Project No. and Title:	S-294 - Postharvest Quality and Safety in Fresh-Cut Vegetables and Fruits
Period Covered:	04-2007 to 04-2008
Date of Report:	20- June-2008
Annual Meeting Date:	4-May-2008

COOPERATIVE STATE STATIONS AND OTHER AGENCIES:

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Brief Summary of Minutes of Annual Meeting:

The meeting convened at 9:45 a.m. on Sunday May 4th, 2008

Review of agenda – accepted as submitted

2007 minutes were approved at the end of the meeting.

The Chair thanked United Fresh for their support of the S-294 Multi-State Research Project and for their help in organizing the meeting.

1. Report from David Gombas, Sr. Vice President, United Fresh Produce Association. Gombas emphasized how important research is to the fresh-cut produce industry, how industry turns to research for answers. He wishes to continue a long-term relationship with the research group. Industry news: Industry has been working actively with Capitol Hill on the new Farm Bill to convince Congress to support specialty crops – may be as much as $230,000,000 for specialty crop research. Most of research need is for food safety, and also diversity, sustainability, shelf-life and food quality. However, industry members feel they do not get all the support for the research: they need solution-based research. Even though S-294 helped organize the Science symposium, the industry does not feel they are getting an answer to their

questions. One issue is in the area of food safety to set standards for produce handling: when they wanted to set food safety standards, they did not have the science base to set the standards. They need assessment of risks to understand microbial cycles. To overcome these difficulties, a research working group is starting this year. A list of food safety research priorities has been made: intervention strategies #1 priority – information on priorities can be found on the UFPA website. John Beaulieu (USDA-LA) will assist in developing/coordinating research priorities on the Food Safety and Technology Council for UFPA. Additional notes of welcome and a description of eligibility of S-294 members for registration at UFPA were provided.

Some discussion followed with respect to the relationship between S-294 and industry:
Jim Gorny: the comments about academia teaming up with industry is appropriate, however, the exchange needs to go both ways. It is important for scientists to understand confidentiality, and how to establish a trusting relationship between industry and research.
Examples of positive relationship between industry and academia were given by Barry Eisenberg (River Ranch Fresh Foods) and Pat Millner (USDA,ARS).

2. Report from Reuben Moore, Administrative Advisor.
Moore complimented the group for its productivity (publications and scholarly activity). Impact is usually greater when there are publications. The Farm Bill is good news for the group. Researchers will have to apply for grants through NSF, NRI or others to get some of these research dollars. He admitted that competitive grants do not have the same priorities than industry solution-based research, but he hopes the researchers follow through to the industry for results. Hatch funds will likely all go competitive, which will negatively impact multistate research projects in the future. He offered assistance if needed. Gorny asked about multistate research funds for travel to this project - 7 or 8 of the attendees indicated they receive travel funds. He noted that UCD did not allocate funds for travel and asked the group how funds were handled. Moore indicated that AES Director has discretion regarding travel and other support. Moore indicated he could find out how much Hatch $$ were sent to each land grant university and offered to send a letter to the Director of the Experiment Station.

3. Election of new secretary. Bassam Annous nominated Mikal Saltveit. Carried unanimously.

4. Planning the 2009 meeting.
Mendonca requested input for UFPA Science Symposium topics. He described the current process for identifying topics. Beaulieu suggested that the request for speakers go out at least 6 months prior to the date of meeting (Sept/Oct). This does not exclude the possibility that an outbreak or crisis occurs, which will need to be addressed. Annous asked about inviting international speakers (example of FAO/WHO project looking at chlorine issues). Lefcourt suggested that technological issues may be more appropriate from in-country speakers. Gombas suggested no limits be placed on Symposium as long as it involves the best of the best research that we as top researchers think the UFPA needs. Mendonca indicated that the executive committee will take up the process earlier, 6 months prior to the meeting as suggested. Motion was made to this effect and carried.

Brecht suggested the executive committee commit to updating the membership list.

5. Research Division – UFPA.
Beaulieu described his activities with the research division of UFPA and initiated a discussion of the relationship between UFPA and S-294. He indicated that the UFPA directors group was large and unwieldy, so working groups were developed. There are now 5 working groups, one of which is the research division. The other four working groups are: education and outreach, microbiology, audits, and standards harmonization. The Center for Produce Safety at UCD will become a clearinghouse for research information; he indicated his interest in assisting that effort. He suggested that contacts with UFPA be utilized for various research-related agendas. He asked that S-294 provide some input as to his role with UFPA. Lefcourt mentioned the difficulty with getting growers and people using equipment and other technologies used in the fresh produce industry involved. Gombas indicated that there really is a good deal of representation across the industry and that within the UFPA, there are 4 sectors: a grower shipper board, fresh-cut processors board, retail food service board, and whole food board. Each board will have their own issues/agenda. Gombas offered his resources to enable appropriate interactions and Beaulieu will work with all 4 groups. Eisenberg provided some descriptions of the realities of the disconnection between researchers and industry, especially with regard to timelines that we both work on. Lefcourt commented on the lack of industry financial support. Brecht reminded that S-294 was created to identify projects and pool our brains/resources to take it to the industry. He thinks the Research Division is a great improvement. He suggested UFPA act as a lobbying group to change funding structure at the governmental level. Gorny suggested the UFPA may provide letters of support for various projects. Moore indicated that government support likely will not directly address UFPA priorities. Gombas agreed, but described success in terms of investment on dollars spent via lobbying. Discussion ensued of the nuances of industry/scientist research relationships. Gombas noted that even political discussions are long-term. Lefcourt indicated there may be a risk regarding the Farm Bill not returning if impacts are not evident.

6. News from UC-Davis.
Gorny described the development of the Center for Produce Safety (Fernandez Exec. Dir.) at UCD including funding ($4.5M) from industry ($4.0M) and California Department of Food and Agriculture ($0.5M). They will have their first advisory board meeting on June 19th, and the Research Committee on May 23rd.

7. Station Reports.
Each participant reported on progress in their stations. Details are available in the complete meeting's minutes posted at: http://postharvest.ucdavis.edu/S294/AnnualMinutes.htm

8. General.
Brecht asked about industry acceptance of irradiation treatments. Fan described recent workshops on food irradiation and motioned some of the concerns and challenges remaining for implementation. Gombas mentioned that consumer resistance may still be significant and that cost is a significant issue. Mendonca described advantages of irradiation on leafy greens in packages, especially with regard to E. coli and its susceptibility to radiation under moist conditions. FDA approval is still somewhat of an obstacle. It was noted that incoming product quality had to be high. Right now, it is cost-prohibitive because of the logistics of the treatment.

New Business:
There was a motion to keep the S-294 meeting before the UFPA meeting. The next S-294 meeting will be scheduled on April 21st, 2009, at 9:00 a.m. The 2009 UFPA meeting will again be here in Vegas.

Next year's CA Conference and Postharvest Conference are in Turkey will be held in the first two weeks of April.

A Gordon conference on Plant Senescence will be held in June 2008.

Saltveit noted that the final version of the USDA Handbook 66 is at the editors.

Brecht noted that the attendance at the S-294 is declining, and lack of funds is an issue. He suggested that a regularly scheduled conference call be developed to enhance interaction, attendance, and planning and offered to organize it. Beaulieu suggested he will take the request for support of the call by UFPA. This was moved (Beaulieu, Beaudry) and motion carried. The first conference call will be scheduled on the first week of July 2008
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Milner suggested UFPA contact land grant university deans to lobby for their making unit involvement a priority. Gorny suggested that National Program Leader on the topic area be included on the call. He provided an update on progress on strategic planning for vegetable industry.

The meeting was adjourned at 4:30 pm.

Accomplishments:

Objective 1. Develop, evaluate, and standardize subjective and objective quality evaluation methods in intact and fresh-cut fruits and vegetables.

- The catechol and nitroso visual assays of Kader and Chordas (1984) were adapted using fresh-cut mango slices to visualize the distribution of PPO and phenolics, respectively, within the mesocarp. (Brecht-FL)

- Digital images of several fresh-cut products were associated with previously developed rating systems for inclusion in the database developed by project participants at UC Davis. (Brecht-FL)

- Methods for comparing analytical and sensory methods of analysis of tomatoes and oranges are on-going. (Baldwin and Plotto-ARS-FL)

- A sensory panel was trained to evaluate tangerine juice from a breeding trial. 54 hybrids were studied by sensory evaluation and volatile analysis, sugars and acids. (Baldwin and Plotto-ARS-FL)

- A sensory panel was trained to study of flavor and aroma profiling of 1-MCP treated versus non-treated tomatoes. The same sensory methodology will be used to study chilling effect on stored tomatoes (Baldwin and Plotto-ARS-FL)

- Untrained/consumer panels were performed to evaluate new selections of strawberries. A briefly trained panel was performed to compare results with consumer panels (Baldwin and Plotto-ARS-FL)

- A method was developed to easily measure the acid content of commodities in the field (Saltveit-CA).

- A new method of volatile analysis using SBSE (sorptive bar solid extraction) instead of SPME (solid phase micro extraction) is being developed to analyze volatiles in cantaloupe (Beaulieu-ARS-LA)

Objective 2. Develop new strategies to maintain fresh-cut product quality

- The effects of the mango hot water quarantine treatment on the visual and compositional quality factors, aroma volatile production, respiration rate, and electrolyte leakage of fresh-cut 'Kent' mango slices stored at 5°C for 10 days was investigated. The results suggest that the quarantine hot water treatment does not significantly affect the quality or shelf life of fresh-cut mango. (Brecht -FL)

- New packaging was designed that significantly decreases weight loss of fruit and nearly doubles the fruit's shelf life in cherry, strawberry and blueberry fruit. (Bai & Baldwin-ARS-FL)

- Post-cutting shelf life and nutritional quality were evaluated for kiwifruit, pineapple, cantaloupe, strawberry, mango and watermelon stored at 5 °C. Light exposure promoted browning in pineapple pieces and decreased vitamin C content in kiwifruit slices, but increased in mango and watermelon cubes (Kader-CA)

- Incorporation of antimicrobial compounds in starch-based edible coatings in combination with MAP increased the shelf-life of fresh-cut squash. (Forney & Fan-Canada)

- Aroma of fresh-cut onion and apples is strongly affected by packaging. Research is in progress to understand mechanisms of action of packaging and internal atmosphere on flavor retention. (Forney, Fan, Bezanson-Canada/ARS-PA)

- Effect of cutting and dipping treatments on quality, volatile recovery, SEM surface appearance, and freshness in cut melon (Beaulieu-ARS-LA)

- Respiration behavior of fresh-cut pears under modified atmosphere and different temperatures was studied. Data provided a basis to predict package permeability to gases

and will help choosing optimum MAP and temperature combinations to prevent anaerobic conditions and maximize shelf-life of fresh-cut pear. (Beaudry-MI)

- Cut pears were treated with ascorbic acid, sodium hexametaphosphate, and calcium chloride solutions. Response to treatments on texture and color varied according to which cultivar was used, Kiefer Seedling, Beurre D'Hardenpont, or Passe Crassane. (Pilizota- Croatia)

- Two strawberry cultivars were treated with different concentrations of H_2O_2, ascorbic acid, citric acid, K-sorbate, or $CaCl_2$ and in different combinations. After 12 days at 4 °C, colour was best maintained with 2.5 % AA, 2.0 % CA, and 2.0 % K sorbate, and texture with 2.5 % AA and 2.0 % and 2.5 % K-sorbate (Pilizota – Croatia)

- The effect of low-dose electron beam irradiation (0, 0.5 and 1.0 kGy) on fruit quality (pH, % citric acid, Brix and total carotenoid content) and populations of *Salmonella* was studied on fresh-cut cantaloupe stored at 5^oC for 12 days. In general, there was no adverse effect of irradiation at low dosage. However, at higher dosage (1.0 kGy), undesirable changes in quality and antioxidant levels may occur. (Woods & Mendonca-AL/IA)

- Irradiation technology is being evaluated to know whether it should be used exclusively in whole fruit prior to fresh-cut process or post sanitizing methods. (Woods & Mendonca-AL/IA)

- The effects of 1 kGy radiation on the quality of thirteen common fresh-cut vegetables was investigated: Iceberg, Romaine, green and red leaf lettuce, spinach, tomato, cilantro, parsley, green onion, carrot, broccoli, red cabbage, and celery. In general, there was no difference in appearance, aroma and instrumental texture between irradiated and non-irradiated samples for most vegetables. When there were differences between samples, appearance was often improved by irradiation. The vitamin C content was similar for most vegetables, except that irradiated green and red leaf lettuce had 24-53% lower vitamin C contents than the controls. (Fan - ARS-PA)

Objective 3. Improve understanding of biochemical, physiological and molecular mechanisms that affect fresh-cut product quality.

- The role of ethylene-action in quality changes and shelf-life of intact versus fresh-cut melon fruit was investigated using pretreatment with 1-methylcyclopropene (1-MCP). Inhibition of ethylene action by 1-MCP reduced tissue watersoaking, improved firmness retention, and suppressed electrolyte leakage. The development of watersoaking or tissue translucency was accompanied by an accumulation of lipases, carbohydrate-degrading enzymes, and up-regulation of genes encoding these and other, unidentified proteins. (Huber-FL)

- The apparent lack of chilling injury symptom development in fresh-cut tropical and subtropical species in terms of more basic physiological responses of the tissues to chilling stress such as textural alterations and aroma volatile production is being investigated using whole and fresh cut 'Kent' mangoes stored at chilling (5°C) and non-chilling (12°C) temperatures. Aroma volatile did not differ among whole fruit and fresh-cut slices stored 5 or 12 °C with the exception of ethanol, which was lower in whole fruit and slices stored at 5°C. There was no difference between storage temperatures for electrolyte leakage. Reduced ascorbic acid content and increased softening at 5°C suggest that the fresh-cut slices did experience chilling stress (Brecht -FL)

- A model was devised to segregate the efflux of CO_2 from segmented tomato fruit into the four components previously described to measure the contribution of each component to the efflux measured. A substantial, but transient increase in CO_2 efflux occurred immediately after segmentation. The rate of efflux did not return to the initial rate but stabilizes at a slightly elevated rate presumably caused by increased respiration resulting from enhanced diffusion of oxygen into the tissue. This method should allow isolating wound-enhanced respiration from other respiratory changes brought on by altering the diffusion of gases into and out of the segmented tissue. (Saltveit-CA)

- Identification of the wound signal in fresh-cut lettuce and measures of physical and physiological effects of wounding on the rate of carbon dioxide efflux are two major studies under progress. (Saltveit-CA)

- Effects of the last irrigation schedule, nitrogen fertilization and sunlight prevalent during the week prior harvest were studied on the quality parameters of intact and fresh-cut lettuce processed in lab and commercial settings. Also, studies are performed involving the effect of sanitizing treatments on chemical/nutritional composition of fresh cuts. (Fonseca-AZ)

- Bioaccessibility, a measure of what components are soluble and therefore available for absorption in the gut after digestion, is measured for antioxidant fractions in fresh cut fruit and vegetables. A method for performing an in vitro digestion of tissue has been developed. Subsequent to digestion, the samples are fractionated to evaluate the antioxidant value of phenolic and non-phenolic fractions of digestate. Results to date indicate that slices from different cultivars of apples have differing characters and that they may show differing responses to application of anti-browning dips. The bioaccessibility of antioxidant constituents is only a fraction (up to 30%) of what is extractable using solvent extraction. This work will help to better quantify relative biological value of fruit or vegetable tissues, based on what can potentially be absorbed as opposed to the current solvent extract analyses which indicate what is present in the tissue before digestion. (Toivonen, Delaquis, Bach & Bezancon-Canada)

- A new project was recently funded to study postharvest flavor loss of whole and fresh-cut fruit and vegetables. The effects of atmosphere modification, interactions with packaging materials, and postharvest treatments on flavor will be assessed to determine mechanisms

of flavor loss and develop technologies to preserve flavour (Forney, Fan & Bezancon-Canada).

- Research is under way to determine how and why certain *Cucumis melo* melons (climacteric, western shippers, eastern shippers, Charentais, Peil de Sapo, Galia etc.) are considered highly aromatic whereas other melons like *Cucumis inodorus* (non-climacteric, honeydew, casaba, some LSL's) are considered non-aromatic or have low volatile production. Methods include amino acid analysis, analysis of volatile precursors, and enzyme assays. (Beaulieu-ARS-LA)

- The apple cultivar 'Eden' has reduced browning upon cutting but is chilling sensitive. Optimum storage conditions need to be determined in order to have these fruit available to industry throughout the year. Studies on 'Eden' under various storage regimes in 2007-2008 were not conclusive. Additional years of postharvest studies are needed to better understand this cultivar and to develop successful storage recommendations. (DeEll & Toivonen-Canada)

- Agriculture and Agri-Food Canada developed a 1-MCP-based new technology (NT) for the packages of fresh-cut apple slices that substantially reduced decay development in all cultivars evaluated. The NT treatment also improved firmness and reduced color change in apple slices, but the level of effect was dependent on cultivar and time in post-slice storage at 5ºC. (DeEll & Toivonen-Canada)

- Storage conditions, pre-storage treatments and maturity at harvest (temperature, time, atmosphere, MCP or DPA treatments) of whole apples affected quality of slices made from stored apples. For short-term CA stored apples, fruit from earlier harvest resulted in slices with less decay than later harvests or fruit treated with MCP. For long-term storage, apples treated with MCP or DPA resulted in slices with less decay and browning. In addition, a few days of holding apples in refrigerated ambient air after CA storage improved slice quality. (DeEll & Toivonen-Canada)

Objective 4. Standardize methods for recovering pathogenic and spoilage microorganisms from intact and fresh-cut produce including tree nuts.

- A method using flow-through immunocapture (FTI) with real-time PCR was developed and demonstrated for detection of several human microbial pathogens on smooth tomato surfaces and in potato salad and ground beef within 8 hours. Food samples were inoculated with an appropriate dilution of a five-serovar *Salmonella* cocktail and enriched for 5 h. Following enrichment, samples were analyzed by the FTI-XLD and FTI-PCR methods. Food samples were also analyzed by a modified U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM) *Salmonella* culture method for comparison. The FTI method demonstrated the ability to isolate presumptive *Salmonella* colonies up to 48 h faster than did the modified BAM *Salmonella* culture method. (Schneider-FL)

- The ecology of human enteric pathogens in packaged fresh horticultural products is being studied using spinach/lettuce and *Listeria/E. coli* 0157 surrogates as model systems. Conventional culture based methods, in vitro enhancement/inhibition assays, fluorescent-stain microscopy, and microbial community profiling are being used to determine the role of plant tissue, its condition (whole, cut) and its native bacterial population in the colonization, persistence, and multiplication of human pathogens on vegetables. (Bezanson et al.-Canada)

- Novel microbiologic and molecular methods to assess the safety of leaf lettuce are being evaluated through lab and field studies designed to detect and estimate the persistence of fecal contamination events by monitoring variations in the ratios of atypical to total coliform or fecal bacteria present on spinach and lettuce and the relative proportions of six major *E. coli* genotypes on plant tissue and in irrigation water and soil (Bezanson, as part of a team funded by the Ontario Ministry of Agriculture, Farming and Rural Affairs-Canada)

- In a program exploring spectroscopy techniques for detection of trace bacteria and biological toxins in foods, efforts continue to develop a Surface Enhanced Raman Spectroscopic (SERS) method as a rapid and routine detection technique, and to fabricate and characterize SERS nano-probes to provide localized and non-destructive SERS identification on surfaces of bulk samples. (Lefcourt, Kim & Chao-ARS-MD)

- Efforts continue in development of sensing technologies for detection of evidence of bacterial and bioagent contamination on food surfaces. Primary targets of interest are produce and food preparation areas. (Lefcourt, Kim & Chao-ARS-MD)

- Development of technologies screening and sorting apples with evidence of fecal contamination. Work continues on the orientation track system: when apples are rolled down a track consisting of two parallel rails, they move to an oriented position where the stem/calyx axis is perpendicular to the direction of travel and parallel to the plane of the track. In addition to the ability to orient apples, the track system could be used to transport apples during processing, thus replacing the current commercial conveyor systems. Work also continues on developing imaging systems and detection algorithms to simultaneously identify quality problems and contamination of apples during processing. (Lefcourt, Kim & Chao-ARS-MD)

- A method involving flow cytometry and fluorescent molecular probes to rapidly detect *Samonella* in bean sprouts is being tested (Mendonca, Boylston, Brehm-Stecher, Reitmeier & Wilson-IA)

- A method was developed, combining a simple adhesive tape-based method of surface sampling with fluorescence *in situ* hybridization (FISH) for the rapid detection of *Salmonella* spp. on tomato surfaces. *Salmonella* spp. were easily detected on tomato surfaces in less than 2 h, with a sensitivity of 10^3 - 10^4 *Salmonella* cells per cm^2. Additional enhancement in sensitivity was obtained after short (8 hr) solid-phase enrichment on selective (XLT-4) agar. This proof of concept work highlights the

potential for tape-FISH to provide rapid, cost-effective and specific detection of *Salmonella* spp. on fresh produce surfaces, even in the presence of non-target organisms such as saprophytic yeasts. (Mendonca, Boylston, Brehm-Stecher, Reitmeier & Wilson-IA)

- While stomaching samples dilutes the microbial population, but includes any that have penetrated into the flesh, washing samples results in less dilution, but may miss some microbes that penetrate the flesh. Surface washes of cut products are preferred at the US-CSP lab. (Narciso, Baldwin & Plotto-ARS-FL).

Objective 5. Evaluate and control unintentional and intentional microbial contamination of intact and fresh-cut produce.

- Survival and growth of *Bacillus atrophaeus* and Salmonella spp. and effectiveness of sanitizer treatments in inactivation of Salmonella on fruit or vegetable surfaces was shown. The effect of moist heat treatment on infesting bacterial endospores was investigated. Recovery studies suggested a less than 2-log10 unit reduction of inoculated *B. atrophaeus* spores after a 3-minute, 85ºC moist heat treatment, and no heat injury symptoms developed on cantaloupe melons during storage for 2 weeks at 5ºC. Increasing treatment temperature from 75 to 95ºC resulted in no increase in efficacy in terms of recovery of indigenous vegetative bacteria. The results suggest that aqueous heat treatment is not a suitable method for reducing populations of the resting structures of spore-forming bacteria from the surface of netted muskmelons. (Schneider&Brecht-FL)

- The effect of ethylene on the survival of Salmonella cells residing on unwounded surfaces of tomato fruit was investigated. Fruit were held at 20ºC and 95% relative humidity after surface inoculation with the low virulence and rifamycin-resistant pathogen *S. enterica* ssp. *enterica* serovar Typhimurium strain LT2 (*S. Typhimurium*). Tomato fruit were treated either with air or with 150 ppm ethylene in air. The results suggest that although Salmonella can survive on the surfaces of tomato fruit in typical ripening rooms, proliferation of Salmonella is neither promoted nor inhibited by ethylene exposure. (Schneider&Brecht-FL)

- New strawberry selections were evaluated for post-harvest decay. The effect of genetics on strawberry shelf-life was obvious. On the other hand, some selections, very sensitive to rain damage, could be improved post harvest by growing under protected cultivation. (Baldwin, Plotto & Narciso-ARS-FL)

- Effect of preharvest practices and irrigation water quality and subsequent intervention mechanisms are examined on microbial population of harvested product (Fonseca-AZ)

- The effect of different sanitizers on the reduction of *Escherichia coli* O157:H7 and *Salmonella* on baby spinach leaves was studied. Inoculated spinach leaves were subjected to a water wash followed by treatment of one of the following sanitizers: 2% lactic acid at 55 C, calcium hypochlorite, peroxyacetic acid, ozonated water, or chlorine gas. The

reductions of *E. coli* O157:H7 and *Salmonella* on spinach leaves were similar to each other and the sanitizers had an average log reduction of 1.1 CFU/g. The treatment that produced the greatest bacterial reduction was lactic acid. This treatment significantly reduced *E. coli* O157:H7 by 2.7 and *Salmonella* by 2.3 log cycles. (Castillo-TX)

- The reduction of inoculated *E. coli* O157:H7 and *Salmonella* on baby spinach by the application of e-beam irradiation was tested. Inoculated samples as well as controls were exposed to 0.79, 1.16 or 2.48 kGy electron beam irradiation and stored for 8 days at 4 C. For *E. coli* O157:H7, the log reduction was 4.1, 6.3 and 6.4 log CFU/g when irradiated at 0.79, 1.16 and 2.48 kGy, respectively. For *Salmonella*, 0.79 kGy resulted in 4.0 log reduction, and 1.16 or 2.48 kGy irradiation reduced initial counts of 7.3 log CFU/g to below the detection limits (< 10 CFU/g). Irradiation did not affect the survival of the remaining microorganisms stored over eight days ($P < 0.05$). (Castillo, with collaboration of the Wintergarden Spinach Producers Board and the Texas Produce Association-TX)

- The impact of wounded generated oxygen radicals on microbial survival and growth will be assessed using both biochemical analyses and confocal microscopy. Initial studies are planned to quantify and localize the production of hydrogen peroxide on cut surfaces over time, after processing. The data generated will allow analysis of the impact of measured hydrogen peroxide levels on specific bacteria in culture systems. Subsequent analysis will be performed, using confocal microscopy to determine whether chronological changes in bacterial populations can be directly associated with levels of hydrogen peroxide. (Toivonen & Pascal Delaquis-Canada).

- Studies will be continued to determine the fate of *E. coli* from manure in soil and Romaine lettuce on two experimental sites. Sampling strategies and methods for the recovery of *E. coli* from lettuce and soil will be adjusted for seasonal differences in plant development and rates of survival for the target bacteria in soil. Test plots will receive liquid dairy cow manure in the spring at least 90 days before planting. Generic *E. coli* and VTEC populations in manure, soil and on lettuce plants will be determined. Lettuce will be examined for internalized *E. coli*; other samples will be cut, washed and packaged to examine the fate of *E. coli* during processing and storage of the crop. Isolates will be retained for characterization to examine the association with and persistence of specific genotypes in each sampled environment. (Delaquis, Bach & Bezanson-Canada)

- The assessment and management of risk to human and environmental health from microbial contamination from animal and human wastes is being assessed through soil and plant applications of liquid dairy manure and naturally contaminated irrigation water to romaine lettuce plots. Standard and chromogenic-media detection and quantification coupled with isolate DNA typing are being used to follow migration, colonization and persistence rates of *E. coli* under field conditions (Bezanson et al.-Canada).

- A project titled “Mitigating dissemination of bioterrorism agents in Canadian food systems” was recently funded by the Canadian Department of National Defense Research and Technology Initiative Program. A part of this project will assess the stability and

behavior of candidate surrogate bacterial, viral and protozoan strains in soil, water and romaine lettuce plants. Selected strains will be introduced via inoculated liquid dairy manure and irrigation water. Substrate optimized isolation, enrichment, and concentration protocols will be developed to provide material for surrogate detection and quantification via culture, immuno-magnetic separation and quantitative PCR-based methods. Trials will be duplicated in BC using different soils, microclimates, plant and cultivar. (Bezanson et al.-Canada)

- Research has been conducted on the efficacy of washing Romaine and Iceberg lettuce with chlorine solutions, and acidic electrolyzed water containing 50 ppm chlorine to reduce surface contamination with *E. coli* O157:H7. None of the wash treatments studied thus far have yielded a log reduction significantly greater than that achieved by simply washing the lettuce with deionized water. Chlorine dioxide gas was tested to reduce surface contamination of Romaine lettuce with *E. coli* O157:H7. No significant log reduction was observed at the concentration-time tested, however less browning of treated leaves over the 8 days of treatment was observed as compared to leaves which were not treated with ClO_2. Further research is currently underway to investigate the efficacy of different concentration-time exposures with chlorine dioxide gas. (Annous & Keskinen-ARS-PA)

- Research was conducted on lettuce plants watered with *E. coli* O157:H7 to determine the possible uptake of the microorganism by the edible portions of the plant via direct plating and via scanning electron microscopy. It was determined that this method of artificially contaminating plants would not be ideal for initial studies evaluating the effectiveness of inactivation of internalized E. coli O157:H7 due to the low numbers of bacteria that are taken up by the plant, if any. (Annous & Keskinen-ARS-PA)

- Spinach inoculation study are underway, collaborator with LSU, Beilie Ge: "Interaction of Escherichia coli O157:H7 with Growing Spinach Plants" (Beaulieu-ARS-LA)

- It was found that ice made from water contaminated with *E. coli* O157:H7 can spread the pathogen to other heads of romaine lettuce in a shipping container as the ice melts. (Harrison & Kim-GA)

- It was determined that lettuce leaves or roots grown in soil contaminated by *E. coli* O157:H7 or *Salmonella* spp. did NOT internalize the pathogens. Conversely, these pathogens on the surface of the lettuce leaves were quickly internalized. (Erickson, Doyle & Beuchat-GA)

- The methods of attachment on romaine lettuce by non-pathogenic *E. coli* ATCC 25922 was studied. This non-pathogenic strain of *E. coli* would be a useful surrogate for *E. coli* O157:H7 for studies involving attachment and recovery from chilled produce. (Harrison & Kim-GA)

- Studies to determine how *Salmonella* attaches itself to raw Roma, round and grape

tomatoes. *Salmonella* grew in the stem scar and pulp tissues of all three varieties of tomatoes at 12° and 21°C, but not at 4°C. It would not grow on the skin of grape tomatoes at any temperature. (Beuchat & Mann-GA)

- The effect of washing practices at Georgia cantaloupe packing houses on microbial contamination is being examined. Although hot water sanitizing was slightly more effective than chlorinated dump tanks in reducing counts, temperatures in the dump tanks could not be sustained. Microbial populations on melons increased slightly after removing melons from the dump tank, suggesting post-process contamination after washing. (Hurst & Harrison-GA)

- In a project to determine subsurface contamination and internalization of *E. coli* O157:H7 in pre-harvested lettuce, studies will determine (1) strain differences among *E. coli* O157:H7 isolates to internalize, colonize, survive and grow on and in lettuce plants; (2) the degree of internalization of *E. coli* O157:H7 and its subsequent survival and growth in different types of lettuce and at different phases of the plant's growth cycle; (3) the role of environmental stress (heat or water) and soil fertility on the level of internalization of *E. coli* O157:H7 in lettuce; the effect of level of contamination on the extent of internalization, colonization, and survival/growth of *E. coli* O157:H7 in and on lettuce plants; (5) the effect of insect damage to lettuce leaf tissue on internalizing *E. coli* O157:H7 and its subsequent survival/growth; (6) survival and possible internalization of *E. coli* O157:H7 in lettuce as a function of location of contamination (abaxial or lower leaf surface vs. adaxial or upper leaf surface); (7) the contribution of soluble organic matter (simulating materials in which *E. coli* O157:H7 can be found) on survival of *E. coli* O157:H7 on lettuce surfaces; and (8) the level and site of contamination of iceberg lettuce when cored by an *E. coli* O157:H7-contaminated knife. (Doyle, Beuchat & Erickson-GA)

- In a project to determine the fate of *E. coli* O157:H7 on fresh and fresh-cut iceberg lettuce and spinach in the presence of normal background microflora, studies will (1) determine the ability of *E. coli* O157:H7 to multiply in the presence of normal background microflora on iceberg lettuce and baby spinach; (2) simulate commercial conditions and practices for handling spinach and lettuce to allow the evaluation of the fate of *E. coli* O157:H7 on produce during typical handling operations; (3) isolate naturally-occurring microorganisms that may be present on spinach and lettuce under different handling conditions that may show inhibitory activity toward *E. coli* O157:H7; and (4) provide insight into how *E. coli* O157:H7 interacts with naturally-occurring microflora on lettuce and spinach such that the knowledge can be used in analyzing what produce handling and packing routines might be beneficial or detrimental in reducing contamination related to this pathogen. (Harrison & Hurst-GA)

- Research is being conducted on applying food grade chemical sanitizers, and low dose irradiation for killing pathogens on the skin surface of cantaloupes to reducing contamination of the fresh-cut fruit. Hydrogen peroxide, selected surfactants or organic acids, alone or in combination, are being tested for destroying human enteric pathogens on cantaloupe, apples, lettuce, seed sprouts and almonds. Sequential applications of

various chemical sanitizers and electron beam irradiation are also being evaluated to destroy pathogens on the outer rind surface of cantaloupe. (Mendonca, Boylston, Brehm-Stecher, Reitmeier & Wilson-IA)

- Research is in progress to determine the fate of pathogens on the outer rind surface of cantaloupe and whole apples following sequential application of chemical washes and low dose irradiation. (Mendonca, Boylston, Brehm-Stecher, Reitmeier & Wilson-IA)

Impacts:

1. S-294 scientists working in food safety have provided the fresh and fresh-cut fruit and vegetable industries with critical information resolve ongoing food safety issues which challenge existence of the industry.

2. Knowledge generated from studies is being used by S-294 scientists in outreach activities for a change in attitude among students towards food safety in general, and food irradiation as an environmental-friendly and safe alternative for food safety.

3. S-294 scientists received two of nine *Fresh Express* competitive research grants awarded in May 2007. This funding targets studies on *E. coli* O157:H7 contamination of spinach and iceberg lettuce.

4. S-294 scientists are looking for industrial partners to commercialize the patent pending technologies of orienting apples based on their inertial properties.

5. S-294 scientists submitted a utility patent application for a hyperspectral/multispectral linescan imaging system that can simultaneously acquire reflectance and fluorescence images using a single camera. Industrial partners are sought to develop the technique at a commercial level.

6. S-294 scientists have signed an agreement with an industrial partner to develop a commercial surface enhanced raman spectroscopy detecting instrument and for collaboration on imaging technologies.

7. S-294 scientists are working with an industrial partner to develop a hand-held device to survey food processing areas for detection of bacterial and bioagent contamination on food surfaces.

8. S-294 members organized the United FreshTech Science Symposium in May 2008, providing leadership to the industry on food safety issues for fresh and fresh-cut produce.

9. The S-294 working group has been involved in the discussions with the Research Division of the UFPA. Research priorities and how to develop better interactions between researchers and industry are being discussed.

10. The S-294 members presented 15 posters at the United FreshTech trade show. These posters generated interest from industry and led to follow-up interactions with researchers.

11. S-294 member are collaborating with industry. Example: Wintergarden Spinach Producers Board

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