School of Food Science

2012 Impact Statements

April 29, 2013

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IMPACT STATEMENTS: RESEARCH

Impact Statements - 2012

Research: IMPROVING FOOD SAFETY

Polymorphic Amplified Typing Sequences (PATS) as an effective Strain Typing System for Bovine *Escherichia coli* O157 Isolates

Summary: Demonstrating the ability of PATS to discriminate unrelated and associate related O157 isolates from cattle.

Situation: Better surveillance and rapid bacterial typing techniques are needed for *E. coli* isolates from cattle. *E. coli* O157:H7 (O157) causes more than 100,000 food-related illnesses, 2,000 hospitalizations and many deaths each year in the United States. As cattle are the primary reservoir for this human pathogen, most human infections occur through food sources that are cattle derived (undercooked hamburger) or uncooked foods contaminated by cattle feces, such as salad vegetables, water, apple cider, and unpasteurized milk. Over the past six years, at least 13 different, multi-state O157 outbreaks were reported, many having a direct link to beef or produce possibly contaminated with bovine manure. With the current mechanization and globalization trends in food production and distribution, the need to monitor produce for foodborne pathogens is critical to the prevention of extensive outbreaks, as is rapid epidemiological surveillance to identify and eliminate potential sources from the food chain.

Response: The PCR-based *Escherichia coli* O157 (O157) strain typing system, Polymorphic Amplified Typing Sequences (PATS), targets insertions-deletions (Indels) and single nucleotide polymorphisms (SNPs) at the *Xba*I and *Avr*II(*Bln*I) restriction enzyme sites, respectively, besides amplifying four known virulence genes (*stx*1, *stx*2, *eae*, *hlyA*) in the O157 genome. Previously, **Carolyn Hovde Bohach a**nd collaborators reported the potential of PATS to effectively discriminate between 46 different O157 isolates associated with various outbreaks and human disease. In the current study, a total of 25 bovine O157 isolates, from different geographic locations across the Northwest United States, were analyzed using PATS, and pulsed-field gel electrophoresis (PFGE). Comparison of the dendograms generated showed that about 80% of the bovine O157 isolates grouped similarly by PATS and PFGE, irrespective of whether it was *Xba*I-based, *Avr*II-based, or based on a combination of enzymes. Isolates that were grouped differently were better matched by location using PATS. The Pearson's correlation coefficient, *r*, calculated at about 0.4, 0.3, and 0.4 for *Xba*I-based, *Avr*II-based and combined PATS and PFGE similarities indicated that these profiles shared a good but not high correlation, an expected inference given that the two techniques discriminate differently.

Impact: PATS has potential for rapidly assessing an isolate's relatedness or unrelatedness, without inadvertent interpretive biases or the need for sophisticated analytical software. PATS effectively complements but does not clone PFGE. Because results can be generated in just 6-8 hours following bacterial isolation, PATS has excellent potential as a convenient tool for early epidemiological, or food safety investigations, enabling rapid notification/implementation of quarantine measures.

Collaboration: Dr. Indira Kudva, USDA ARS, Iowa.

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Comprehensive Review of *Yersinia pestis* Ail: A Protein with Multiple Roles

Summary: A comprehensive review was compiled that describes the current knowledge regarding the role of a specific virulence factor in *Y. pestis* pathogenesis and virulence.

Situation: *Yersinia pestis* is one of the most virulent bacteria identified. It is the causative agent of plague, a systemic disease that has claimed millions of human lives throughout history. *Y. pestis* survival in insect and mammalian hosts requires fine-tuning to sense and respond to varying environmental cues. Multiple *Y. pestis* attributes participate in this process and contribute to its pathogenicity and highly efficient transmission between hosts. These include factors inherited from its enteric predecessors: *Y. enterocolitica* and *Y. pseudotuberculosis*, as well as phenotypes acquired or lost during *Y. pestis* speciation. Representatives of a large Enterobacteriaceae Ail/OmpX/PagC/Lom family of outer membrane proteins are found in the genomes of all pathogenic *Yersiniae*. Understanding the role of Ail in virulence will contribute to the development of effective therapeutics and/or vaccines.

Response: The review discussed (1) inhibition of the bactericidal properties of complement, (2) attachment and *Yersinia* outer proteins (Yop) delivery to host tissue, (3) prevention of PMNL recruitment to the lymph nodes, and (4) inhibition of the inflammatory response. Finally, Ail homologs in *Y. enterocolitica* and *Y. pseudotuberculosis* are compared to illustrate differences that may have contributed to the drastic bacterial lifestyle change that shifted *Y. pestis* from an enteric to a vector-born systemic pathogen.

Impact: OmpX (Ail homolog) is a key virulence factor for a number of Gram negative pathogens. In depth analyses by **Carolyn Hovde Bohach** and **Scott Minnich** have looked at gene expression patterns under various environmental conditions, its role in invasion, its contribution to serum resistance, contribution to outermembrane integrity and in vivo requirement for virulence. These studies have shed light on potential aspects of this surface protein in Gram negative bacterial virulence. This complex area of study in infectious disease and pathogenesis was in need of an accurate and comprehensive review. The publication, released just a few months ago, has been viewed by more than 2,000 people worldwide (tracked by *Frontiers* publisher).

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The Role of a New Intimin/Invasin-Like Protein (Ilp) in *Yersinia Pestis* Virulence

Summary: A new *Y. pestis* surface protein (Ilp) was discovered by analysis of a comprehensive transposon library and shown to be required for cellular invasion and virulence in both *in vitro* an *in vivo* analyses. A new plasmid for light expression was developed to track plague infections in vivo that has potential use in other Gram negative animal infection models.

Response: Scott Minnich and colleagues constructed a comprehensive Tn*phoA* mutant library in *Yersinia pestis* KIM6 to identify surface proteins involved in *Y. pestis* host cell invasion and bacterial virulence. Insertion site analysis of the library repeatedly identified a 9,042-bp chromosomal gene (YPO3944), intimin/invasin-like protein (Ilp), similar to the Gram-negative intimin/invasin family of surface proteins. Deletion mutants of *ilp* were generated in *Y. pestis* strains KIM5 $pgm^-/pCD1^+$, KIM 6 $pgm^+/pCD1^-$, and CO92. Comparative analyses were done with the deletions and the parental wild-type for bacterial adhesion to and internalization by HEp-2 cells *in vitro*, infectivity and maintenance in the flea vector, and lethality in murine models of systemic and pneumonic plague. Deletion of the *ilp*

had no effect on bacterial blockage of flea blood feeding or colonization. The *Y. pestis* KIM5 *Δilp* had reduced adhesion to and internalization by HEp-2 cells compared to the parental wild-type strain ($p < 0.05$). Following intravenous challenge in mice, *Y. pestis* KIM5*Δilp* had a delayed time-to-death and reduced dissemination to the lungs, livers, and kidneys as monitored by *in vivo* imaging using a *lux*-reporter system (IVIS) and bacterial counts. Intranasal challenge in mice with *Y. pestis* CO92*Δilp* had a 55-fold increase in LD_{50} (1.64×10^4 CFU) compared to the parental wild-type strain LD_{50} (2.98×10^2 CFU). A novel virulence factor, Ilp, was identified in *Y. pestis.*

Impact: *Yersinia pestis* is on the top-five list of potential bioweapons due to its ability to be spread by aerosol. Understanding the mechanism of virulence and host interaction is important in developing new strategies for interdiction.

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Genomic Reduction of Flagellin Expression in *Yersinia pestis* affects Virulence

Summary: Understanding the evolution of bacterial pathogenicity using *Y. pestis* as a model organism.

Situation: The highly virulent *Yersinia pestis* diverged recently from its mildly virulent *Y. pseudotuberculosis* progenitor. Understanding this divergence offers a good model to explain the evolution of pathogenicity. A salient difference between these two organisms is the significant genomic reduction of *Y. pestis*. **Scott A. Minnich** and others have hypothesized that gene loss may be a mechanism to increase virulence because loss of certain molecular pattern molecules, like flagellin, might facilitate evasion of host innate immunity.

Response: To test this hypothesis, *flhD* sequences were compared among *Y. pestis* and *Y. pseudotuberculosis* genomes. All sequenced *Y. pestis* strains from diverse geographies lost motility by a single conserved thymine insertion in *flhD*, a required regulator for flagellar gene expression. This indicated that loss of motility was an early event. Second, ectopic expression of a flagellin-β-lactamase reporter was recognized and translocated by the *Y. pestis* plasmid-borne (pCD1) virulence type III secretion system into human neutrophils or mouse macrophages. Third flagellin translocation elicited increases in mouse macrophage IL-6, MIP-2, and IL-1β and increased macrophage cell death as indicated by the release of lactate dehydrogenase (P values <0.05). Two different *Y. pestis* CO92 constructs expressing *Y. pestis* or *Y. enterocolitica* flagellin were tested for lethality in a mouse infection model. Mice challenged with either construct died significantly earlier than wild-type controls ($P < 0.05$). This suggested increased virulence was due to flagellin-induction of caspase 1 which may have led to rapid septic shock, because the same results were not found by either strain in caspase-1 deficient mice.

Impact: Understanding the evolution of bacterial pathogenicity is a key concept in medical microbiology. Because phylogenetic evidence shows the highly virulent *Y. pestis* (bubonic plague) is a relatively recent (<20,000 yrs) derivative of the low virulence food-borne pathogen, *Y. pseudotuberculosis*, understanding the differences in these two closely related pathogens is important. We and others have shown that the primary difference between these two organisms is loss of genetic information by *Y. pestis*. Our experiments show why this loss may be paramount in the evolution to high virulence (innate immune system evasion). Importantly, this is an emerging paradigm shift in our understanding of bacterial pathogenesis.

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Defining Host Physiological Conditions affecting *Escherichia coli* O157:H7 Colonization in Cattle

Summary: Reducing *E. coli* O157:H7 gut colonization and fecal shedding from cattle.

Situation: *E. coli* O157:H7 is the most common bacterial etiologic agent in outbreaks associated with beef products. *E. coli* O157:H7 produces Shiga toxins which cause serious diseases and even death. The bovine large intestine is the primary reservoir and the principle site of O157:H7 colonization. Eliminating or reducing O157:H7 colonization in the GI tract is the key to minimize its contamination in foods and further human infection. However, our understanding of mechanisms regulating *E. coli* O157:H7 gut colonization remains very limited.

Response: Meijun Zhu and colleagues demonstrated that inflammation has a critical role against *E. coli* O157:H7 epithelial colonization; mucins, a previously underappreciated factor, may have key roles in mediating *E. coli* O157:H7 gut colonization. Increasing mucin expression decreased *E. coli* O157:H7 gut colonization.

Impact: The gastrointestinal tract of cattle is the major source of contamination in foods. Previous studies have been focused on characterizing *E. coli* O157:H7 and identifying surface proteins of the pathogen which are responsible for gut colonization. Studies suggest focusing on the physiological conditions of cattle which are responsible for *E. coli* O157:H7 colonization. These studies will impact the beef industry, by providing insight into methods to reduce or eliminate *E. coli* O157:H7 gut colonization, improving the safety of beef.

Funding: Supported by USDA AFRI 2010-65201-20599 and Agricultural Experiment Station at University of Wyoming.

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Risk Assessment and Intervention Strategies for the Emerging Food Safety Threat of Ochratoxin in the United States

Summary: Determining the current status and risk of ochratoxin to protect the public from this harmful contaminant in foods.

Issue: Ochratoxin is a potent carcinogenic substance and toxic to kidney, immune system, and fetus. It occurs frequently in a wide variety of agricultural commodities and foods but there is not enough data from the U.S. where the toxin is not regulated unlike in Europe and Canada.

Response: A national survey, led by **Dojin Ryu** and colleagues, will be completed over two consecutive years to determine the incidence and levels of ochratoxin in the U.S. food supply. Commodities included are cereal grains (wheat, barley, & corn), breakfast cereals, coffee, cocoa, meat, dried fruits, nuts, wine, beer, milk, baby foods, and infant formula. Samples will be collected from five areas in the U.S. – San Francisco, Fargo/Minneapolis, Lincoln/Omaha, Dallas, Chicago, and Pittsburg.

Impact: The first year survey was completed. Oat based products, including breakfast cereal and infant cereal, were the most frequently contaminated food product (>50% positive). The highest level of contamination was also found in oat based products at 11.84 ng/g while the regulatory limit in E.U. is 3 ng/g. The U.S. food supply should not be assumed to be free of this common mycotoxin and may prompt a need for adopting regulations for a safer food system. Second year survey and risk assessment will be carried out before a final recommendation is made.

Funding: Agriculture and Food Research Initiative, National Institute of Food and Agriculture, USDA.

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Research: FUNDAMENTAL APPROACHES TO IMPROVE FOOD QUALITY

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

Summary: Starch reactivity was studied to understand the relationship between modified starch properties and end-use functionality.

Situation: Modified starches with novel properties are needed to meet the diverse functional demands of complex food and industrial applications. Though modified starch products are industrially produced, starch reactivity at the granular and molecular levels is not yet understood. There is need to understand: 1) how reaction system conditions impact starch granular and molecular reaction patterns (on starch chains), and 2) how differing granular and molecular reaction patterns relate to modified starch properties and functionality. The long-term goal is to generate information needed to facilitate more precise control of starch modification practices to enhance the capability of strategically tailoring modification conditions to match the desired end- use and to achieve novel starch functionalities (within the current regulatory restraints).

Response: The project utilizes a novel "model" reaction system employing a fluorescent probe (DTAF) as the chemical reagent. This novel approach allows for direct determination of granular locale of reaction (CLSM) and molecular reactivity of starch chains (amylose [AM] and amylopectin [AP] branch chains via SEC). **Kerry Huber** and collaborators are the first to successfully link granular- and molecular-level reaction patterns with starch properties and functionality. In light cross-linking reactions, pasting viscosity became more inhibited as reaction patterns within granules became more homogeneous, further coinciding with an increase in AM reactivity at the molecular level. Overall findings suggest that granule architecture not only impacts the relative reactivities and reaction patterns of AM and AP branch chains, but that both granular and molecular reaction patterns impact the physical (pasting) properties of modified starches. The hypothesis that granular and molecular reaction patterns may be manipulated to control starch functionality represents a new paradigm in starch modification research.

Impact: As reported by Frost and Sullivan, modified starch (chemically enhanced) represents roughly half (46.8%) of the total U.S. starch market. The U.S. starch market value is estimated at $1.63 billion, with growth to $2.58 billion projected by 2014. The ability to elucidate starch reactivity on a molecular level is unique, and will allow researchers to understand how starch molecular reaction patterns

ultimately relate to starch functionality (experiments are underway). The substantial change in reactivity due to level of hydration provides evidence of a glass transition (which phenomenon has been disputed for granular starch among researchers). These findings also provide new information regarding the arrangement of AM and AP at the starch granule surface (AP appears to be more prevalent than AM). Thus, this research contributes new fundamental understanding of both starch granular and molecular structures and reactivities needed to enhance starch modification practices.

Funding and Collaboration: This research was funded by the USDA-NRICGP and included Dr. James BeMiller (Dept. of Food Science, Purdue Univ.) as a co-PI.

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Whole-Tissue, Potato-Based Resistant Starch Materials with Moderated Glycemic Response

Summary: Generation of resistant starch (RS) within potato products to moderate the rate of starch digestion (postprandial glycemic response) and promote colonic health (prebiotic function). A novel aspect of our approach is that RS products are prepared using whole-tissue materials (i.e., potatoes), rather than simply pre-isolated starch.

Situation: There is increasing scientific evidence supporting a potential link between high postprandial glycemic response and risk for obesity and human disease (e.g., type II diabetes, heart disease, etc.). Potato products, which undergo heating/cooking prior to consumption, are categorized almost exclusively as high glycemic foods, or foods to avoid for those with need to control blood sugar levels.

Response: **Kerry Huber** and collaborators are generating sufficient quantities of the potato RS4 (chemically modified) ingredient to accommodate pilot-scale application testing by the J.R. Simplot Co. within their commercial products. Efforts are being undertaken to partner with a third-party starch company to successfully scale up the bench-top procedure to a pilot-scale level to generate amounts needed to support application testing and future animal and clinical studies. Laboratory efforts have continued to focus on diversifying whole-tissue potato RS ingredients to encompass a broader range of product types (RS1/starch shielded from digestion; RS2/retention of native starch resistance to digestion; RS3; formation of starch molecule structures that resist digestion).

Impact: Development of potato products with enhanced RS content and moderated glycemic response expands traditional uses of dehydrated potato products, allowing their entry into market areas that are at present inaccessible to traditional potato products. Potatoes provide the largest source of crop revenue within the state, with a base crop value of $796 million reported for 2009. Direct sales from Idaho potatoes and processed potato products totaled $3.4 billion in 2002, with the majority of this total ($2.3 billion) generated by the frozen/dehydrated potato processing industries. In 2002, the total impact of the Idaho potato industry, including both direct and indirect (i.e., supporting) business activities, accounted for more than 5% of Idaho's gross state product, as well as 39,500 jobs, $6.7 billion in sales, and $1.3 billion of income within the state.

Funding and Collaboration: Funded by the Higher Education Research Council (Idaho State Board of Education) and J.R. Simplot.

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Evaluation of Tannin Additives to Red Wine

Summary: Study on efficacy of adding exogenous tannins to red wines.

Situation: Tannins contribute to wine quality and are frequently added during winemaking. Tannins are added to wine for a range of reasons including effecting the sensory properties of the wine, deactivating enzymes or bolstering the amount of antioxidants. Tannin additives and their impact on wine are poorly documented.

Response: James Harbertson and collaborators characterized a range of enological tannins and their contribution to wine quality. Enological tannins were analyzed for protein precipitable tannins and iron reactive phenolics. One tannin product was added to a Merlot wine during barrel ageing, at a range of concentrations from 60 to 300 mg/l. Condensed and hydrolysable tannins were added to Cabernet Sauvignon wine post-pressing at a recommended and excessive rate. Wines were analyzed for anthocyanin, small and large polymeric pigment, precipitable tannin, iron reactive phenolics and sensory character. Enological tannins contained 12–48% tannin and recommended additions had little impact on wine tannin. High tannin additions were readily measured in the wines and were discriminated in sensory analysis with lower intensities of most parameters except brown color, bitterness and earthy character. Recommended addition rates are too low to impact the measured tannin concentration of Merlot and Cabernet Sauvignon wines from Washington (USA). High enological tannin additions that had a measureable impact on final wine also had a negative impact on sensory character.

Impact: Tannins are added to wines for a range of reasons and represent one of many input costs in an industry increasingly seeking efficiencies in response to global economic circumstances, over-supply and an ongoing price point squeeze. This research suggests many tannin additions may be unjustified and have limited or negative impacts on quality. Practitioners will have better information about adding tannins to wine and may not do so.

Funding and Collaboration: Collaborators were Chateau Ste. Michelle, Department of Primary Industries Victoria, Australia and UC Davis. Funded by the Washington Wine Advisory Committee.

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Evaluation of Rootstock and Scion on Grape Wine Composition in Eastern Washington

Summary: Rootstock did not alter grape or wine composition.

Situation: Because the world's major wine grape (*Vitis vinifera* L.) cultivars are susceptible to damage and decline by the root louse phylloxera (*Daktulosphaira vitifoliae* Fitch) and various species of nematodes, they are typically grafted to crosses of North American *Vitis* species, which have resistance to or tolerance of these pests. Rootstocks are also utilized to adapt to adverse soil conditions such as salinity, pH (alkaline and acidic), and extremes in water availability (drought and flooding). Very few studies have been conducted to evaluate the effect of rootstock on wine composition due to the necessary additional experimental design and logistical requirements. Because phylloxera has been cited as the cause of some of the largest losses to wine grapes in European and American history, research was begun in Washington state in 1999 where vineyards are exclusively own-rooted to evaluate the

impact of rootstock on vine performance and fruit and wine composition. The main additional environmental factor that must be accounted for in eastern Washington is its arid climate due to the limited annual rainfall (152-254 mm).

Response: **James Harbertson** and collaborators evaluated the performance of Chardonnay, Merlot and Syrah on five rootstocks (5C, 140Ru, 1103P, 3309C, and 101CU) or on their own roots using ten field replicates over three vintages (2007-2009) in the Yakima Valley AVA. At harvest two field replicates each were combined to make five winemaking replicates; 45 kg of grapes were made into wine. The grape variables measured were soluble solids, titratable acidity (TA), pH, potassium (skin, seeds and pulp), tannins (skin and seeds), and anthocyanins. Wine variables measured were ethanol, TA, pH, potassium, tannins, total iron reactive phenolics, anthocyanins and polymeric pigments. The grape variety and vintage were the most significant variables and rootstock did not ultimately alter fruit or wine composition.

Impact: Results provided vineyard managers and winemakers in Eastern Washington several rootstock-grafting choices if the need ever arises. There are about 35,000 acres of wine grapes in Eastern Washington that would cost an estimated $15,000 per acre to reestablish. An estimated replant of the entire state due to phylloxera outbreak would be ~$525,000,000. Although this type of catastrophic crop failure is unlikely, this type of devastation has taken place in the 1860s in Europe and California. Thus our work provides important information about rootstock selection if replants due to root louses are ever required.

Funding and Collaboration: This work was conducted in collaboration with Markus Keller. Funded by the Washington Wine Advisory Committee and Northwest Center for Small Fruits Research.

Evaluation of Timing and Severity of Regulated Deficit Irrigation on Wine Composition and Quality

Summary: The use of deficit irrigation altered the composition of anthocyanins and tannins in wine.

Situation: All vineyards in Eastern Washington are irrigated due to its arid climate, which in some areas receives less than 10 inches of rainfall annually. Due to the expense of water usage many vineyards are evaluating the use of regulated deficit irrigation to either improve the wine or alter the wine style. Both timing and severity of regulated deficit irrigation are unknown areas of research with regards to altering wine composition and quality.

Response: In a study led by **James Harbertson** and colleagues, regulated deficit irrigation was applied to a 30-year old own-rooted Cabernet Sauvignon vineyard in Mattawa, Washington in attempt to evaluate the effect of altering the timing and severity of deficit irrigation on grape and wine phenolic composition. Evapotranspiration (ET) was used to estimate full vine ET and manage the irrigation applications and mid-day water potentials. The experimental deficits applied were 1) early, 2) full season 3) 100% ET and 4) control (70% ET). The control received 70% of ET whereas all of the treatments are 25% of ET when the deficit is applied. Further in the early deficit which is applied at 25% ET it will return to 100% ET at véraison.

Impact: Significantly greater concentrations of anthocyanins were found in the fruit and wine of the 25% ET treatments whereas only enhancements in the fruit tannins were observed. However the extended maceration treatments showed that the 25% ET and 25%-100% had significantly greater tannin concentrations than the control and 70% ET. Sensory evaluation of these wines will begin in spring. It is expected that a better understanding of water reduction and wine quality will be understood as the project continues.

Funding and Collaboration: Funded by the Washington Wine Advisory Committee and the Fullbright Scholar program.

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Analytical and Chemical Aspects of Wine

Summary: Wine matrix components had a significant impact on the sensory and chemical quality of wine.

Situation: There is an important link between wine quality and economic success. Decisions made during the winemaking process influence the final quality of the wine, with winemakers having the ability to manipulate wine composition.

Response: Carolyn Ross and colleagues studied the influence of different aspects of wine composition (matrix components), their interactions with aroma and flavor compounds and how this influences the sensory and chemical quality of the wine. They also evaluated the influence of different winemaking practices on the final quality of the wine and studied wine finish.

Impact: This research was the first to demonstrate important three-way interactions between different wine matrix components. The influence of the matrix components on wine aromas can be used by winemakers to craft wines that encourage certain aromas or flavors. The study of wine finish, an understudied research area, has provided the industry with information regarding how different flavor/sensory attributes influence wine quality.

Funding and Collaboration: Collaborators included Charlie Edwards, Marc Evans, Jim Harbertson and Jeff Bohlscheid. Research was supported by funds from the Northwest Center for Small Fruits, Washington State Wine Commission and the WSU Sensory Evaluation Facility.

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Controlling the Growth of Spoilage Yeast in Wine

Summary*:* Limiting the growth of a spoilage yeast in wine, *Brettanomyces*.

Situation*: Brettanomyces* is a challenging yeast that spoils countless number of wines in Washington. However, little is known or understood regarding ways to limit its growth.

Response*:* **Charles Edwads** and colleagues conducted experiments to determine the impacts of SO_2 x temperature, Velcorin®, chitosan, and other antimicrobial additives on the growth of *Brettanamyces.*

Impact*:* Winemakers have gained a much better understanding of what can be done to limit this yeast under vinification conditions. If we can give winemakers different methods for control, then the quality (and price) of regional wines will increase.

Collaborators and Funding: Collaborators include regional wineries, Lallemand Inc., and several graduate students. Funded by Washington Wine Advisory Board, Fulbright Program, and Lallemand.

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Inhibition of *Listeria monocytogenes* on cold-smoked salmon using potato processing waste-based edible films containing oregano essential oil

Summary: Bioactive packaging of cold-smoked salmon

Situation: Idaho ranks first in potato production and potato processing in the nation, accounting for over 50% of all potatoes processed in the U.S. Idaho's agricultural processing industry produces over 593 million pounds of potato waste annually, with significant disposal costs and environmental concerns. Potato peel waste (PPW) is an under-utilized renewable biomass and finding viable use for it is a challenge to the potato processing industry. There are now about 2,000 cases of listeriosis reported every year, with 500 fatalities. *Listeria monocytogenes* poses a serious health threat to high-risk populations including the pregnant, newborn, elderly and immuno-compromised individuals. The estimated annual costs and productivity losses for human listeriosis in the U.S. is $209 to $233 million. These figures do not include the economic losses to food companies caused by the recall of *L. monocytogenes*-contaminated product, as well as *Listeria* control costs. There has been increasing interest in developing novel ways to protect foods against contamination. Antimicrobial edible films and coatings have been shown to be feasible for improving the microbial safety of ready-to-eat (RTE) foods. Biologically-derived antimicrobials are gaining interest in the food industry because of their greater acceptance by the growing "natural foods" market. Thus, research efforts are focused on the incorporation of the antimicrobials into edible films and coatings. Essential oils and their components, such as oregano oil, are becoming popular as natural antimicrobial agents for food use.

Response: Gulhan Unlu, Caleb Nindo and collaborators have developed PPW-based edible films with antimicrobial properties. Specifically, the team has been working on the development of PPW-based films with incorporated oregano essential oil (PPW-OO films) and using the newly developed films to improve microbiological safety and quality of cold-smoked salmon. They have also been optimizing the tensile, barrier, and sensory properties of the newly developed films using innovative approaches.

Impact: Conversion of low-value PPW into high value edible films with antimicrobial properties offers multiple benefits, including: 1) enhanced profitability and sustainability for potato growers and processors; 2) the development of sustainable technology utilizing renewable resources and waste streams to produce valuable products; and 3) helping ensure food safety, extend the shelf-life and maintain quality of perishable food products.

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Development of Fish Gelatin Films with Enhanced Physical, Antioxidant and Antimicrobial Properties

Summary: Development of fish gelatin films

Issue: Worldwide production of gelatin is nearly 326,000 tons annually. Terrestrial animal gelatins often do not comply with Halal and Kosher requirements, and, there has been a concern that they may be contaminated with prions. Therefore, alternative sources of gelatin are sought. There is vast potential for fish skins to be used as a source of gelatin. Fish processing waste can account for up to 75% of the weight of the total catch and about 30% of this waste is skins and bones with collagen. About 1.7 million metric tons of seafood processing waste per year is generated in Alaska alone. Fish processing companies have regulatory and cost issues with disposing their waste in sea or on land.

Response: Research by **Gulhan Unlu** and **Caleb Nindo** is directed toward the development of trout gelatin-based edible films with antioxidant and/or antimicrobial properties. Specifically, the team has been working on the development of trout gelatin-based films with incorporated antioxidants (EGCG and green tea powder) and incorporated antimicrobials (nisin), then using the newly developed films to extend the shelf life of foods prone to oxidation or to improve safety of trout fillets. They are also optimizing the tensile, barrier, and sensory properties of the newly developed films using innovative approaches.

Impact: Conversion of fish gelation obtained from low-value fish skins/bones into high-value edible films with antioxidant and/or antimicrobial properties offers multiple benefits, including: 1) enhanced profitability and sustainability for the fish processors; 2) the development of sustainable technology utilizing renewable resources and waste streams to produce valuable products; and 3) helping ensure food safety, extend the shelf-life and maintain quality of perishable food products.

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Removal of Micropollutants from Wastewater

Summary: Engineering design and development of a tertiary wastewater treatment process to remove trace chemicals of emerging concern, such as hormones and antibiotic residues, as well as nutrients like phosphorus.

Situation: Nutrient impacts on surface water from point sources continue to challenge environmental quality. Many bioactive micropollutants, such as pharmaceutical and personal care products, escape the municipal wastewater treatment process, and are released into natural waters. There compounds are now regularly detected in surface and sometimes groundwaters, and some have been shown to cycle into drinking water. Wildlife in and near aquatic ecosystems impacted by discharged wastewater have been shown to have biochemical and physiological effects including feminization of males. There are also concerns for croplands irrigated by treated wastewater and from dairy lagoon waters. Nutrient removal

from wastewater is an ongoing concern due to surface water quality concerns and regulatory compliance. Regulated dischargers in highly nutrient impacted areas require low cost, high efficiency removal of nutrients from wastewater.

Response: Three patents were issued for a novel reactive filtration water treatment process demonstrating a new approach for very low nutrient removal that is cost effective and efficient, at demonstrated water treatment rates of millions of gallons per day. Additional patents were issued for a novel catalytic oxidation water treatment process and licensed to industry. **Greg Moller** and colleagues completed an exploratory 10 gpm trial of catalytic oxidation. Preliminary findings suggest promise for efficient economic operation. They are exploring this approach to also develop high efficiency P removal in algae laden water. A full scale catalytic oxidation project is planned, although some aspects of the technology have already been placed into commercial water treatment.

Impact: The reactive technology is in commercial practice across the US and in several foreign countries, serving over one million people. The technology is deployed for treatment rates exceeding 10 million gallons per day, and also at the municipal wastewater treatment plant with the lowest total P discharge permit in the United States. The work was recognized in 2009 by the *Harrison Prescott Eddy Medal*, the highest research award by the international Water Environment Federation and appeared on a list of *25 Innovations That Changed the World* produced by the Association of University Technology Managers. The patents are licensed by Blue Water Technologies of Hayden, ID.

Funding and Collaboration: USEPA, USDA Hatch Project and Blue Water Technologies, Inc.

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Research: DEVELOPING HEALTHY AND SUSTAINABLE FOODS

Cheese as a Source of Probiotics and Prebiotics with Effects on Gut Microbial Communities

Summary: Aging of American cheese influences the synthesis of bioactive compounds with anti-obesity properties.

Situation: Cheese consumption is declining due to reports linking cheese consumption to obesity.

Response: Giuliana Noratto and her research group are currently evaluating the content of bioactive compounds that are synthesized during the aging process of Cougar Gold cheese due to biochemical and enzymatic reactions. These bioactive compounds in cheese (prebiotics) can reach the colon and exert some health beneficial effects to the host due to their growth-promoting activity on probiotics (health beneficial bacteria). Their goal is to demonstrate that fermented aged cheese is a source of prebiotics and probiotics that can modulate the relative proportions of bacteria population in the colon and protect against low level chronic inflammation induced by obesity.

Impact: Once the current study is concluded, the dairy and cheese industry will benefit, particularly the Washington dairy stakeholders due to the increased consumer awareness about the health benefits of cheese consumption.

Funding and collaboration: Faculty startup and the WSU Creamery.

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Bioactive Compounds in Plant Foods that can Modulate Gut Microbiota and Protect from Obesity and Obesity Related Diseases

Summary: Evaluation of foods with enhanced content of non-digestible compounds that can be metabolized by the colon microflora and promote the growth of health beneficial bacteria to protect the host against obesity and obesity related diseases.

Situation: The dramatic increase in obesity during the last 20 years in the United States has contributed to increased health costs. Growing evidence suggests that gut microbiota can be modulated by diet and play an important role in preventing obesity and obesity-related diseases.

Response: Giuliana Noratto and her research group are currently investigating food plants as the source of non-digestible bioactive compounds that can help to modulate gut microflora balance and diversity as a strategy to influence host physiology and prevent obesity.

Impacts: These studies will provide critical information for the selection of plant food cultivars and development of food ingredients with enhanced health properties. In addition, knowledge of the anti-obesity effects of plant foods with enhanced content of non-digestible bioactive compounds will contribute to improve the sustainability of U.S. agriculture due to their increased demand.

Funding and collaboration: Faculty startup funding.

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Dietary Grape, Gut Microbiota and Intestinal Epithelial Health

Summary: Dietary polyphenolic compounds associated with grape ingestion improve gut microbiota and overall health.

Situation: Grape production in Washington State has increased at an exponential rate during the past 30 years. Accompanying this growing grape and wine industry is the availability of grape skin and seed. Effective utilization of this by-product will generate additional revenues for the grape and wine industry in Washington State. Grape seed extracts (GSE) contain high amounts of polyphenolic compounds that are known for their anti-oxidative and anti-inflammatory effects. Gut epithelial integrity and barrier function is a central predisposing factor to inflammatory bowel diseases, autoimmune and related allergic diseases. Thus, improving gut epithelial barrier function is critical for health. We hypothesize that GSE have beneficial roles on epithelial inflammation and barrier function through modulating gut microbiota.

Response: Meijun Zhu and colleagues have demonstrated that dietary polyphenolic compounds associated with grape consumption ameliorated gut inflammation and improved overall health in mice, including the increase of colonic goblet cell number, the down-regulation of inflammatory cytokine

expression and inflammatory signaling in the large intestine and further altered gut microbiota composition.

Impact: Polyphenolic compounds derived from GSE (by-product of wine industry) improved gut barrier function and reduced gut inflammation. The demonstration that grape polyphenolic compounds have beneficial effects on gut health will likely promote grape skin and seed utilization, and stimulate wine and grape product consumption, thus benefiting the Washington State grape and agriculture industry.

Funding: This project was supported by Washington State University New Faculty Seed Grant and NIH R15HD073864.

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Protein Enriched Cassava can be used to Produce Fufu:
An often Consumed Food in Sub-Saharan Africa

Summary: Evaluating the feasibility of using protein enhanced cassava materials in fufu.

Situation: Cassava is a major source of calories for many in underdeveloped countries but is a protein deficient dietary source. Significant improvement of cassava protein content has been achieved by gene transfer and expression of storage proteins such as ASP1, zeolin (a chimeric protein made of portions of zein and phaseolin) and sporazein (a chimeric protein made of portions of zein and sporamin).

Response: Protein enhanced cassava materials were evaluated by **Joseph Powers** and colleagues for incorporation into fufu, a lactic acid fermented product commonly consumed in Africa. A selected strain of *Lactobacillus plantarum* provided by the Max Rubner-Institut (Karlsruhe, Germany) was used as starter for fufu, using protein (zeolin, sporazein, sporazein plus pro-vitamin A) and pro-vitamin A fortified cassava flours and low protein wild type cassava flours in lab-scale fermentation trials. The added strain rapidly increased titratable acidity (TA) and lowered pH with all cassava types tested. Gas chromatography was used to evaluate aroma profiles. Volatiles were collected using headspace-solid phase microextraction. A similar aroma profile was found in all samples suggesting that protein fortification does not have a detrimental effect on the aroma of fufu. No sensory impact of cassava type was observed in cooked fufu aroma.

Impacts: This study represents the first attempt to evaluate high protein GMO cassava material in a product form commonly consumed in Africa. Fermented cassava products constitute an important part of many diets in Sub-Saharan Africa where cassava is a staple food. Cassava is high in starch, but low in protein content. Preparation of acceptable fufu, a common cassava-based fermented product, using protein fortified cassava material demonstrates suitability of a more nutritious product.

Collaborators and Funding: Collaborators include John Fellman, Kerry Huber and Gerhard Munske. The project was supported by state funds.

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Determining the Sensory Aspects of Different Horticultural Products

Summary: The sensory aspects of cherries, apples, raspberries and beer were examined.

Situation: Carolyn Ross and collaborators have provided fruit and vegetable breeders and growers with information regarding sensory acceptance of new cultivars, pre- and post-harvest handling on the final sensory properties and consumer acceptance of different products.

Response and Impact: A novel package developed by researchers at Michigan State University extended the sensory properties of cherries during storage. This research is of significance to the cherry industry as the use of this package could extend the fresh cherry consumption season, thus increasing cherry consumption.

Consumers prefer the WSU Apple Breeding Program's releases, WA2 and WA38 over Gala apples. As these apples are in the final commercialization phase, we also identified 3 new apple selections of potential commercial importance. The apple breeders will use this information to further these selections into Phase III or Phase IV of the commercialization cycle.

Different pest pressures resulted in differing beer sensory properties. Not all pest pressures decreased the acceptability of the beer thus providing interesting research to the industry when considering how to handle hops that have experienced pest pressures during the growing season. Research led to the development of a novel method to isolate beer volatile compounds.

Certain sensory attributes of frozen raspberries influence consumer acceptance more than others. Results will be used by the raspberry breeders (in OR, CA and WA) to better understand what sensory qualities of raspberries consumers desire. The breeders can then target specific genes responsible for various traits. This will help the raspberry industry economically by providing what consumers want.

Funding and Collaboration: Collaborators included Matt Whiting, Kate Evans, Pat Moore, Catherine Daniels, and Doug Walsh. Research efforts were supported by three USDA-SCRI grants and 1 grant from the Washington Tree Fruit Research Commission.

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IMPACT STATEMENTS: TEACHING

Communicating Technical Information

Summary*:* A new exercise was added to FS 460 Food Chemistry to improve student comfort at expressing "informed opinions" on controversial topics.

Situation*:* One of the core competencies in the IFT Education standards is for students to achieve competency in communication skills (*i.e.*, oral and written communication, listening, interviewing, etc.). Students should not only be able to search for and condense information, but also be able to communicate technical information to technical and non-technical audiences. If writing is a form of thinking, and if students write better, they will think better too. In addition, better writing requires better thinking which generates deeper understanding." The SFS External Advisory Board indicated a desire for students to have additional experiences in which a series of debates might help.

Response: Charles Edwards assigned students to teams of 4 to 6 based on answers OPPOSITE to those in an initial opinion survey. For instance, students who were opposed to ingredients from GMO were assigned the group who supported their use. The goal of this exercise was to develop "informed opinions" regarding controversial topics where each student may or may not agree with the assigned position. Students considered all opinions (technical, political, moral, ethical, economic, and medical). Teams worked together to develop general themes or ideas; however, each paper reflected the work of the individual student. Each student's paper was graded for both technical and written quality according to a rubric. Once the papers were completed, members of each team worked together to develop a single oral presentation discussing their position. The teams representing each side of a single issue gave presentations to the class on one day. Students provided an evaluation of each team using another rubric. Of 21 students who responded, 20 indicated that the exercise should be maintained in FS 460.

Impact*:* Will be assessed over time.

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Improving Learning Outcomes of FS 522/422 Based on Assessment

Summary: Additional learning objectives were incorporated into the Sensory Evaluation of Food and Wine lecture (FS 522/422) and lab (FS423).

Issue: According to a SFS faculty survey, students need more experience with identifying, defining and solving real-world problems.

Response: **Carolyn Ross** created new assignments in both FS422 and 522 by incorporating two learning objectives identified as high priority by SFS, oral presentation skills and problem solving. In FS422, students formed groups and profiled a sensory product. The students analyzed data and prepared a report. In the FS522, additional real-world scenarios were presented for student discussion.

Impact: The students benefited from following the sensory profiling for start to finish and gained experience in presenting scientific research.

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Principles of Sustainability: A Cinematic Doculecture Course for a Global Audience of Learners

Summary: A new online course, *Principles of Sustainability*, was developed for upper division students.

Situation: Sustainability is a broad area of inquiry, rapidly changing as we develop new knowledge on human practices that are more sustainable or less sustainable. Seek a hard vision of sustainability and you will surely be disappointed. Our gaps in knowledge are great, but the task of growing a more sustainable global community is greater. We are faced with immense challenges that grow greater by the day. The social, political, economic, and environmental complexity of the task often confounds and defeats simplistic approaches.

Response: The *Principles of Sustainability* course, developed by **Greg Moller**, provides students with a broad understanding of sustainability across multiple human dimensions. Upper division and graduate students from many disciplines will find the courseware of broad interest, intense in some areas and introductory in others, but complete in a desire to present the landscape of a rigorous study in sustainability. The course attempts to synthesize linkages and commonalities of understanding through a presentation of the major elements in the field. Other specialty courses in a student's disciplinary area may give a sidebar or complete disciplinary treatment to sustainability that will help organize a more complete understanding in a particular focus area of sustainability, and thus complement this course. *Principles of Sustainability* is a global online open course that uses multiple modes of new media to inform and inspire students. Normalized for upper division/graduate students of any disciplinary background, cinematic HD/surround-sound doculectures in each of the fifty learning areas form the basis of this experimental pedagogy. A doculecture is a PowerPoint-free presentation, formalized in content, and supported with media such as subject supportive film, photographs, animations, music, and text. The approach couples the information intensity of a university lecture with the audiovisual warmth and dynamics of a documentary film. The course leverages our understanding of the cognitive neuroscience of learning and memory and uses dynamic imagery, sounds, and virtual spacial effects. *Over 100 filmmakers and scholars across the globe, and numerous students, contributed to the course.*

Impact: The Creative Commons 3.0 licensed doculectures are *loaded almost 2000 times per week in 80+ countries across the globe*. The course site is already the *Google search rank #1 (of 36 million)* for the disciplinary important term "principles of sustainability," and *#1 or 2 (of 122 million)* for the search term "sustainability course". The course site has 8 of the top 10 search results for the term "doculecture" – the title of this experimental pedagogy. In their positive review of the course, "Teaching Sustainability in an Unsustainable World", PBS and Scientific American Films producers wrote: "*And if sustainability is hard to learn about, just imagine what it's like to teach people - young people, at that - how to better understand and develop a sustainable economic infrastructure in world with so many highly unsustainable systems in place. How do you tell them what they need to know when so much of the information that contextualizes each issue is either inaccessibly technical or under-informed and biased*?"
The Chapter One Part Six doculecture, "*A Planet in Peril*" won the prestigious El Capitan Award from the Yosemite International Film Festival in 2011 and a Bronze Telly Award in 2012.

Funding and Collaboration: UI Greening the Curriculum grant; USDA Hatch funds. Over 100 filmmakers and scholars across the globe, and numerous students, contributed to the course.

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IMPACT STATEMENTS: INTERNATIONAL

Improving Global Food Safety

Summary: Advise international and national boards on technical and legal issues in agriculture and resource management.

Issue: A significant overhaul of food safety law is underway in the United States for the first time in over sixty years. This is leading a great demand for competent assessment of the impact of these new laws on domestic food production and international trade.

Response: **Barbara Rasco** participated in an expert panel advising the FDA on hazard analysis and risk assessments for high and medium risks foods, the recommendations of which formed the basis for pending regulations and guidance pursuant to the Food Safety Modernization Act (2011) the first major overhaul of US food safety law since 1938. On an international level, Dr. Rasco serves as a presidential appointee to the US/Pakistan/Afghanistan Trade Corridors Working Group. This commission has furthered the development of regional, national and provincial agricultural policies across Central Asia assisting with food policy development and regulatory reform, including regulations compliant with international best practices and with the Food Safety Modernization Act. As a result of the passage of the Pakistan-Afghanistan Transit Trade Agreement and the 18th amendment in Pakistan which devolves federal authority to the provincial level, consistency in regulations across this country have become more precarious. Regulatory and legislative initiatives in this region are fraught with problems, but are moving along due to pressure from the private sector for transparency and consistency.

Dr. Rasco assisted in the development of a new program for technical capacity building under the UI USDA FAS grant and with a recently launched food inspectors certification program funded in part by UNIDO in Central Asia designed to improve the professionalism and competency of food regulators across the region. Eventually these efforts should improve the credibility of regional producers in international markets. She also helped draft food safety and commercial law statutes for foreign and tribal governments, most recently the agricultural products law for the Kingdom of Cambodia, and food safety laws promoting jurisdictional sovereignty for Native American tribes compliant with WTO and international market and global food safety standards.

Impact: Drafts of statutes for fresh produce and horticultural crops important in export trade, white papers for trade associations regarding compliance issues, recommendations to the US FDA and industry stakeholders on preventive controls for high and intermediate risk foods; these have been submitted for adoption by the FDA. Training programs for food safety professionals (over 500 in the past three years, with 30+ professional in-country trainers) provide continuing education in developing nations in Central, South and South East Asia on food safety. These programs will lead to improved food safety across the globe.

Funding: USDA Foreign Agricultural Service, US Agency for International Development.