumulation. Although capsinoids show pharmacological activity, no published data is available to discuss the variability in the relative concentrations of these compounds in the *Capsicum spp.* Our group has developed analytical methodologies to identify and quantify E-capsiate and dihydrocapsiate in both sweet and pungent peppers obtained from the USDA's germplasm collection. Extraction of the compounds was done using acetonitrile. Identification and quantification of the capsinoids was conducted using a high performance liquid chromatography system equipped with diode array detector (HPLC-DAD) and a monolithic column. The wavelength was monitored at 280 nm, and the mobile phase used was acetonitrile and water (60:40). Identities of the compounds were confirmed with a gas

chromatograph equipped with mass spectrometer (GC-MS) and a HP-5 MS capillary column. The most efficient extraction was done with 25 mls of acetonitrile. In most of the cultivars of *Capsicum spp.* analyzed, E-capsiate was more abundant than dihydrocapsiate. The immature peppers contained higher levels of the capsinoids than the mature fruit. With increased interest in pharmacologically-active compounds in foods, data relating to the quantities of capsinoids in peppers will be of benefit to the food and pharmaceutical industries, as well as consumers.

Identification of Phenolic-Maillard Reaction Mechanisms: Implications on Flavor Development in Processed Foods

Vandana Totlani, Yuko Noda, Marlene Moskowitz, and Devin G. Peterson, Department of Food Science, Pennsylvania State University, University Park, PA 16802

The Maillard reaction is a ubiquitous chemical reaction in life and a well documented critical food (flavor, color, nutritional value, toxicity) and biological reaction (aging, inflammation, cardiovascular disease, etc.).

The overall objective of this project was to investigate the reactivity of food phenolic compounds, specifically the flavan-3-ols, on the mechanisms of product generation in Maillard reaction model systems with a focus on flavor development.

The reactivity of phenolics on the mechanisms of the Maillard reaction was investigated in both model (aqueous and low moisture) and food systems. Carbon-13 and nitrogen-15 labeling studies were utilized to define the reactivity of phenolic compounds in simple aqueous and low moisture model systems. The structural properties of an epicatechin-methylglyoxal adduct reaction product was also characterized by NMR.

Under aqueous conditions, epicatechin was reported to form adduct reaction products with C_2, C_3, and C_4 sugar fragments, whereas under low moisture roast conditions, epicatechin formed adduct reaction products primarily with C_6 sugar-moieties (i.e. 3-deoxyglucosone). The addition of epicatechin to glucose glycine model systems also had a strong inhibitory effect on the generation of Maillard-type flavor compounds which would be anticipated as sugar fragments are known to be key transient precursors of the Maillard reaction. The structural properties of an epicatechin-methylglyoxal adduct was reported to consist of covalent linkage between the C1 position of the methylglyoxal and either the C6 or C8 position of the epicatechin A-ring; presumably generated by hydroxyalkylation, aromatic substitution reactions. Analysis of phenolic structure-reactivity relationships on the mechanisms of flavor compound generation indicated that phenolic compounds with a more ‘activated’ benzene ring for electrophilic aromatic substitution reactions were the most reactive in reducing the concentration of sugar fragments and MRPs in these model systems.

In summary, our findings from this USDA project indicated that flavanols quenched sugar-fragments (key transient intermediate precursors of MRP) primarily by ionic electrophilic aromatic substitution reactions. Other phenolic structures, such as the ferulic acid, also altered Maillard chemistry but by unique reaction mechanisms. These findings identified a novel connection between phenolic and Maillard chemistry. Phenolic-Maillard reactions provide an improved understanding of the chemistry and fate of phenolics in processed foods and related implications on product quality.

Author Information: Peter Pfromm, Mary Rezac, Kansas State University

Annual U.S. consumption of partially hydrogenated vegetable oil is nearly 14 billion pounds. One side-effect of the traditional hydrogenation process is the production of *trans* fatty acids (TFA) that may increase the risk of coronary heart disease. The research presented here demonstrates a reactor system on the laboratory scale which can hydrogenate soy oil to practical levels with only minimal TFA co-production.

Our membrane reactor approach relies on adding hydrogen directly near the catalytic sites of known hydrogenation catalysts located on the skin of a polymeric high-performance asymmetric gas separation membrane. Oil is pumped past the skin side of the membrane while hydrogen permeates the membrane by diffusion though the defect-free skin layer from the (opposite) substructure side of the membrane. More hydrogen permeates the membrane as hydrogen is consumed at the catalytic sites. Hydrogen transport due to the chemical potential gradient from the hydrogen side to the oil side of the membrane is self-regulating. High concentrations of hydrogen near the catalytic sites (compared to conventional slurry catalysts) enable a significant decrease in temperature which promotes the hydrogenation reaction over the *cis* to *trans* isomerization.

At an iodine value of 100 the membrane reactor (70ºC, 3.4 atm H_2) produced less than 2 wt% TFA while the conventional system produced nearly 10 wt% TFA (Pt as catalyst). Platinum, palladium, nickel, and alloy catalysts have all shown reduced TFA production in our membrane reactor as compared to their conventional slurry counterparts.

CSREES Award Number: 2005-35503-16153

Interaction of Flavors with Macromolecules: Tannins and Proteins

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The physical-chemical interaction of flavor with nonvolatile food components can dramatically affect the intensity and perception of the flavoring agent. We are utilizing 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. While the interactions of wine aroma compounds with wine polyphenols and proteins are fairly well studied, little is known about how wine haze influences wine aroma. Depending on concentration ratios, wine proteins can interact with polyphenols in solution and create soluble complexes, hazes or sediments. In a model wine solution we observed that formation of a protein-polyphenol haze and precipitate lowered the concentration of available aroma compound by up to 50 percent, depending on the structure of the aroma compound. This is significantly more than the effects the protein and polyphenol had when present separately in solution and suggests an irreversible incorporation of aroma compounds in to the wine haze followed by sedimentation and removal from the wine.

Role of heme crevice microenvironment in oxidative processes

Project Director:
Mark P. Richards

CSREES Award No. 2005-35503-16134

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Abstract

The underlying stereochemical mechanisms for the dramatic differences in autooxidation and hemin loss rates of fish versus mammalian hemoglobins (Hb) have been examined by determining the crystal structures of perch, trout IV, and bovine Hb at high and low pH. The fish Hbs autooxidize and release hemin ~50 to 100-fold more rapidly than bovine Hb. Five specific amino acid replacements in the CD corner and along the E helix appear to cause the increased susceptibility of fish Hbs to oxidative degradation compared to mammalian Hbs. These sites are CD3/CE3, CD4, E10, E11 and E14. The mechanisms by which autooxidation and hemin loss occur more rapidly in the fish Hbs involve i) steric displacement of bound ligands, ii) weak anchoring of the heme propionates to the globin, iii) larger channels for solvent entry into the heme pocket, and iv) weakened interactions with the distal histidine.

Ile is present at the E11 helical position in most fish Hb chains whereas a smaller Val residue is present in all mammalian α and β chains. The larger IleE11 side chain sterically hinders bound O_2 and facilitates dissociation of the neutral superoxide radical, enhancing autooxidation. Lys(E10) is found in most mammalian Hb and forms favorable electrostatic and hydrogen bonding interactions with the heme-7-propionate. In contrast, Thr(E10) is present in most fish Hbs and is too short to stabilize bound heme, and causes increased rates of hemin dissociation. The larger gaps for solvent entry into the heme crevice of the fish Hbs occurs near βCD3 and E14. There was no electrostatic interaction between αHis(CE3) and the heme-6 propionate in only perch Hb.

The heme crevice surface areas differed little when comparing bovine and fish Hbs. Currently deoxyHb, oxyHb and bovine myoglobin crystals are being farmed to further elucidate mechanisms of discoloration and rancidity in muscle foods.

EFFECT OF PLANT GENOTPYE AND PROCESSING TECHNIQUES ON CAROTENOID STABILITY IN TOMATO PRODUCTS BY INFRARED SPECTROSCOPY

Daniel Rubio-Diaz, Thais De Nardo, Steven Schwartz, David M. Francis, V.M. Balasubramaniam and Luis Rodriguez-Saona

Tomatoes are the second most produced and consumed vegetable in the U.S., and represent a rich source of dietary carotenoids. Genetic resources that include naturally occurring variants of genes affecting both the structure and regulation of key enzymes in the carotenoid biosynthesis pathway make tomato an excellent model food for nutritional studies. The development of new high throughput analytical techniques for the efficient measurement of diverse carotenoids will facilitate the study of the physiological function of these compounds in large genetically characterized populations. These new techniques could also help to clarify conflicting evidence reported on carotenoids chemical modifications occuring during food processing and storage which could help to optimize their potential health benefits in final products.

Our objectives include the development of new IR spectroscopic methods to quantify and characterize tomato carotenoids, and monitoring of chemical transformations of carotenoids during thermal and high pressure processing to help elucidate non-invasively and in real-time the effects of processing methods on these biologically active compounds.

Thirty tomato varieties that included *trans*-lycopene, *cis*-lycopene, β-carotene, δ-carotene and a low carotenoid control were grown and harvested in a replicated trial. The oleoresins from these carotenoid-rich tomato pastes was extracted using hexane and directly applied to a ZnSe crystal plate for attenuated total reflectance infrared (ATR-IR) analysis. The use of a temperature controlled ZnSe crystal coupled to an infrared spectrometer allowed to collect spectra (600 to 4000 cm^{-1}) on real-time from samples exposed at 60, 80, 100 and 120°C during 95 minutes. Soft independent modeling of classification analogy (SIMCA), a multivariate analysis technique, was used to classify tomatoes based on unique infrared spectral information.

Pattern recognition (SIMCA) models exhibited tight and well-separated clusters (inter-class distances >3.0), and demonstrated the capability to classify based on tomato variety and type of carotenoid. Major discriminating bands were 957cm^{-1}, 960cm^{-1}, 964.5cm^{-1}, and 968cm^{-1} associated with bending HC=CH out-of-plane deformation vibrations of carotenoids. Genetic characterization of the tomato varieties allowed assignment of spectral bands to specific carotenoids. Also, infrared spectra resolved unique absorption profiles that allowed for real-time monitoring of carotenoid transformations during thermal treatments. Spectra collected at 60°C did not showed noticeable variations indicating carotenoid stability. Above 80°C several spectral changes were observed with marked shifts at the 956 cm^{-1} *trans* lycopene band with time towards a higher frequency (966 cm^{-1}) that were consistent with heat-induced isomerization reactions. Increasing temperatures resulted in complex band trends associated with oxidation and breakdown of carotenoid and other lipids.

ATR-IR combined with multivariate analysis provided a simple, rapid and high-throughput tool for the identification and quantification of dietary carotenoids and for real time monitoring of

thermally-induced changes. This technique will allow for effective and efficient selection of tomato varieties with specific pigment content, improving the process of screening for carotenoid-rich products and provide tools for understanding thermal behavior of carotenoids. Ultimately, it will help food processors to develop carotenoid-rich products for functional foods and may return value to growers, processors and consumers.

ABSTRACT
Sathe, S. K. and Roux, K. H.

Project Title:
Development of Monoclonal Antibody Based Immunoassays for Tree Nut Detection and Quantification (Proposal No.**:** 06-00957, 2006-2009; Sathe, S. K. PI; Roux, K. H. Co-PI).

Background:
Currently available immunoassays for almond (*Prunus dulcis*) and cashew (*Anacadium occidentale*) nut seed proteins are inadequate with respect to the specificity, robustness, and in some cases, sensitivity.

Project Objectives:
1. To produce mAbs specific for stable proteins and stable constituent epitopes in almonds and cashew nuts.
2. To develop mAb-based immunoassays which are specific, sensitive, and robust for the detection of trace amounts of almonds and cashew nut seeds.
3. To investigate the influence of various processing methods on the selected stable epitopes on the targeted proteins using mAb-based immunoassays.

Results:
1. We have identified amandin a 13S major storage globulin to be a stable and robust marker for almond detection. In cashew nut seeds, Ana o 1 (a 7S globulin), Ana o 2 (an 11S globulin), and Ana o 3 (a 2S albumin) have been similarly identified to be stable markers for cashew nut detection.
2. We have developed several amandin specific mAbs (e.g. 1F5 and 4C10) as well as mAbs targeting the three cashew antigens Ana o 1 (e.g. 1H2, 4B7), Ana o 2 (e.g. 4C3, 4H9), and Ana o 3 (e.g. D2). We have several additional mAbs that are currently being screened for specificity and target identification. These mAbs have been used to develop immunoassays for specific and robust detection of almond and cashew nut proteins. We can detect the targeted proteins with a sensitivity of 20-100 ng/ml.
3. We have subjected almond and cashew nut seeds to a variety of processing treatments including autoclaving, blanching, cooking, frying, γ-irradiation, microwave heating, pH exposure, and roasting and have demonstrated the antigenic stability of amandin, Ana o 1, Ana o 2, and Ana o 3.

ABSTRACT
Roux, K. H. and Sathe, S. K.

Project Title:
Characterization of tree nut allergens. **(Proposal No.** USDA NRICGP CREES# 2003-01212, 10/1/2003-9/30/2007; Roux, K. H. PI, Sathe, S. K. Co-PI)

Background:
At the inception of the project available information on epitopes of tree nut allergenic proteins was, at best, scant. Virtually nothing was known about cashew nut allergens in terms of molecular properties of cashew nut allergenic protein.

Project Objectives:
To clone and characterize allergenic proteins from various tree nuts.

Results:
1. We have developed cDNA libraries for almond, cashew, pecan, pistachio, and walnut.
2. Following allergenic proteins have been identified, cloned, and sequenced to date:
   Almond: A 13S globulin in almond (amandin), profiling (Pru du 4), lipid transfer protein (LTP)
   Cashew Nut: Ana o 1 (a 7S globulin), Ana o 2 (an 11S globulin), and Ana o 3 (a 2S albumin), (LTP)
   Pistachio: Pis v 3 (7S globulin)
   Pecan: 11S, 7S, and 2S
3. Linear epitope mapping of Ana o 1, Ana o 2, Ana o 3 is completed and that for almond and cashew LTPs is underway.
4. We have discovered a new class (60S ribosomal protein) of tree nut allergens in almond and walnut.
5. We have demonstrated that mutation of targeted immunodominant epitopes in cashew allergen Ana o 2 results in hypoallergenic Ana o 2 mutants.

ABSTRACT

Soymilk was prepared from dehulled soybean by Gaulin homogenizer or produce sterile soymilk using microfluidizer-throttling or continuous flow high pressure throttling (CFHPT) to retain essential soybean solids. The soymilk was characterized for particle size distribution, rheological and ultrastructural properties, to establish an empirical model to characterize the distribution of particle size of particles in the soymilk, and to evaluate consumer acceptability. Whole dehulled soybeans were blanched, mixed with deionized water, and comminuted coarsely in a food processor. An intermediate comminution step in Megatron or Stonemill or Fitzmill was followed by homogenization at selected pressure using Gaulin homogenizer or microfluidizer-throttling or CFHPT system. The combined process of megatron with CFHPT at highest pressure was considered best treatment. Therefore, high pressure throttling process will allow utilization of whole soybean to produce excellent quality soymilk with high emulsion stability. The increase in the CFHPT flow rate significantly affected size reduction of particles of soymilk. The empirical model were established which can be used to predict the size of particles in soymilk, at different volume fraction, processed using high pressure throttling process at various pressure and flow rates. Consumer acceptability test showed that more research is needed to make a soymilk that appeal to the taste of the American consumer before the CFHPT process can be used commercially to produce soymilk.

Effect of the high pressure throttling (HPT) process on the shelf life of soymilk and injured microorganisms was also investigated. Soymilk was pressurized at 207, and 276 MPa at four different exit temperatures (85, 121, 133 and 145^{o}C) and (102, 121, 133, 145^{o}C) respectively and three different flow rates (0.75, 1.0, and 1.5 L/min). Pressure, time, and temperature showed significantly different effect in the inactivation of *C. sporogenes* in soymilk.

Inactivation of *C. sporogenes* in soymilk was higher at 276 MPa as compared to that at 207 MPa when the exit temperature was 121^{o}C and hold time was 20.8 s. However, when temperature was increased to 145^{o}C more than a 5 log reduction occurred at both pressures and all the three hold times (20.8, 15.6, and 10.4 s). There were more injured cells (0.5 log) at 207 MPa than at 276 MPa. When the temperature and time was increased, there were fewer injured cells implicating that spores were completely inactivated rather than injured. The D_{121} value of *C. sporogenes* by heat alone was 3 folds more in soymilk than in 0.1% peptone water.

INDEX WORDS: high pressure throttling, Soymilk, particle size distribution, rheological, ultrastructural, microbicidal effects, injured spores

Title: Identification of molecular mechanisms of stress resistance in turkeys to improve meat quality
Investigators: Gale Strasburg (PD), Wen Chiang, John Linz, and Al Booren
Institution: Michigan State University

Abstract: One of the aims of this study was to evaluate the effect of heat stress on thyroid hormone (T3 and T4) response and meat quality traits in two turkey lines: a growth-selected commercial line and a genetically unimproved control line. Birds were subjected to heat stress for different durations before harvest. The commercial line had higher pH at 15 min post mortem, and higher lightness values, but lower cook loss and marinade uptake than control line during the heat stress. There was no difference in drip loss between the two lines. The T3 concentration was positively correlated with cook loss and was negatively correlated with marinade uptake. The thyroid hormone response during heat stress was less stable in the commercial line than in the control line and the unstable thyroid hormone response in commercial turkeys caused by heat exposure might influence the consistency of meat quality. Results of this study may provide an application in selecting turkeys which yield consistent meat quality.

Using Multiscale Fluid Transport Theory to Predict Stress-Cracking in Corn Kernels During Drying

(Grant No: 2006-03846)

ABSTRACT

Food grains such as corn often develop cracks during various stages of processing. Cracked grains are generally considered of inferior quality, are prone to dust formation, and may undergo insect and microbial damage. The objectives of the study are to develop a hybrid mixture theory (HMT) based mathematical model for predicting stress-crack initiation, solve the model using finite-element method and validate the predictions by conducting drying experiments. The HMT was modified to model dual-porosity systems such as corn kernels. This allows modeling moisture loss during drying at a faster rate through high permeability channels around the germ and endosperm, and low permeability channels in the cellular matrix. Since, viscoelastic properties play a critical role on moisture transport and crack formation, dynamic viscoelastic properties of corn kernels were measured using dynamic mechanical analyzer. The results showed that the corn kernels undergo glass-transition in the temperature and moisture content range applicable to drying. This also provided vital information for modeling Fickian and non-Fickian drying profiles. The dual moisture diffusivities were measured using the IsoSorp technique. This led to determining diffusivity values for germ, endosperm and macro flow channels between germ and endosperm, and between endosperm and outer pericarp. It is a novel contribution as in the past only average diffusivity values were used for corn. Multiple diffusivity values for different parts of corn kernel shows that it is important to account for transport properties variation for heterogeneous food materials. This allows predicting experimental drying and cracking behavior more reliably using computational tools, which saves time and cost of doing expensive experimental trials. Numerous continuous and time-varying drying experiments were conducted, and time-temperature combinations in relation to glass-transition regimes that cause fewer stress cracks were identified. The work on solving the model with finite element method using Comsol Multiphysics package is currently in progress.

Abstract for PD Progress Reports 2008 --- Hyperspectral Fluorescence Imaging to Detect Black Walnut Shell Fragments (MD-ENBE-0609, NRI Grant#: 2005-35503-16213)

Differentiation of walnuts' shell and meat has great potential application in harvest walnuts industry. The purpose of this project is to automatically detect the walnuts shell fragments from meats in hyperspectral fluorescence imagery. The black walnuts after harvested were provided by USDA AMS.

We have developed a new technology that uses hyperspectral fluorescence imaging technique analyze the difference of walnut shell and meat. This technique utilizes the fluorescence emitted light responses under hyperspectral wavebands and high-dimensional imaging algorithms to identify the shell fragments and nut meats. Our hyperspectral image consists of about eighty contiguous spectral bands. From the hyperspectral images, spectral information from concentrated bands is used to separate the target from the other objects. Notably, the fluorescence imaging technique is a powerful tool in chemical component identification. Fluorescence occurs when a short wavelength light hits an object with fluorescent components. The emitted light has the spectrum and intensity that is related to its composition. Because the nut meat and the shell are of different chemical compositions, the hyperspectral fluorescence imaging method is found effective in discriminating the shell fragments from nut meat. Through intensive experiments, our laboratory has proven the concept and demonstrated that the method is able to detect the hard-to-detect shell fragments, regardless of the inner or outer shell fragments, color, shape or location in the stream of walnut meats.

A labor-prototype of corresponding real-time processing system has been built in our lab, which included hyperspectral camera settings, hyperspectral images grabber and nuts conveyer controller. Next stage is to build an automated system technology, with tasks including instantaneous on-line hyperspectral image processing, nuts-adaptive dynamic hyperspectral data mining algorithm optimization, and real-time tracking and rejection system controls. These necessary research tasks will be a step further toward developing a mature automated walnut shell fragment detection system technology with high precision and throughput.

Publication List:

[1] Jiang, L., B. Zhu, X. Rao, G. Berney, and Y. **Tao**. 2007. Discrimination of Black Walnut Shell and Pulp in Hyperspectra1 Fluorescence Imagery using Gaussian Kernel Function Approach. *J. of Food Engineering*. Vol. 81(1): 108-117.

[2] 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.

[3] Zhu, B., L. Jiang, F. Jin, L. Qin, and Y. **Tao**. 2008. ICA-kNN based Optimal Wavelength Selection and Walnuts Shell and Meat Differentiation under Fluorescence Hyperspectral Imagery. *J. of Sensing and Instrumentation for Food Quality and Safety*. Springer. Vol.(1):123–131.

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[6] 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.*

U.S. Patent:

[7] **Tao, Y.**, L. Jiang, and B. Zhu. Methods and Device for Automated Walnut Shell Fragment and Meat Differentiation. *Provisional Patent No. 60/884,501.*

Fundamentals, Effects, and Consequences of Deliquescence in Multicomponent Food Systems

Lynne S. Taylor, Lisa J. Mauer and David E. Nivens
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Deliquescence is a first order phase transition from solid to solution that occurs at a relative humidity (RH) that is characteristic to the solid ingredient. In blends containing more than one component with deliquescent behavior, the RH of the solid-solution transition will be lowered. The phenomenon and implications of deliquescence lowering in blends of common food ingredients are not well understood. The specific objectives of this project are to: 1) obtain a fundamental understanding of the important factors affecting the extent and RH of deliquescence in powder blends; 2) investigate hetero and homo particle-to-particle contact stresses as a function of RH and to probe the potential role of capillary condensation in inter-particulate voids as an initiator of deliquescence lowering; 3) determine the effects of deliquescence in mixtures on powder physical structure; 4) determine the effect of deliquescence in food ingredient blends on chemical stability of the individual ingredients; and 5) determine the effect of non-deliquescent macromolecules/biopolymers and anticaking agents on the mixture deliquescence behavior and water transfer in closed systems. Phase diagrams illustrating the relationship between solids ratio and water activity have been determined experimentally and theoretically modeled. While characterizing the deliquescence phenomenon in ingredient blends, we have proven that deliquescence significantly impacts both chemical and physical stability and that storing blends at RHs below the deliquescence point of the blend can preserve the chemical integrity of ingredients. Some anticaking agents have been identified that can reduce the impact of deliquescence, and the mechanism of this effect is currently under investigation.

Atherosclerotic effects from degradation products created during the pressurized hot water extraction of milk thistle fruit

Sunny N. Wallace, Danielle J. Carrier, Roy Penney, Jennifer Gidden, Jack Lay, and Edgar C. Clausen

This study investigates the generation, identification, and quantification of thermal degradation products formed during the extraction of *Silybum marianum* fruits with pressurized hot water (PHWE). To generate thermal degradation products from the S. marianum flavonolignans, 5 mg of each of the individual flavonolignans (silichristin, silidianin, silibinin and isosilibinin) were first dissolved in 2 mL of methanol and added to 198 mL of deionized water, and were then exposed to pressurized hot water at 500 kPa and 413 K for 0.5 hr to generate thermal degradation products. LC/MS/MS characterization of the subcritical water-extracted flavonolignans showed the presence of multiple degradation products. Extracts were evaluated for their athero-protective effects by the thiobarbituric acid-reacting substances (TBARS) assay. PHWE of silibin A and B resulted in the formation of two degradation compounds. Silichristin occurs in three forms, A, B, and C. PHWE of silichristin resulted in a complete loss of A, and the formation of one degradation compound. PHWE of silidianin resulted in the formation of three degradation compounds. PHWE extraction of isosilibin A and B created six degradation products. The TBARS and ROS assays were used to evaluate the ability of the flavonolignans, both before and after subcritical water extraction, to exert anti-atherosclerotic effects. A bench-scale countercurrent extractor is being constructed for the PHWE of silymarin fruits in an effort to minimize flavonolignan degradation by minimizing both the solids and liquid retention times in the extractor. Samples will be collected from the extracts and evaluated for their degradation profiles and anti-atherosclerotic effects.

IMPROVED QUARANTINE TREATMENTS FOR PERSIMMONS USING WATER ASSISTED RADIO FREQUENCY ENERGY

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Abstract

California, a major producer and exporter of persimmons, is subject to incursions by Mexican fruit fly, *Anastrepha ludens* (Loew). Mexican fruit fly, a serious economic and quarantine pest threatens California's persimmon exports. Persimmons require a quarantine treatment to control potential egg or larval infestations when Mexican fruit fly is present in production areas. Methyl bromide fumigation is the only quarantine treatment available presently, but its future use is uncertain because it has been recognized as an ozone depleting substance under the Montreal Protocol and its use may be severely restricted or become uneconomical. Our research explores the use of water-assisted radio frequency (RF) heating for controlling Mexican fruit fly and other insect pests in persimmons as an alternative quarantine treatment to methyl bromide fumigation because it may overcome heating non-uniformity problem associated with RF treatments in fresh fruits.

The objectives of our study were to identify preheating times at each target temperature required for RF heating uniformity, to develop a treatment using water-assisted RF heating to control Mexican fruit fly larvae in persimmons, and to evaluate the quality of RF-treated persimmons under ambient (22°C) and cold storage (4°C) temperatures.

Based on preliminary tests on heating uniformity and thermal mortality data for Mexican fruit fly, three holding times were selected for each of three treatment temperatures , one time at, one above and another below to achieve 100% mortality of Mexican fruit fly third instars. Heat treatments included preheating the fruit in 40°C water followed by heating in a 12 kW, 27.12 MHz RF system and holding at the target temperature for the selected time. The treatment was followed immediately by hydrocooling at 4°C for 30 min. The preheating time at 40°C was selected because it provided RF heating uniformity over the fruit cross-section. Quality parameters, including weight loss, firmness, soluble solids, titratable acidity, peel and pulp color, and calyx browning of persimmons, were evaluated after 7 days storage at of ambient and cold temperatures.

Our results showed that all RF treatments except 48°C + 8 min holding either significantly improved or had no effect on the overall quality of persimmons. Slight calyx browning was observed in the treated samples and the degree of browning increased with treatment time for each treatment temperature. We observed an increase in the firmness of cold stored treated fruits that extended the storage life of persimmons. RF treatments using 46°C + 25 min holding, or 48°C + 6 min holding or 50°C + 2 min holding have potential to provide 100% mortality of Mexican fruit fly with acceptable fruit quality. Confirmatory tests with infested persimmons are needed to establish the quarantine treatment parameters for the water- assisted RF heating technique.

Title: Structure/functionality relationships of granule architecture and leaching/swelling of starch

Project Director: Ya-Jane Wang, Department of Food Science, University of Arkansas, 2650 N. Young Avenue, Fayetteville, Arkansas 72704

Award No.: 2005-35503-15399

Proposal Type: Standard Strengthening Grant

Effective period: 01/01/2005 – 12/31/2007

The overall goal of this research was to investigate the contributions of starch components, molecular structure, and granule structure to its leaching characteristics, swelling behavior, and granule integrity. The rheological properties of starch in different concentrations of urea, a hydrogen bond-breaking agent, were measured by a dynamic rheometer. The maximum values of storage modulus and loss modulus were greater for long-grain rice starch and increased with increasing urea concentration when compared with those of waxy rice starch. Starch was also gelatinized to various degrees with 13 M LiCl, and the surface gelatinized starch and ungelatinized remaining granule were separated and characterized for amylose content, water absorption, thermal properties, and molecular structure. The remaining granule after surface removal exhibited a lower gelatinization temperature and enthalpy, but swelled to a greater extent upon heating when compared to its native counterpart. The amylopectin in granule envelope remnants obtained at a higher temperature had larger weight-averaged molar mass and z-averaged gyration radius than those in remnants obtained at a lower temperature. The results suggest that the interactions between amylose and amylopectin are stronger than those within amylopectin molecules. Starch periphery is not responsible for maintaining starch granule integrity during gelatinization and swelling. The composition and structure of granule envelope remnant that maintains granule integrity are not constant but dynamic during gelatinization. Amylopectin with larger molecular weight and size were not as easy to be solubilized during heating. The formation of semi-permeable membrane-like surface structure during gelatinization is a result of molecule entanglement after gelatinization.

Sonochemically-Assisted Conversion of Chitin to Chitosan

- A Progress Report for USDA NRI project "Ultrasonic Extraction and Sonochemical Modification of Chitin and Chitosan" -

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The ability of high-intensity ultrasound to aid in the conversion of chitin to chitosan, a process that typically requires long processing times and high solvent concentrations (concentrated NaOH). Chitin flakes from NAS byproducts were submerged in 0.25M NaOH and sonicated for 5, 15, and 30 minutes at 25, 44 and 65 Wcm^{-2}. Another set of chitin flakes were sonicated at $65W/cm^2$ for 5, 15, and 30min at 28, 60 and 90°C (0.25M NaOH). Samples were washed lyophilized and filter yield, purity and liberated proteins determined. Chitin flakes were re-suspended in 45% NaOH for 10, 20, 60, 100 and 160 minutes for further deacetylation. Protein liberated into solutions was determined by Lowry. Purity of treated chitin was determined from the total amount of glucosamine. DDA was determined by Fourier Transform Infrared Spectroscopy (FTIR) and direct titration.

In samples sonicated at 25, 44 and 65 Wcm^{-2}, filter yield decreased, e.g. at an ultrasonic intensity of 65 $Wcm^{-2,}$ filterable solids decreased from 81.9 wt% for untreated samples to 67.6 wt% (based on initial mass) after 160 min. of deacetylation in 45wt% NaOH, which was attributed to increased solubility of ultrasonically decetylated chitosan and additional loss of protein. Chitin yield upon suspension in 45% NaOH for 160 min slightly decreased from 73.2% to 70.7% and from 74.9% to 60.2% after 15 and 30 min sonication at 90°C, respectively. However, DDA of samples increased significantly after pretreatment with ultrasound in 0.25 M NaOH *e.g.* DDA of samples sonicated at 60°C increased from 8.8% to 9.3%, 19.7% and 26.1% for 0, 5, 15 and 30 min of sonication, respectively. Subsequent suspension in 45% NaOH led to further deacetylation at significantly increased rates. For example, DDA of not-sonicated samples increased from 7.8% to 42.5% and 63.6% after suspension in NaOH for 60 and 160min but increased from 17.3% to 83.7% and 87.7% after 15 min sonication at 28°C. Amount of proteins liberated increased from 0.17 to 7.18 and 43.79 mg/g after sonication with 25, 44 and 65 Wcm^{-2} for 30 min, respectively, suggesting additional removal of contaminating proteins after sonication. FTIR scans showed only slight increases in chitin DDA after HIU pretreatment. However, during the subsequent deacetylation treatment, HIU accelerated removal of acetyl groups, e.g. after 60 min of deacetylation following the application of ultrasound for 15 min at 44 and 64 Wcm^{-2}, the DDA increased to 63.6% and 84.1%. Efficiency of HIU to accelerate the reaction decreased with increasing temperature and little improvements in the reaction kinetics were found at 90°C.

Our results indicate significant reductions in time of deacetylation which may lead to cost savings and an increased production rate. Results suggest that reaction

temperatures need to be carefully controlled when using HIU as a pretreatment due to decreases in efficiency and increases in yield losses at higher temperatures. A brief pretreatment with HIU can dramatically reduce time required for chitosan conversion. Together with the previously reported results, this study demonstrates that high intensity ultrasound, a low cost, non-thermal processing technique, can be applied to shorten processing times required to extract chitin from crustaceans as well as yield chitin (and thus subsequently chitosan) of higher purity compared to traditional processing conditions. Application of ultrasound also promoted the conversion of chitin to chitosan possibly due to better accessibility of solvents to the reactive sites, generation of new surface areas as well as generation of free radicals that contribute to enhanced reactivity of the reagent in radical-mediated reactions. For the production of chitin and chitosan, ultrasounds thus has the potential to lower production cost, decrease processing time and allow for a better control of the production process.

USDA COOPERATIVE STATES RESEARCH, EDUCATION AND EXTENSION SERVICE

Award No. 2004-35503-114824
Project Title: Purification Process Influences on Structural and Nutritional Function of Grain Sorghum Lipids
Principal Investigators: C.L. Weller, T.P. Carr, V.L. Schlegel, S.L. Cuppett, K.T. Hwang, and L. Wang
Funding Amount: $338,000
Period of Support: 10/1/04 to 9/30/08

Abstract

The specific goal of this project was to create an alternative health-impacting food source capable of lowering cholesterol in humans by investigating the active compounds in grain sorghum, a product currently used in livestock feed and ethanol production. The approach was to (1) characterize the hexane extract of grain sorghum by designing and applying relevant physiochemical analytical methods, (2) develop well-characterized processes capable of effectively separating and purifying individual lipid compounds from grain sorghum, and (3) identify and characterize the individual lipid compounds responsible for lowering cholesterol by using animal models.

Physiochemical characterization of extracts showed that grain sorghum, whether in whole kernel form, ground form, as DDGS or as part of an anatomical part of the sorghum plant contained various lipids. These lipids included hydrocarbons, wax and steryl esters, α- and γ- tocopherols, aldehydes, triacylglycerides, diacylglycerides, fatty acids, policosanols, free sterols, monoacylglycerides, in various profiles and quantities. Palmitic, oleic and linoleic acids were three main free fatty acids supercritical CO_2-extracted and constituted about 94% of all free fatty acids.

Hexane extracts from leaves contained more alcohols and sterols whereas extracts from stalks and whole kernels contained the greatest amount of free fatty acids and fatty aldehydes, respectively. The lipid profiles of extracts from the whole kernels, leaves, and stalks from the same plant differed. The lipid profiles of extracts from each anatomical part of the sorghum plant differed.

Total lipid yield for three methods of extraction (refluxing, bench-scale recirculating solvent and Soxtec) on the grain samples ranged from 0.04 to 3.59%. The method of extraction affected the total lipid recovered and the composition of the extract. The Soxtec method yielded greater total lipid content and yielded greater amounts of policosanols than the refluxing method and bench-scale recirculated solvent methods.

Lipid yields from DDGS were the highest among all the forms of the grains used. Sorghum DDGS contained the highest levels of plant sterols and policosanols. The amount of lipids recovered increased from 6.7 to 7.5 g /100 g of dry DDGS as the time of extraction increased from 0.5 to 6 h. Conversely, the amounts of plant sterols and policosanols extracted by the reflux

method were not influenced by the times of extraction investigated. Total plant sterols (sum of sitosterol, stigmasterol and campesterol) averaged 67.2 mg /100 g of dry DDGS and total policosanols (sum of C26, C28, C30, C32) averaged 71.6 mg /100 g of dry DDGS.

The amounts of lipids extracted as well as content of plant sterols and policosanols increased significantly as the temperature of the Soxtec heating fluid increased. The temperature of the heating fluid had a significant linear effect on the lipid and total plant sterol yields. Total policosanol yields were also affected by the temperature of the heating fluid following a quadratic trend.

A maximum lipid yield of 150 g/kg DDGS was achieved for supercritical CO_2 extraction with a mass ratio ~ 45, an extraction pressure at 27.5 MPa, an extraction temperature at 70℃ and an extraction time of 4 hr. Under these extraction conditions, the contents of tocols, phytosterols, policosanols and free fatty acids were 0.44, 15.6, 31.2 and 155.3 mg/g in the extract. Shorter extraction time and higher flow rate of CO_2 may achieve higher contents of tocols, phytosterols and policosanols but lower content of free fatty acids in the lipid extract. Extraction conditions had no observed effects on the composition of free fatty acids in the extract.

The total yields of lipids obtained by supercritical CO_2 extraction at 27.5 MPa and 70.7℃ were 150 g lipids/kg DDGS, while the yield obtained by bench-scale recirculating hexane extraction at 69.7℃ was only 85 g lipids/kg DDGS. The contents of four high-value compounds, i.e., policosanols, phytosterols, free fatty acids and tocols, in the lipids obtained by supercritical CO2 extraction were 31.2, 15.6, 155.3 and 0.50 mg/g at 27.5 MPa and 70.7℃, compared to 26.6, 9.6, 57.3 and 0.03 mg/g for bench-scale recirculating hexane extraction with hexane at 69.7℃. The profiles of phytosterols and free fatty acids in the sorghum DDGS lipids were relatively independent of the extraction methods and operating conditions.

Plasma non-high density lipoprotein (non-HDL) cholesterol concentration was significantly reduced in hamsters fed one percent grain sorghum lipids compared to hamsters fed a placebo diet. Liver cholesterol concentration was also significantly reduced in hamsters consuming the minimum amount of grain sorghum lipids at 0.5 percent diet. Consistent with these results was the finding that dietary grain sorghum lipid reduced cholesterol absorption efficiency.

Findings from this research will 1) further the development of grain sorghum as an alternative health-impacting food source, 2) promote whole grain consumption so as to allow consumers to maximize their health benefit at low cost, 3) justify using grain from a cereal crop in the diet of United States citizens that consumes less water and is more drought tolerant than corn, and is accepted in other parts of the world as a food crop and 4) enhance the export marketing position for a United States' cereal grain.

CSREES Award Number: 06-35503-17600
Project Title: Phenolic composition and antioxidant activity of almond skins in ground top round beef and chicken breast as affected by electron beam-irradiation
Project Director and Co-PD: Lilian Were and Christine Hughey
University: Chapman University

Effect of electron-beam irradiated (10, 20 and 30kGy) almond skin powder (ASP) on phenolic content and antioxidant properties in meat was evaluated against non-irradiated (0kGy) ASP. Phenols were quantified and characterized using Folin Ciocalteu method, Liquid chromatography with DAD, FLD and negative ion electrospray mass spectroscopy. Fifteen phenolic compounds were identified and quantified including: flavan-3-ols, flavanones, flavanols, phenolic acids. Lipid oxidation (LOX) was measured by monitoring peroxide values, conjugated dienes, TBARS values and hexanal content over time. Addition of ASP to beef resulted in lower Hunter a*values, and for ground chicken breast (GCB) lower L*values with no effect on a* and b* values were observed. During two weeks of refrigerated storage of un-cooked beef, samples containing ASP resulted in lower formation of LOX products with 30kGy irradiated ASP being the most effective. For un-cooked GCB during two weeks of refrigerated storage, samples treated with ASP resulted in a greater reduction of LOX products as compared to samples without ASP. Un-cooked GCB during 7 months of frozen storage resulted in a 3-82% reduction in LOX products with irradiated ASP resulting in increased antioxidant activity. For cooked GCB, irradiation at 10kGy resulted in lowest formation of LOX products as compared to samples without ASP over one week of refrigerated storage. In general, meat with irradiated ASP resulted in lower LOX products as compared to samples without ASP. Almond skin powder could thus be used to extend the shelf life of meat.

Project title: β-Glucan Structure and Interactions Modulate Physicochemical, Physiological and Sensory Functions of Oats
Year I: Interactional effects of oat components on viscosity, and of β-glucan molecular weight on bile acid binding and viscosity

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Understanding the role of oat components, especially that of β-glucan, on viscosity will help develop oat-based food products with desirable health benefits and sensory qualities. Seven experimental oat lines with high (6.2-7.2%), medium (5.5-5.9%) and low β-glucan (4.4-5.3%) concentrations were evaluated for contributions of β-glucan, starch, and their interactions, to pasting properties of oat flours by using Rapid Visco Analysis. The viscosities were measured under four different conditions: 1) autolysis (sodium buffer for dispersion), 2) inhibition (silver nitrate solution for dispersion), 3) enzymatic hydrolysis of β-glucan by lichenase, and 4) enzymatic hydrolysis of starch by amylase. The correlation ($r= 0.8289$, $P<0.05$) between β-glucan concentration and peak viscosity (PV) after amylolysis demonstrated β-glucan's importance to viscosity. Further, the impact of β-glucan molecular weight (MW) on viscosity and texture in muffins and on *in vitro* bile-acid binding was explored by adding to muffins, water-extracted β-glucan treated with different amounts of lichenase enzyme (1,3-1,4-β-D-glucanase) to yield three β-glucan MW fractions: 1) low molecular weight (LMW, $1.57x10^5$ daltons), 2) medium MW (MMW, $2.77x10^5$ daltons), and 3) high MW (HMW, $5.60x10^5$ daltons). The lower the MW of the β-glucan fraction, the less impact on batter viscosity, allowing more addition of β-glucan. Thus, resulting concentrations in the muffins were 1.64% for LMW, 1.31% for MMW, and 0.52% for HMW β-glucan treatments. Per 30-g muffin, there were 0.49 g, 0.39 g, and 0.16 g of β-glucan, respectively. Current FDA guidelines require > 0.75 g fiber per serving for a health claim. Texture profile analysis showed LMW and MMW β-glucan muffins bound more BA than did the HMW treatment. Also, LMW and MMW extracts bound more BA than did the HMW extract on an equal-weight basis. These results add further evidence to the importance of fine-tuning β-glucan structure to provide maximum health benefits while maintaining high product quality.

Mechanism of Oxidation-Induced Functionality Changes of Myofibrillar Protein
CSREES NRI Grant No. 2004-35503-14122
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Muscle proteins are susceptible to oxidation that occurs even in a normal meat processing and storage environment. The overall objective of this study is to determine the molecular mechanism involved in the alteration of pork muscle myofibrillar protein (MP) functionality under normal ionic, pH, temperature, and two oxidative conditions that are commonly encountered in meat processing: 1) Iron-induced hydroxyl radical oxidizing system (IOS) with 0.01 mM FeCl3, 0.1 mM ascorbic acid, and 0.0~5.0 mM H2O2, and 2) H2O2-catalyzed MetMb oxidizing systems (MOS) with 0.0~0.5 mM MetMb and H2O2. Oxidized MP was subjected to chymotrypsin digestion that produces s-1 and rod fragments of myosin followed by SDS-PAGE for the elucidation of cross-linking sites. Dynamic rheological measurements and Instron penetration tests were performed to determine the influence of extent and type of oxidation on MP gelation.

Oxidation promoted protein covalent cross-linking through the tail (rod or light meromyosin) of myosin, rather than through the head (s-1 or heavy meromyosin) portion of myosin. Disulfide bonds were the major cross-linker, but other covalent linkages (dityrosine; malonaldehyde) also exhibited some roles. During heat-induced gelation, the aggregation of the s-1 fragment was suppressed by IOS and MOS, but that of the tail was enhanced ($P < 0.05$), leading to a significantly improved gel elasticity (G'). 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.

Development of novel food packaging for controlled release of active compounds: polymer blend morphology and composition effects

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Packaging that delivers active compounds such as antimicrobials and antioxidants into food over time to replace those consumed or degraded in the package contents offers potential for significantly extending shelf-life and quality of foods. However, limitations in conventional film properties and fundamental understanding of factors that control release from polymer films have impeded application of this new technology.

To systematically develop *controlled release packaging (CRP)* that releases active compounds at differentiable rates suitable for food applications, we propose a conceptual framework for elucidating how film composition and processing can be manipulated to achieve desirable film structures and properties, and, thereby, controlled release rates of active compounds. Linking these characteristics to release rates required by individual foods can then direct tailoring of films for specific applications.

To determine effects of polymer composition and processing on release rates, films were generated from various combinations of LDPE, HDPE, PP, PS, and EVOH in conventional cast and blown film extrusion as well as smart blending (an innovative technology based on chaotic advection). Antioxidants (mixed tocopherols, sesamol) were impregnated into the films during processing. Physical and release properties of films were measured by standard methods, and film morphology was monitored by scanning electron microscopy. Tocopherol decomposition was evaluated by HPLC-MS. Target release rates for preventing oxidation of linoleic acid were determined using a syringe pump model system.

A greatly increased range of release rates has been achieved by manipulating polymer composition, processing, and morphology. Tocopherol release from LDPE can be effectively slowed by blending the polymer with PP in conventional extrusion and by generating multilayer film morphologies via smart blending. Tocopherols are stable during both types of processing. Non-volatile tocopherols and volatile sesamol have been successfully incorporated into films and used to extend shelf life of test food products.

Effects of muscle fiber morphology on optical scattering

Our previous studies have shown that sarcomere and collagen structure have different effects on optical scattering. In this study, we investigated optical scattering changes during the rigor process in *Sternomandibularis* muscles. The measured optical scattering parameters were analyzed along with the simultaneously measured passive tension, pH value, and histology analysis. We found that the temporal changes of optical scattering, passive tension, pH value and fiber microstructures were closely correlated during the rigor process. These results indicated that in addition to sarcomere and collagen structures, the muscle fiber morphology also has a significant impact on light scattering. These studies further clarify the complicated mechanisms involved in light propagation in muscle.

Impact of Non-equilibrium and Non-quiescent Conditions on the Processing of Biopolymer Mixtures

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Foods are multi-component, multi-phase materials. The structure, texture, stability and flavor delivery in foods depend on the physical-chemical interactions of biopolymers in the food matrix. Non-equilibrium and non-quiescent conditions charaterize food processing, which can induce phenomena such as phase separation, aggregation, complex formation or gelation. This project addresses the basic mechanisms of interaction (both segregative and associative) between biopolymers in aqueous media with the goal to more accurately recognize, explain, predict, avoid or design processes that involve non-equilibrium and non-quiescent phenomena. Our approach involves the study of a model system of ι-carrageenan and maltodextrin in which pertinent parameters such as shear rate, cooling rate, and temperature are varied to influence the final morphological and rheological properties. The major transitions under study are gelation (largely in the case of ι-carrageenan) and phase separation.

During this first year we have first puried commercial ι-carrageenan by dialysis and ion exchange. Molecular weights of ι-carrageenan and maltodextrin samples have been determined by gel permeation chromatography. The melting/gelling temperatures for separate ι-carrageenan and maltodextrin dispersions as a function of polymer concentration and KCl concentration have been measured using DSC and rheology. The equilibrium phase diagram of the ι-carrageenan/maltodextrin system at 80, 85 and 90 ºC and KCl concentrations of 0.1, 0.2 and 0.3 M have been established. Tie-lines have been determined by compositional analysis of phases using FTIR methods.