Maine Agricultural Center Integrated Research/Extension Grants: 2012–2013

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MAC128: Food Safety Research and Bottling Guidance to Reduce Maple Syrup Contamination

Principal investigator(s): Seanna Annis, Kathy Hopkins and Beth Calder

Background:

Occasionally maple syrup producers have found their bottles or containers of maple syrup have floating masses or obvious surface mold. Over the last three years, we have found 32 out of 34 samples of maple syrup turned into the University of Maine Cooperative Extension have been contaminated with fungi. These samples and other reports of contamination have come from Maine, Minnesota, Rhode Island, Connecticut and Vermont. Previously, the maple industry assumed that microbial growth could not occur in syrup processed to a minimum of 66° Brix (66% sugar) because of the high sugar content and any contamination that occurred was likely due to low Brix. The industry also assumed that heating syrup to 85°C before bottling would kill off any microbial contamination, and if the syrup was contaminated, boiling the syrup for 15 minutes would kill any contamination and threat to human health. Determining the cause of the contamination and developing processes to prevent contamination will protect both the consumer and the integrity and value of a $91 million specialty forest crop industry in the United States. It will also increase consumer confidence in local food systems. According to the Center for Food Integrity in their 2011 report, every sector of the food system whether farmers, manufacturers, branded food companies, grocery stores or restaurants is under increasing pressure to demonstrate they are operating in a way that is consistent with stakeholder values and expectations[1].

Preliminary research conducted by Dr. Seanna Annis and undergraduate students in her lab have identified several fungal genera (*Penicillium, Aspergillus, Cladosporium* and *Wallemia*) within or on the surface of contaminated syrup. Some of these fungi have the potential to produce mycotoxins, which may be a food safety concern. Dr. Annis' lab has also conducted preliminary heat tolerance studies with fungi isolated from contaminated maple syrup. Spores of these fungi may survive temperatures as high as 75°C (167° F) for 3 minutes and may survive higher temperatures for shorter periods of time. Heat tolerance studies need to be completed to determine the temperature range these fungi can survive and provide information as to whether higher bottling temperatures are necessary to prevent fungal contamination.

Maple producers commonly produce syrup and package their product initially into barrels. They will reheat the syrup and repack this product into containers for retail at approximate temperatures of 85°C (185° F). Research conducted by Whalen and Morselli[2] found that syrup packed in retail containers at 93°C did not develop fungal mats. However, at this temperature, niter or sugar sand will precipitate out, thus rendering syrup packed in glass jars visually unappealing. What are lacking are studies on how rapidly maple syrup cools in different containers. This information along with the temperature tolerances of maple syrup associated fungi will determine what bottling temperatures are necessary for maple syrup to be heated to prevent fungal contamination. The rate at which syrup cools from 85°C to temperatures that fungi may survive will depend upon the container size, composition and the temperature in the surrounding environment. These data will provide guidance to developing processing and repackaging protocols in order to prevent contamination and ensure safe maple products for consumers. This will be helpful to the maple syrup industry and the general public.

Research and/or extension education activities:

A work-study student under the guidance of Dr. Annis will isolate fungi from new incoming contaminated maple syrup samples, and identify fungi using morphology and comparison of their DNA sequences to known sequences. The student will run DNA extractions and PCR (Polymerase Chain Reaction) analysis to amplify genes to identify new isolates. Thermal studies will also be conducted on new isolates to determine the heat tolerance of these fungi.

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[1] Expressions of Trust in the U.S. Food System 2011. *Center for Food Integrity. Accessed March 8, 2012.* www.soc.iastate.edu/sapp/soc415RecreancyTrust.pdf

[2] Fungi Associated with Pure Maple Syrup Packed at the Minimum Recommended Reheating Temperature. Whalen, Mary Lynn and Morselli, Maria Franca. *Journal of Food Protection*. Vol. 47. No. 9. 1984. p. 688-689.

Dr. Beth Calder and Kathy Hopkins will conduct cooling curve studies with Eric Ellis, owner of Maine Maple Products, in Madison, Maine at his facility. Ambient temperature will be maintained at approximately 70 ± 5° F. Syrup will be heated for 10 minutes in a maple syrup canner to several temperature ranges: 91° C (195° F), 88° C (190° F), and 85°C (185° F). Thermocouples will be inserted to record syrup temperatures and be placed into six standard types of containers commonly used in the retail of maple syrup. The volumes and container types will be as follows: glass maple leaf 100 mL, glass maple leaf 250 mL, glass maple leaf 500 mL (pint), plastic jug 100 mL, plastic jug 500 mL (pint), and plastic jug 946 mL (quart).

Containers will be filled and temperatures will be recorded every 1-minute for 30 minutes. Temperatures will be then be monitored every 10 minutes until bottles reach ambient temperatures. Cooling curve data will be calculated based on Newton's Law of Cooling. The research will help provide guidance to the maple industry on how fast maple syrup cools down in certain types of containers, and the recommended syrup reheating temperatures to reduce the risk of microbial contamination. Anticipated completion date of the cooling curve research will be completed by October 1, 2012. The fungal research will be ongoing and all research will be completed by May 1, 2013.

Expected outcomes and method for sharing outcomes:

Based on fungal heat tolerance studies and cooling curve results, syrup repacking protocols will be written for maple syrup producers. These protocols may vary depending upon the container size. For example, if the maple producer is repacking syrup into small, glass maple leaf containers, he/she should heat the syrup to a certain temperature for a recommended period of time to reduce the risk of fungal contamination. Research results, including the cooling curve data will be shared with the maple industry at several venues. Kathy Hopkins and Dr. Beth Calder will present the research results and processing recommendations directly to Maine maple producers at the Maine Agricultural Trades Show in Augusta, Maine. The research results will be incorporated into the curriculum of the IMSI Maple Grading School presented annually in North America by Kathy Hopkins and colleagues and will be published in an extension bulletin to be posted on the Cooperative Extension's maple syrup webpage (http://extension.umaine.edu/forestry-wildlife/maple-syrup-production/). The research will also be presented at a future NAMSC (North American Maple Syrup Council) and IMSI (International Maple Syrup Institute) Annual Meeting in a research poster presentation. This annual conference is the premier event at which current research on maple issues is presented to a combined North American audience.

Final Report

Original objectives that were met and significant findings:

1. Cooling Curve Studies of Maple Syrup Bottled in Different Types of Containers:

We determined the rate of syrup cooling from typical bottling temperatures (85°C to 90°C) for common maple syrup bottles. Our study included pint (473 mL) and quart (946 mL) plastic containers, and the following glass bottles: 40 mL nip, three sizes of maple leaf (50, 100 and 250 mL), 100mL and 250mL square marasca-style, and handle (250 mL) bottles. Maple syrup was heated in a syrup canner (12" x 20" stainless steel canner, W.F. Mason Custom Welding, Porter, ME), to bottling temperatures of 85°C or 90°C, which are within the range that producers bottle their syrup. Thermocouples (Datalogger Thermometer model RDXL4SD; Omega Engineering, Stamford CT) recorded temperatures every 10 seconds at two points: the top in the bottle shoulder and another at 6 mm above the bottom of the container until the syrup cooled to 65°C. Previous work had found spores of fungi isolated from maple syrup survived heating at temperatures of less than 70°C for 3 minutes. Each trial used two bottles of the container being tested, and two trials were conducted for each container size at each bottling temperature.

Our work has shown that container size has a role in determining potential contamination. The quart and pint plastic containers held syrup at higher temperatures longer than any other containers examined. Containers of one pint size and smaller did not have enough mass of hot syrup to maintain a temperature of 82° C for at least three minutes after bottling with 85°C syrup. The smaller 40 ml nip, 50 and 100 ml glass maple leaf and 100 ml marasca bottles had the fastest cooling and took less than a minute for the syrup to cool to below 70^0 C. It is likely that the maple shaped glass containers cooled syrup more quickly because of their larger surface area to volume ratio. Pre-heating of the 40 ml nip, 50 and 100 ml glass maple leaf bottles in the oven at 149 °C for 30 minutes or in a boiling water bath (4.5 cm depth of water) for 5 minutes increased the time syrup remained at temperatures greater than 70^0 C to at least 3 minutes. This study showed that while bottling with syrup at 82°C to 85°C is a best management recommendation, modifications of bottling practices may need to be made to ensure smaller containers maintain a high enough temperature for a long enough period of time to kill fungal contamination.

2. Continuation of Fungal Isolation and Identification Work from Contaminated Maple Syrup Samples:

To date, 74 isolates have been collected from 46 maple syrup containers from the Northeast. The most common genera have been *Penicillium*, *Aspergillus*, *Wallemia* and *Eurotium*. Some *Penicillium* and *Aspergillus* species are known to produce mycotoxins. Isolates of *Penicillium brevicompactum* collected from maple syrup were found to produce the immune system suppressing mycotoxin, mycophenolic acid, when grown in optimized nutrient medium and in maple syrup. Further studies on mycotoxin production by other isolates is being conducted.

3. Heat Tolerance Studies on Fungal Spores:

Fungal species were tested for their tolerance to heating at temperatures from 60^0C to 82^0C and most were found to survive heating at 75^0C for 3 minutes. *Penicillium* isolate (SVT11A) was heated in maple syrup held at 70^0C for different time intervals to determine if longer times at lower temperatures would kill spores. All spores were killed after 7 minutes at 70^0C. Future studies will investigate whether holding syrup for longer periods of time at lower temperatures will kill *Penicillium* and *Aspergillus* spores.

Original project objectives that were not met:

Specific bottling protocols have not been written yet pending completion of temperature studies on fungi and of work on mycotoxin production by fungi isolated from maple syrup.

Methods used to evaluate outcomes:

Kathy Hopkins has presented and will continue to present research updates at several different avenues for both Maine maple producers and international audiences through the North American Maple Syrup Council and International Maple Syrup Institute annual conferences. This information has also been incorporated into the curriculum of the International Maple Syrup Institute Maple Grading School conducted by Kathy Hopkins and Henry Marckres. This information has been welcomed by attendees at industry presentations. Our research has drawn attention and interest from the maple industry from inspectors in Ontario, to maple producers of varying sizes and large maple syrup packers, such as Butternut Mountain Vermont Maple Sugar Company which has cited our research in their HACCP plan. Maine maple producers will be surveyed at a later date once the full research project findings have been presented including re-bottling recommendations to determine research impacts, knowledge transfer and actual actions taken as a result of this project.

Integration of research and extension activities:

Maple producers asked for help in understanding what was causing and why maple syrup was, on rare occasions, contaminated by floating masses. Kathy Hopkins and Beth Calder conducted preliminary research and determined the floating masses were not bacteria, and then contacted Seanna Annis to investigate if these masses were fungi. This has lead to a collaboration where applied questions of how to avoid contamination of maple syrup during bottling and what effect this contamination may have on consumers has been informed by basic research into what fungi are causing this contamination, whether they can produce toxic metabolites and what are their temperature tolerances.

Research findings have been presented to several different audiences across the U.S., which includes professional meeting presentations to academic and maple conferences, as well as maple syrup producer meeting .

External funding both sought and received:

- Detection of Toxins in Contaminated Maple Syrup -$9,245 from the North American Maple Syrup Council in 2013.
- Evaluating Microbial Maple Syrup Contamination - $7345 from the North American Maple Syrup Council in 2012.
- Evaluating Microbial Maple Syrup Contamination and Potential Food Safety Risks for $8216 from the North American Maple Syrup Council in 2010.
- Maple Syrup Microbial Contamination and Potential Food Safety Risks for $40,809 Specialty Crop Grant Program – not funded.

Educational material, publications, and programs:

This work will be later written as a short Extension publication of best management practices for bottling maple syrup, which will be included in the Maple Grading School and future HACCP and good manufacturing practice guidelines/recommendations for maple syrup producers. Eventually, a UMaine Cooperative Extension Maple web page will be developed with these educational materials and web sites will be distributed to the maple industries to find this information. The investigators of this project have also been invited to write a food safety chapter in the next edition of the North American Maple Syrup Producers Manual.

Research publications, abstracts, and presentations:

Professional Meeting Presentations

- Annis, S.L. **(presenter),** B. Calder, R. Garcia and K. Hopkins **(presenter).** 2013. Identification of Fungal Contamination in Bottled Maple Syrup. Technical session presented at the North American Maple Syrup Council/International Maple Syrup Institute Annual Meetings in Moncton, NS, Canada.
- Annis, SL (**presenter**), Garcia, R, Calder B, Perkins, B and Hopkins, K. 2013. The Production of Mycotoxins by Fungi Isolated From Maple Syrup. Mycological Society of America. Annual Meeting. Poster presentation, Austin, TX.
- Hopkins, K, Annis, S, Calder, B (**presenter**), Marshall, W, Garcia, R. 2012. Identification of Microbial Spoilage in Maple Syrup. Technical session presented at the North American Maple Syrup Council/International Maple Syrup Institute Annual Meetings in Mystic, CT.

Industry Presentations

- Hopkins, K. (**presenter**) July 11, 2013 “Fungal Contamination in Maple Syrup and Canning Protocols” Ontario Maple Syrup Producers Association Annual Meeting.
- Hopkins, K. (**presenter**) Jan. 24-25, 2013 “Fungal Contamination in Maple Syrup” and “Canning Protocols - What Works?” Annual Ohio Maple Days Winter Conference held in Morrow, Wayne/Holmes and Geauga Counties, OH.
- Hopkins, K. (**presenter**) Jan. 19, 2013 “Fungal Contamination in Maple Syrup” New Hampshire Maple Producers Annual Meeting held in Lebanon, NH.
- Hopkins, K. (**presenter**) Jan. 4-5, 2013 “Fungal Contamination in Maple Syrup” Annual New York State Maple Producers Winter Conference held in Verona, NY.

MAC129: Food Safety Research to Support Maine Artisan Cheese

Principal investigator(s): Beth Calder, Gary Anderson, Katherine Davis-Dentici and Jason Bolton

Background:

Fresh cheeses, such as chevre, queso fresco and ricotta, are common value-added products sold at farmer markets in Maine and across the nation. Currently, we have approximately 30 licensed cheesemakers in Maine and that number is growing. Farmers often incorporate fresh chopped herbs, garlic, green onions and other spices to season these cheeses. However, it is not a common practice to wash fresh herbs with water or chlorinated dips before being added to cheeses. This practice may transfer potential pathogens or microorganisms that could cause foodborne illness. Herbs have been associated with food recalls due to pathogen contamination and it is quite possible a fresh cheese associated food borne illness outbreak could occur in the future. Basil has been recently implicated in a food recall in September 2011 suspected to be contaminated with *Salmonella* (FDA 2011a). Another recall in June 2011 involved queso fresco, which was possibly contaminated with *Staphylococcus aureus* (FDA 2011b). Queso fresco was also recalled in July 2010 being suspected to contain *Listeria monocytogenes* (FDA 2010). No research studies have addressed this potential public health risk and washes should be evaluated to determine if they reduce the microbial load of fresh herbs and produce commonly added to fresh cheese.

Another issue that arises during farmers market season is maintaining ice and a refrigerated environment of 40° F (4° C) or below for cheese and dairy products. Travel coolers and ice are commonly used to transport perishable food products to the farmers market. One innovative Maine farmer has been using laminar foil to maintain ice. However, research has never been conducted to determine if foil does significantly decrease ice loss and maintain cooler temperatures during warm summer months.

The safety of soft and hard artisanal cheeses when subjected to warm summer temperatures has never been evaluated. Time and temperature curves to assess bacterial growth in soft and hard cheeses subjected to temperature ranges of 70-90° F (21-32° C) would be helpful to understand how quickly bacteria grow at these ranges and how long cheeses should be kept at these temperature ranges. It is common that soft cheeses are kept at refrigerated temperatures, but samples for consumers may not. Most cheesemakers assume that hard cheeses due to the low moisture content, salt and aging process allow these cheeses to be safe at room temperature even during the warm market season.

References:

- FDA 2011a, internet: http://www.fda.gov/Safety/Recalls/ucm271762.htm
- FDA 2011b, internet: http://www.fda.gov/Safety/Recalls/ucm259411.htm
- FDA 2010, internet: http://www.fda.gov/Safety/Recalls/ucm217902.htm

Research and/or extension education activities:

Three separate studies will be conducted.

1. **Herb study:** This study will investigate 5 types of washes to reduce overall bacteria, yeast and mold levels on the surface of basil, garlic cloves and green onions:
    - Dry control (initial count of bacteria, yeast and molds)
    - Distilled water control
    - Hot water (160° F or 71° C for 15 seconds)
    - Tsunami 100® (peroxyacetic acid, commercial fruit and vegetable wash product)
    - Chlorinated water (100ppm)

Preliminary studies will be conducted to determine an adequate dip time (5, 10 and 15 minutes) for herbs to determine if contact time has any effect in reducing microbial counts. After dip treatments, herb samples will be serially diluted and plated to determine: total aerobic (bacteria) plate counts, total coliforms/*E. coli*, *Listeria*

monocytogenes, *Staphylococcus aureus*, *Salmonella sp*, yeast and mold counts. Bacteria, yeast and molds will be calculated as log CFU/g.

2. **Cooler study:** This study will investigate the use of laminar foil bubble wrap and a new polypropylene fabric as additional insulation to reduce ice loss in coolers during warm summer months. Three treatments will be evaluated:
    - Cooler control
    - Laminate foil covering ice inside cooler
    - Laminate foil on outside and covering ice inside cooler
    - Radiant reflective material covering ice inside cooler
    - Radiant reflective material on outside and covering ice inside cooler

Five 75-quart travel coolers will be purchased. A known volume of ice will be weighed and added to each of the coolers under full sun exposure. Thermocouples will be placed in coolers to monitor temperatures every 15 minutes during a 4-hour period to mimic farmers market conditions. Solid ice mass will be weighed at the conclusion of the study.

3. **Accelerated Shelf-life Study:** This study will investigate the bacterial growth curve of Maine artisan soft and hard cheeses exposed at three different temperatures at 80% relative humidity (average summer relative humidity):
    - 1) 70° F (21° C)
    - 2) 80° F (27° C)
    - 3) 90° F (32° C).

The accelerated shelf-life of these cheeses will be evaluated by using the shelf stability chamber in the Dr. Matthew Highlands Pilot Plant at the Department of Food Science & Human Nutrition. The cheeses will also be exposed to full spectrum light. Cheese samples will be plated initially and after incubation at 1, 2, 4, 8, 12 and 24 hours and plated to determine total aerobic bacterial counts. Bacterial counts will be calculated as log CFU/g and growth curves will be calculated from the data.

Expected outcomes and method for sharing outcomes:

The research results will be used primarily for educational purposes for the Maine Cheese Guild members. The intent is to bring awareness to cheesemakers to evaluate current cheesemaking practices and incorporate new methods to reduce food safety risks. Gary and Beth will share research results with Guild members at a Maine Cheese Guild meeting and will also be posted on their web site. The results will also be incorporated into the Dairy Sanitation curriculum, which is a popular UMaine Cooperative Extension training (taught by Gary, Beth and Jason) for cheesemakers and raw milk processors. Other avenues of outreach will include posting a report on the Maine Agricultural Center web site and an online fact sheet for UMaine Cooperative Extension. The herb data will be presented as a research poster to the Institute of Food Technologists Annual Meeting and a manuscript will be prepared and submitted to the *Journal of Food Science*.

Final Report

Original objectives that were met and significant findings:

1. Herb dip study:

With some soft cheeses, herbs are added for flavorings; we investigated the bacterial load of fresh untreated herbs and herbs dipped in various treatments to evaluate the change in bacterial load. This study investigated three different types of vinegars, 4 different lemon juice concentrations, 2 different chlorine concentrations, several hot water treatments, Tsunami 100®, and distilled water for dip times of 30 seconds, 1, and 5 minutes compared with fresh basil purchased at local Hannaford grocery stores as the control. Basil was selected for use in the dip treatments due to its year round availability and volume needed to conduct the research. Herbs treated with vinegar produced

an unacceptable flavor when added to soft cheeses and were not included after initial trials. Peeled garlic cloves and green onions were plated to determine an average bacterial load, but were not used in the dip studies. We suspected fresh herbs would have high bacterial loads. Basil (5.34 log CFU/g) and green onions (6.00 log CFU/g) had extremely high counts, while garlic had much lower counts (2.15 log CFU/g) most likely due to the protection of the outer sheath layers and possible antimicrobial properties. During our first two basil trials, the 30-second chlorine dip (200ppm) provided a 3.8 log reduction in bacterial counts, while the hot water dip (145 deg F) reduced bacterial counts by 2.5 logs. The 75% lemon juice and distilled water treatment provided a reduction of 1.9 logs and the 25% lemon juice lowered counts by 2.3 logs.

2. Cooler study:

This study investigated the ability of 4 modified, insulated coolers compared with an unwrapped (stock) control to reduce ice loss when exposed to 3 warm summer days (average temperatures ranged from 78 to 90 deg F in direct sunlight) for 4 hours. The goal was to simulate environmental temperatures that would be experienced at a summer farmers market. Two coolers were insulated with laminar foil bubble wrap or a polypropylene reflective fabric covering all six external sides of the cooler. The other two treatments utilized unwrapped coolers with an inside layer of foil bubble wrap or reflective fabric just under the lid with an air space over the ice. Coolers were filled ¼ full with 20 lb. bags of nugget ice cubes (Artic Penguin Ice, Getchell Bros). Inside cooler air temperatures were continuously monitored every 10 minutes by thermocouple dataloggers near the top of the cooler and just above the ice. The coolers used in this study (Coleman Xtreme®, 70 quart) were quite effective and all coolers had less than 1 lb. of ice loss even though the inside temperatures ranged from 66-86 deg F at the top of the air space in the cooler to 37-61 deg F over the ice. Coolers were opened every 15 minutes to mimic farmers market conditions and positioned in direct sunlight which provided outside air temperatures in excess of 100 deg F in the direct sun with one maximum temperature reading at 123 deg F. The insulated wraps provided some temperature control advantages during the hottest temperature study day and better retention of ice compared with the control during most studies. The foil bubble wrap had a slight advantage by maintaining 37 deg F over the ice, but this observation could not be repeated in the other studies. Because the coolers were well insulated, larger differences may be seen with less insulated coolers.

3. Accelerated Shelf-life Study:

This study investigated the bacterial growth of Maine artisan soft (chevre) and semi-soft (Monterey Jack) cheeses exposed at 80 deg F and 70% relative humidity for a 9-hour period in a shelf-stability chamber. The cheeses were purchased directly from Maine cheesemakers. The treatments included cheeses inoculated with *Staphylococcus epidermidis* prepared in sodium citrate, a medium control (sodium citrate) and untreated control cheeses. Cheeses were plated at 0, 3, 6, and 9 hours for aerobic bacterial plate counts, detection of *Staphylococcus*, and *E. coli*/coliforms. Although the cheeses were placed in an environment to mimic farmers market summer conditions, no large bacterial spikes in growth of total aerobic bacteria, coliforms or *Staphylococcus* occurred during the study for either cheese. Comparatively, the chevre cheese had lower total aerobic bacterial counts than the Jack over the experimental storage time and actually decreased by 1 log CFU/g in 9 hours. The chevre cheese had a lower pH (more acidic) than the Jack, which may have lowered bacterial counts over time and/or the lactic acid bacteria present in the cheese provided competitive bacterial inhibition. The Monterey Jack had overall higher *Staphylococcus aureus* and coliform counts, but coliforms decreased and were not detected by 9 hours. However, the *Staphylococcus aureus* remained present in the Jack cheese over time and in some chevre samples which is a food safety concern. *Staphylococcus* is known to be a slow growing bacteria which was also indicated in this study.

Original project objectives that were not met:

We met most of the three objectives, but had to make the following modifications due to budget and time constraints. For objective 1, we only focused on the bacterial load (aerobic plate counts) of the herbs and did not plate out yeast/molds or specific pathogens. The garlic and green onions were plated for aerobic bacterial plate counts, but dips were not conducted on these two items, once again due to the costs associated with running these experiments. For objective 3, only 80 deg F was selected for the accelerated shelf-life study since it is close to the average summer temperature in Maine.

Methods used to evaluate outcomes:

The herb studies showed that basil had on average a bacterial load of 969,500 bacterial colony forming units (CFU) per gram of fresh basil and 1,183,750 CFU/g for green onions. Some cheesemakers may chop fresh herbs and add them to finished fresh cheeses to enhance flavor, but this can also possibly affect consumer safety by adding bacteria into the cheese. Chlorine (200ppm) was shown to be effective and reduced fresh basil bacterial counts to under 50 CFU/g. Other effective options for cheesemakers would be to heat water to boiling for 5 minutes and to cool it down to 145 deg F for 30 second to 1-minute dips and then place herbs in a clean salad spinner to dewater the herbs. However, we found that the effectiveness of the dips to sanitize is based on the source of water. We found our distilled water in Hitchner Hall was contaminated and provided higher counts during some of our later studies in August 2013, which then inflated our results in some of our studies. Therefore, clean potable water is important for effective dip treatments and using commercial distilled water may be an option for cheesemakers when creating chlorinated dips.

Purchasing well-insulated coolers will also help cheesemakers to reduce ice loss during the farmers market season. Wraps may also help to decrease ice loss, but starting with a well insulated cooler will be most practical approach. Based on our cooler results, we recommend that product be submerged in ice when packed in the cooler, opposed to sitting on top of the ice.

From our preliminary research, it appears that cheeses may naturally control bacterial growth if samples are left out at room temperature at the farmers market. However, total coliforms and *Staphylococcus aureus* may be present on the cheese. It is still recommended as best practices that cheese not be left out longer than 2 hours at room temperature and be kept under refrigerated temperatures.

Integration of research and extension activities:

From our collaborations and interactions with Maine Cheese Guild members, we developed these objectives with artisan cheesemakers in mind to help answer their questions in regards to cheese food safety at the farmers markets and how to safely incorporate herbs in soft finished cheeses. I met with the Maine Cheese Guild at their annual meeting in November 2013, and I asked to host a guild meeting at UMaine in October 2014 to present our data/findings at this meeting.

Educational material, publications, and programs:

No programs or educational materials have been developed at this time. However, the results will be incorporated into our Dairy Sanitation workshop that we offer every other year (2015) to the dairy industry. UMaine Extension fact sheet will also be developed on best practices for herb dip treatments/sanitizers. We intend to write the project findings into a MAFES technical bulletin.

Research publications, abstracts, and presentations:

A research poster was presented at the Institute of Food Technologists Annual Meeting in July 2013 titled “Microbial Characteristics of Fresh and Semi-soft Cheeses Exposed to Accelerated Shelf-life Conditions to Simulate a Farmers Market Environment” in Chicago, IL.

MAC130: Maine Spotted Wing Drosophila 2012 Field Survey, Real-time Observations and Predictions for Grower Decision Support

Principal investigator(s): Glen W. Koehler, James F. Dill

Background:

Spotted Wing Drosophila (*Drosophila suzukii*) (SWD) were first detected in Connecticut in August 2011, and were subsequently found in all six New England states, including Maine in September 2011. In the first weeks after detection, lost crop value due to SWD infestations in CT, MA, NH, RI are estimated at $500,000 – 1,000,000 (Richard Cowles, CT Agric. Res. Sta., George Hamilton, Univ. of NH; Jon Clements, Univ. of MA; personal communication).

There is great uncertainty and concern about the potential for SWD to cause damage in Maine in 2012 because many high-value Maine crops are on the list of susceptible hosts (Beers et al., 2011, Dreves et al. 2011). Observation of active SWD on warm days in January 2012 in CT has increased concern (Lorraine Los, Univ. of CT personal communication). Some NH berry growers who had to shut down harvest in fall 2011 because of SWD infestation are considering going out of business for 2012. A repeat of the 2011 SWD damage fiasco could push Maine berry growers to that extreme

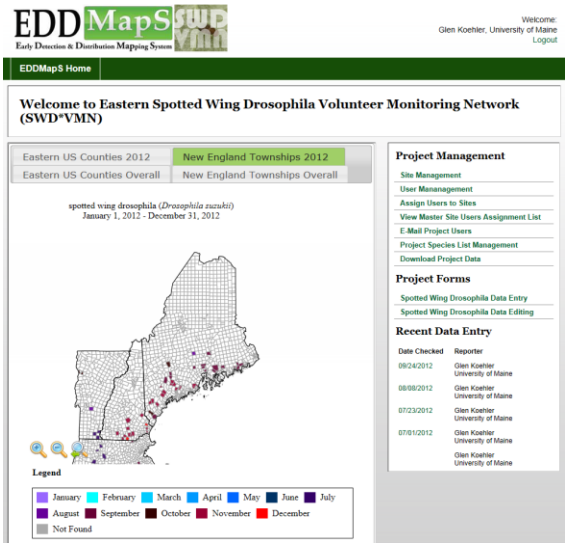
Maine has over $70 million annual crop value at risk of damage from SWD infestation in 2012: lowbush blueberries $55 million, apples: ($12.7 million), strawberries ($3.4 million), highbush blueberries ($1.4 million), and raspberries ($0.8 million) (NASS 2011a). Yield losses of up to 80% were observed in SWD-infested fruit crops in California, Oregon, and Washington in 2009 (Walsh et al. 2011).

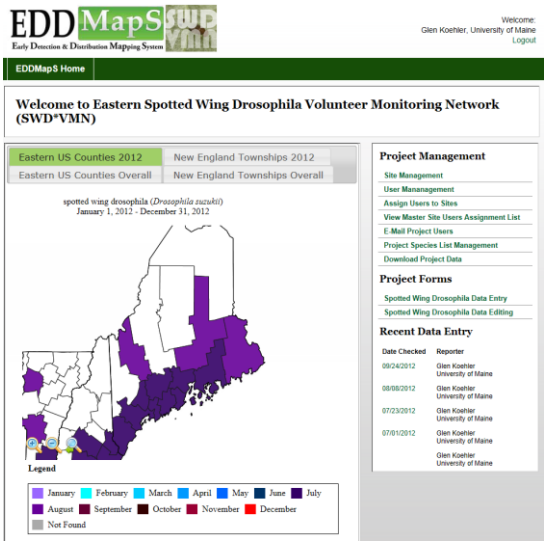
With funding from the Northeastern IPM Center, Koehler organized a meeting in March 2012 of New England research and Extension staff to design a coordinated survey protocol. Survey activity on lowbush blueberry is being covered by blueberry research grants, but there are no funds to conduct SWD survey on southern Maine raspberry, strawberry, highbush blueberry, and apple fruit crops. There are also no funds to operate a SWD data system to make observations easily available to growers, or to provide degree day forecasts for when key events in SWD population events (i.e. first egglaying by overwintered females). We need financial resources to buy materials and pay for staff time to collect and publish information to help Maine growers deal with a new, unknown, unpredictable, and potentially devastating pest. Unless we receive funding to monitor this pest, growers will be left to fend for themselves individually to determine how and when to protect their crops. The foreseeable outcome from that scenario is a steep increase in insecticide use.

Unlike other vinegar flies which can only oviposit into existing breaks in the skin, SWD is able to lay eggs in intact fruit, starting at beginning of coloring through harvest maturity, and continuing on fruit leftover after harvest. SWD oviposition scars are too small for reliable visual detection, and egg hatch and larval development occurs over just a few days. The inability to identify damaged fruit during the egg-hatch latency period means that growers cannot rely on selective harvest to prevent delivery of infested fruit to consumers. The lost revenue from crops left unharvested due to SWD damage is compounded by the risk of damage to reputation and future markets from harvest and sale of apparently wholesome produce that soon becomes riddled with maggots from undetected SWD larvae.

Because SWD is a new pest in Maine (and the rest of Northeast) there is no experience upon which estimate when and where to expect SWD populations to develop, and no other other monitoring network to support threatened crop growers in making spray decisions. In the absence of information about the status of the SWD threat in their

area, growers wary of the potential for damage are likely to assume SWD are present and rely on multiple insecticide applications as soon as vulnerable crops begin reaching maturity, and thus become susceptible to SWD oviposition.





SWD infestation is especially problematic because the greatest potential for damage occurs just before harvest. Insecticide application close to harvest complicates field access for harvest operations and increases the risk of above-tolerance residue on the harvested crop. Widespread use of "insurance sprays" would disrupt over 30 years of IPM progress in minimizing insecticide use on the affected crops through pest monitoring and thresholds and the economic, environmental and social benefits derived from IPM. The association of local fresh Maine fruit crops with dietary health and environmental sustainability could be undermined by increased insecticide use against an unspecified SWD threat. Our survey won't solve the SWD problem, but it will at least give us a view of what is or isn't happening where and when versus shooting in the dark.

References

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Research and/or extension education activities:

1. Purchase trap supplies – May 10, 2012. (1000 Hefty Style Clear Colors 18 oz. cups (each cup used twice); 2,000 disposable lids; 250 9"x12" yellow sticky cards to cut into 3"x4" strips; 2000 feet 24-gauge galvanized steel wire; cider vinegar, sugar, yeast for bait solution). Koehler.
2. Construct 1000 traps: May 21-22, 2012. (each trap used twice, number of trap "sets" is 15 sites x 6 traps per site x 22 weeks = 1980). Hired scout & Koehler.

3. Set traps: May 23-25, 2012. (Estimated date for potential of start oviposition by overwintered SWD in southern Maine is May 30). Hired scout.
4. Build data management system. May The system can be adapted from an existing system used to publish apple pest forecast models (see Orchard Radar at http://umaine.edu/ipm/programs/apple/apple-pest-forecasts%20/). Adapting this system to auto-publish the 2012 southern Maine (raspberries etc., this grant) and downeast Maine (lowbush blueberry traps funded elsewhere) SWD observations will be relatively simple, but does involves developing PHP code to access free temperature data available from Weather Underground.com to operate the degree day forecasts. Once established, data system operation primarily consists input of trap counts, which is conducted as part of the scout duties. Koehler.
5. Continue trap monitoring – Weekly checks: June 1 – October 31, 2012. Daily uploading of trap data into data management system. Hired scout until August 31. Koehler in September and October.
6. Daily automated data system updates of table showing current SWD trapping data and degree day model estimates: June 1 – October 31, 2012. Automated with oversight by Koehler.
7. Project report shared with Maine Vegetable and Small Fruit Growers Associoation at the at the Maine Agricultural Trade Show in January 2013. Final project report to Maine Agricultural Center, Januray 2013 by Koehler.

A logic model for project inputs and outputs is available online at:
http://pmo.umext.maine.edu/apple/AppPestRept/ME-SWDsurveyLogicModel.pdf

Expected outcomes and method for sharing outcomes:

1. Data collected to inform raspberry, strawberry, highbush blueberry, and apple growers in southern Maine on SWD population status during the 2012 growing season.
2. Those growers, plus lowbush blueberry growers more aware of SWD status in southern and downeast Maine through daily updates of SWD trap observation web site. Information also available to growers through Extension newsletters.
3. Growers more aware of need to check their own fields through degree day model estimates for key SWD population dates (start of oviposition by overwintered adults, start of 1st generation adult emergence, start of 1st generation oviposition, peak adult emergence and peak oviposition by overwintered and 1st–5th generations).
4. Grower and Extension staff enter 2013 with a record of how SWD populaitons developed in 2012.

Final Report

Original objectives that were met and significant findings:

1. **Objective:** Purchase trap supplies to build traps to monitor Spotted Wing Drosophila (*Drosophila suzukii*) (SWD) on Maine fruit farms in 2012.

Accomplishment: Trap and lure design completed, trap supplies purchased.

2. **Objective:** Construct 1000 traps.

Accomplishment: Actual number of Maine traps was 500. We originally planned to use 6 traps per site, but this proved impractical after discussion with cooperators, so we adjusted the number of traps to 3 per site. We also thought that traps would only last 2 weeks, but most traps were serviceable for the full trapping season.

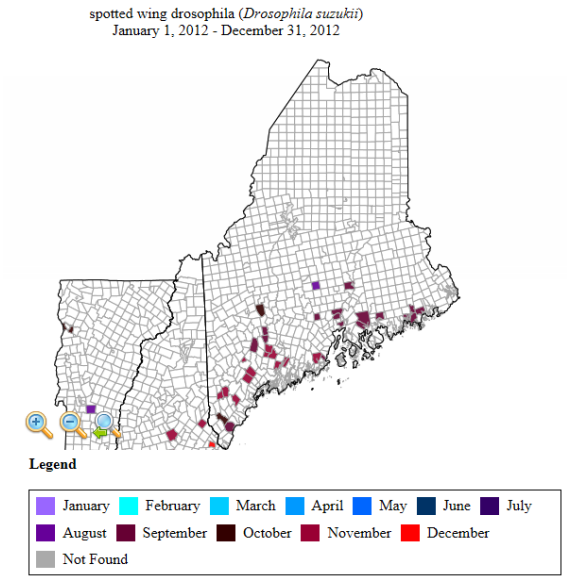
The original proposal called for trapping at 15 sites. The actual number of Maine sites was 111.

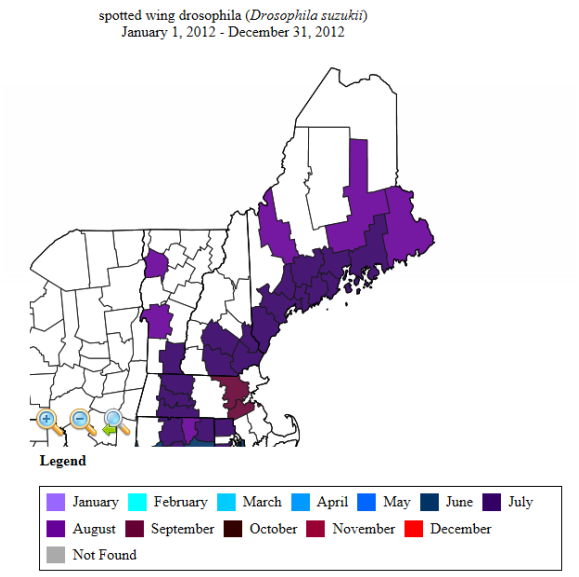
3. **Objective:** Set traps in May 2012. (Estimated date for potential of start oviposition by overwintered SWD in southern Maine is May 30).

Accomplishment: Traps were set in June and July. This turned out to be acceptable as first SWD were caught on the first check date at 7 out of 78 sites.

4. **Objective:** Hire scout.

Accomplishment: Scout started visiting orchards May 2012.





5. **Objective:** Build data management system for 15 sites.

Accomplishment: A system to display Maine SWD trap data in real time was built upon the EDDMAPS (Early Detection and Distribution Mapping System, http://www.eddmaps.org/project/project.cfm?proj=9) with assistance from Joseph LaForest of the University of Georgia. Without funds in the grant to pay for development time, it was not possible to complete a system to forecast degree day values associated with SWD life cycles. And it was determined that real time observations for 78 sites was more useful. Additional traps were monitored with observations communicated to growers with written and verbal scouting reports.

6. **Objective:** Monitor traps at 15 sites through October

Accomplishment: 84 sites (75 of the database sites + 9 others) were monitored into September, 24 (15 in database + 9 others) into October, and 22 (13 in database + 9 others) into November. Additional traps were monitored through August.

7. **Objective:** Project report shared with Maine Vegetable and Small Fruit Growers Association at the Maine Agricultural Trade Show in January 2013.

Accomplishment: Project data shared with growers during the growing season, in December 2012 after growing season, in January 2013 at Ag Trade Show, and in March at Maine Apple IPM Meeting.

Original project objectives that were not met:

Objective: Daily automated data system updates of table showing current SWD trapping data and degree day model estimates: June 1 – October 31, 2012.

Outcome: Constructing such system was beyond the funds provided in the grant after the budget was cut eliminating all funds for Professional staff. It was also determined that real time display of trap observations was more valuable than projections from an untested phenology model.

Methods used to evaluate outcomes:

Real time information on SWD presence and population level provided to growers and crop advisors in real time from many sites in Maine. Evaluation procedures to measure the value of this information was beyond the scope of the project budget which was limited to colleting survey information.

Integration of research and extension activities:

While primarily an Extension project to collect and disseminate critically important information on a new and potentially devastating pest, the project also served to collect baseline information to better understand SWD phenology in Maine, i.e. to have a better understanding of when populations reach an economically damaging level.

Educational material, publications, and programs:

- *Spotted Wing Drosophila 2012 Season Summary for Maine Berry Growers*, David Handley, Vegetable & Small Fruit Specialist; James Dill, Pest Management Specialist; Kaytlin Woodman. December, 2013. (see attachment)
- Issues of the Maine Tree Fruit Newsletter and newsletters to Maine vegetable and small fruit growers.

Research publications, abstracts, and presentations:

- Presentations at Maine Vegetable and Small Fruit Growers Association twilight meetings and at Maine Agricultural Trade Show.
- Presentations to Maine State Pomological Society at Maine Agricultural Trade Show and Maine Tree Fruit IPM Meeting.

MAC131: Food Grade Astaxanthin from Lobster Shell Discards

Principal investigator(s): Denise Skonberg, Jason Bolton

Background:

Last year, Maine's catch of American lobster was 47,117 metric tons, valued at over $330 million and contributing 70% of the commercial fishing income in this state. Although Canadian firms import about 50% of the catch, the rest is processed primarily at small and mid-sized facilities in Maine. Waste from these processes can reach 75-80% of the raw lobster by weight, consisting of shell, viscera, and residual meat. One of Maine's larger processing facilities uses an emerging technology called high hydrostatic pressure, which causes tissue membranes to separate from the inside of the shell, and results in shell discards that are almost completely free of meat.

Lobster shell waste represents a potentially valuable, underutilized resource. Additional incentive to use this material is provided by high disposal costs. For a facility processing 15,000 pounds of lobster per day, these costs can run upwards of $4000/month. Some small composting operations accept lobster shell waste, but the vast majority is sent to landfills. Alternative uses for lobster shell waste would help with rising disposal costs and would extract additional value from a plentiful resource.

Astaxanthin, a naturally-occurring carotenoid pigment in lobster shell, imparts a rich red color that has potential application as a natural colorant for food products. Carotenoid pigments including astaxanthin are also known for their anti-inflammatory and antioxidant properties and have many applications in the nutraceutical, functional food, and cosmetic industries. Much of the astaxanthin in crustacean shell is bound within protein complexes called carotenoprotein. Extraction of astaxanthin from lobster shell requires: 1) demineralization of the shell, 2) removal of chitin, and 3) separation of astaxanthin from the protein complex. Although previous researchers have successfully extracted carotenoprotein from crustacean shell for application in salmon feeds, the methods and reagents used were not appropriate for human food use or for commercial scale-up.

The objective of this proposed research project is to extract astaxanthin from lobster shell waste using commercially available, food grade reagents. This study will create a foundation for the development of a commercial process creating a high-value food ingredient from Maine lobster shell discards.

Research and/or extension education activities:

Each of the three processing steps (demineralization, chitin removal, astaxanthin separation) will initially be evaluated using lobster shell waste obtained from a high pressure processing (HPP) operation. This shell material is relatively devoid of attached muscle tissue, and since the HPP method does not involve cooking the astaxanthin may be easier to extract and of higher quality. Once appropriate methods have been successfully developed using HPP shell material, they will be tested on shell from conventional boiled lobster processing facilities.

The three processing steps will be evaluated in three separate experiments. Demineralization, or removing the minerals (primarily calcium carbonate) from the shell, is frequently carried out with sulfuric acid. Instead, we will assess the efficacy of food grade EDTA (Ethylenediaminetetraacetic acid) and gluconic acid in this step using a multifactorial design to test various reagent concentrations, shell:reagent ratios, and processing times. For step two, chitin will be separated from the carotenoprotein complex by enzymatic proteolysis. A number of inexpensive, food grade enzymes are commercially available that have fungal, bacterial, or plant origins. Variables to be tested in this second experiment include specific enzyme, enzymatic activity, and processing time. Appropriate reaction temperatures and pH will follow supplier recommendations. In the final step, the pigment can be readily separated from protein by benzene, hexane, and other organic solvents. However, for our end purpose, an ethanol:water extraction will be more suitable. Experimental variables will include ethanol:water ratio, solvent:sample ratio, and processing time. For all three experiments, we will assess the effects of treatment variables on the yield and composition of the intermediate (or final) product. The final product will also be assessed for a number of quality attributes, including color (Hunter colorimeter), purity, and antioxidant capacity. Although this is not a process optimization project, this series of experiments should result in an effective method suitable for a food-grade

ingredient. It will also provide a good foundation for a follow-up process optimization proposal which will be submitted to the Maine Technology Institute for funding.

Expected outcomes and method for sharing outcomes:

We expect to establish methods necessary for the development of a commercially feasible process to extract astaxanthin from lobster shell for use in human food products. Establishing a high-value use for lobster shell will add an income stream, reduce waste disposal costs at Maine's lobster processing facilities, and improve utilization of the resource. This process will also result in two lower value product streams (chitin and hydrolyzed protein) which have the potential to increase revenue. Extension outreach to the seafood community will take place at the Lobster Institute's annual Canadian/US Lobstermen's Town Meeting and through publication in The Lobster Bulletin. Results will be shared with seafood scientists at the Institute of Food Technologists Annual Meeting, and through publication in the Journal of Food Science or similar peer-reviewed outlet. Results will also be used in the development of a proposal to the Maine Technology Institute to investigate commercial scale up of the process.

Final Report

Original objectives that were met and significant findings:

1. 1) Each of the three processing steps (demineralization, chitin removal, astaxanthin separation) was evaluated using lobster shell waste obtained from a commercial high pressure processing (HPP) facility. HPP lobster shell waste was obtained from Shucks Maine Lobster and ground in a Paoli meat/bone separator, from which the shell fraction was graded into two particle size ranges, 1.2-2mm, and 0.3-1.2mm.

Demineralization: Both particle sizes were exposed to several demineralizing solutions and processing regimens: citric acid, disodium EDTA, and sodium gluconate at two concentrations (0.2 and 0.5M) and three ratios (1:5, 1:10, 1:20, shell:solution w/v). Samples were then sonicated or shaken at 8C for 2h to assess the effect of agitation method. In subsequent tests, enzyme addition (bromelain and papain, for disruption of protein shell matrix) was explored. When agitation was finished, samples were filtered. Final solution pH was measured and filter containing demineralized filtrate was combusted in a muffle furnace at 550C for 12h. Efficiency of demineralization was determined by difference in ash content (shown below).

Fig 1. Effectiveness of demineralization for 0.3-1.2mm particles. Error bars represent ±1SD.

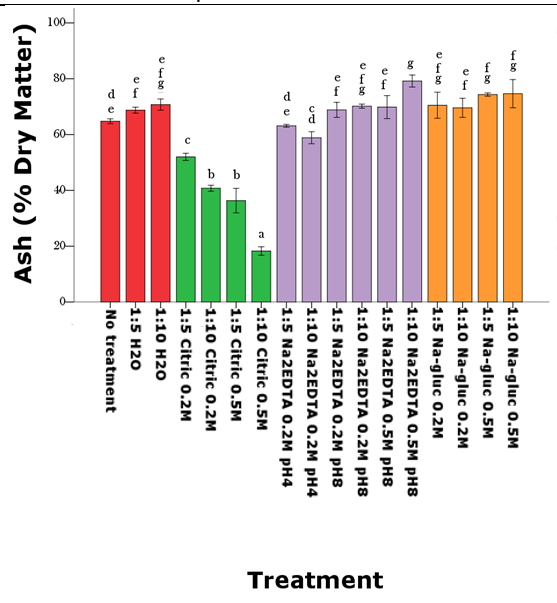
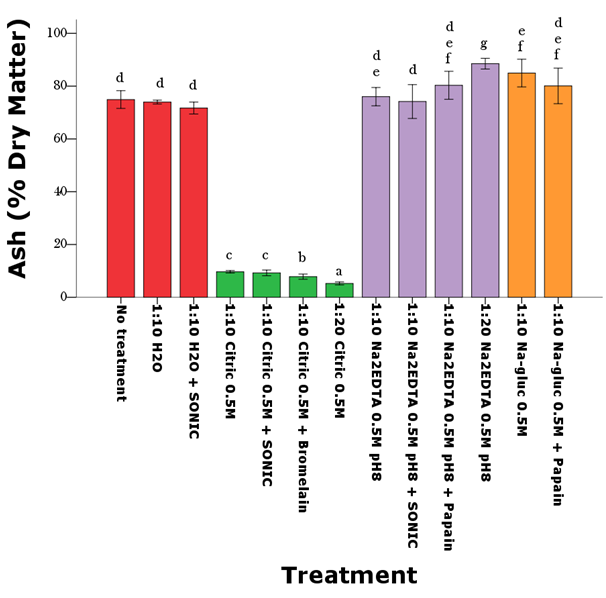


Fig 2. Effectiveness of demineralization for 1.2-2mm particles. Error bars represent ±1SD.



Particle size of 0.3-1.2mm and citric acid at 0.5M and 1:10 ratio was the most cost-effective treatment for purposes of demineralizing lobster shell under conditions minimally destructive to bioactives. With this treatment, shell ash was reduced from 75 to 10% of dry weight.

The filtering process resulted in no visible leaching of shell pigment into the wash water/demineralizing solution, and the filtrand retained its bright red color, ready for future astaxanthin extraction (figure 3).

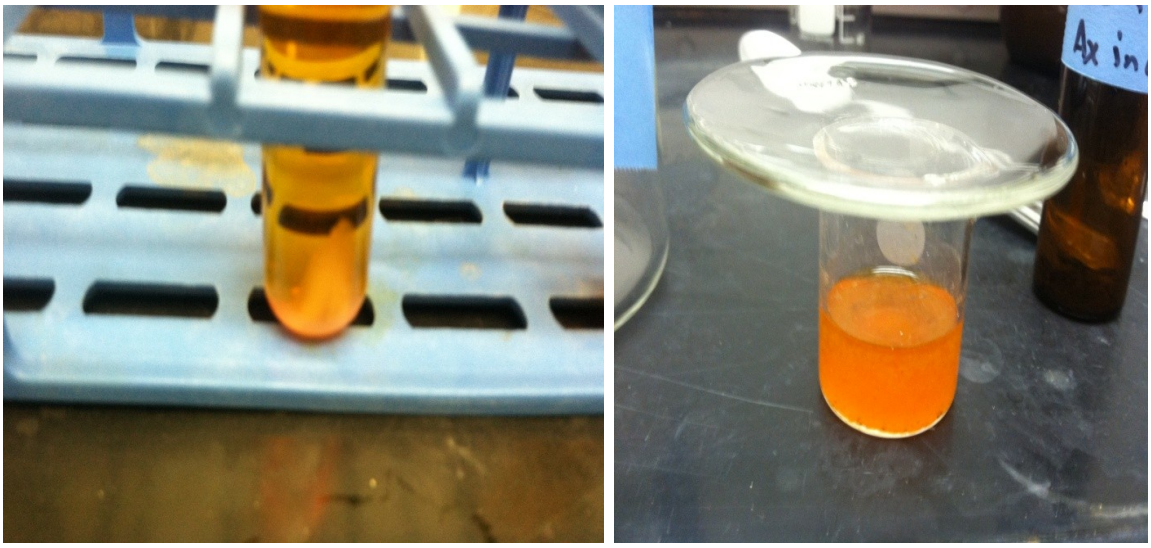
Figure 3. Cooked demineralized shell (orange/red color visible through filter) and wash water/demineralizing solution (clear) (left) and HPP demineralized shell (right)

After initial experiments in extracting and quantitating total astaxanthin with ethanol, acetone, water, and pet ether, it became evident that protein and chitin were not present at significant levels in the extracted product, and their removal as proposed was therefore unnecessary. Standard extraction with acetone showed HPP shell to contain approximately 40µg/g astaxanthin. A different method of grinding shell (with a Hobart food cutter) that generated less friction was tested for grinding a batch of cooked shell (Figure 4).

Figure 4. Cutting and grinding cooked lobster shell for subsequent astaxanthin extractions.

Astaxanthin extraction: For astaxanthin fluid extraction, we focused on the use of pressurized extraction using an accelerated solvent extraction (ASE) apparatus. Pressurized fluid extraction is an emerging technology for high-pressure, low temperature extraction, which shows better efficiency than extractions done at room temperature. Food grade ethanol and ethyl acetate were tested as solvents in the ASE system for extraction of astaxanthin from cooked shell (0.3-1.2mm particle size). Ethanol and ethyl acetate at 725 psi and 70% flush and 70C and 85C respectively yielded 26.0(±2.5) and 22.8(±0.2)µg/g astaxanthin from under-mineralized cooked shell. Demineralized cooked shell yielded results with much higher variation, 18-20 µg/g, and future experiments are planned to optimize the process for both demineralized and undemineralized shell. See Figure 5 for pictures of the extracts.

Figure 5. Astaxanthin extracts



Original project objectives that were not met:

Color, purity, and antioxidant activity of the final extract have not been fully characterized, since evaluating the carotenoid extraction processes took longer than anticipated. Also, the initial antioxidant activity method tested in our laboratory in Summer 2013 was not effective. To replace this method, two other antioxidant activity methods will be investigated during the 2013-2014 academic year. Purity will be assessed in terms of carotenoid composition of the extract. In addition to astaxanthin content, canthaxanthin and zeaxanthin will also be determined by HPLC this Fall, 2013. Evaluating color of the extract is the last objective we will accomplish, as the measurement will be done on the final product.

Methods used to evaluate outcomes:

The results of these experiments provide useful information for further work using pressurized fluid extraction technology. This information will be valuable to the lobster industry, the food industry, and the salmonid farming industry (which is a heavy user of natural astaxanthin and carotenoids). The results of the demineralization study can be used by lobster processors to stabilize lobster shell waste for reduced odor and removal of minerals which may make sale of the demineralized product for subsequent chitin extraction more feasible. The shell grinding method developed in this study is superior to our original method for gentle processing of the shell and will be sufficient for future work without further adaptation. The total astaxanthin quantitation method adapted for this study is highly replicable and easy to conduct in future experiments.

Integration of research and extension activities:

This was a collaborative project between researchers in Food Science and Human Nutrition and UMaine's Cooperative Extension. Dr. Bolton's contacts and interactions with the lobster processing community were integral to carrying out this project and in establishing critical research questions to be addressed in our recent proposal to NOAA. Various starting shell materials were obtained from Cozy Harbor Seafood, Maine Fair Trade Lobster, and Shucks Maine Lobster, who continue to be our partners in this research program. Extension outreach to the seafood community will occur at the Lobster Institute's annual Canadian/US Lobstermen's Town Meeting in February 2014, and results of these studies will be published in the Fall 2014 edition of The Lobster Bulletin.

External funding both sought and received:

This research team, Denise Skonberg, Jason Bolton, Balunkeswar Nayak, and Beth Fulton, submitted a $220,173 proposal titled "Green Production Methods for a High Value Product From Lobster Shell Waste" to the NOAA Saltonstall-Kennedy program in October 2013.

Educational material, publications, and programs:

No education materials or publications as yet. Graduate student Beth Fulton (Food and Nutrition Sciences) is carrying out this research as part of her PhD program.

Research publications, abstracts, and presentations:

- Fulton, B, Bolton, J., and Skonberg, DI. 2013. Demineralization methods for subsequent extraction of bioactives from High-Hydrostatic-Pressure (HPP) processed lobster shell waste. Abstract, Institute of Food Technologists Annual Meeting, Chicago, Illinois; July 2013.