Research Project: Development of New and Improved Systems to Enhance Food Safety Inspection and Sanitation of Food Processing

Location: Environmental Microbial and Food Safety Laboratory

2006 Annual Report

1.What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? Why does it matter?

The National Program this project is aligned to is NP 108, Food Safety (animal and plant products), to develop effective, reliable, and cost-effective methods to control or eliminate pathogens in/on food-producing animals throughout production and processing, and to address the recently recognized problems of pathogens in fruits and vegetables. This research comes under Section 2, Postharvest Food Safety, Activity 2.1 (Methodology), Section 2.1.1, "ARS should take the lead to develop methodologies that have both regulatory, industry and research uses: a commonality of interests between government and stakeholders". It addresses Objective 2.1.1.2, "Develop methods that operate or function in real-time, including on-line detection; validate process intervention strategies; can be used by both large and small processors; and, where appropriate, can be incorporated into a HACCP program," and Objective 2.1.1.7, "Develop methods for the automatic identification of pathophysiological abnormalities, lesions, fecal contamination, ingesta, and processing damage on animal carcasses."

Today, almost 8 billion chickens go through federally inspected processing plants annually, compared to less than 3 billion chickens 40 years ago, when inspectors from the United States Department of Agriculture (USDA) began inspecting every chicken intended for sale to the general public. Because of the rising demand, the industry currently needs processing lines that run at speeds as high as 140 birds per minute (bpm). However, an inspector's visual and manual inspection speed is limited to 35 bpm. The need to develop new technology that will not only improve inspection speed but also minimize problems of human error and variability, and improve the effectiveness of federal food safety inspection programs, drives the research at the Instrumentation and Sensing Laboratory (ISL).

Working closely with the Food Safety and Inspection Service (FSIS), the ISL has successfully tested two poultry inspection subsystems, a visible/near-infrared (Vis/NIR) spectrophotometric subsystem and a multispectral imaging subsystem, as stand-alone units in conventional commercial poultry processing environments. All tests were conducted in the least intrusive manner possible on the processing lines, to avoid interrupting normal plant operations. However, recently, FSIS has been testing the HACCP-based Inspection Models Project (HIMP) in 16 volunteer plants that process young chickens in the U.S. To facilitate commercialization of the ISL poultry inspection system, we need to implement the system online in commercial plants operating under HIMP, and to demonstrate that the system can sort and divert wholesome and unwholesome birds to separate processing lines.

Thus, Objective 1 of this project is to fine-tune systems for detecting pathophysiological abnormalities in poultry with a goal of commercial implementation as part of existing or new poultry processing systems. Before the systems can be commercialized, the multispectral imaging system must be fine-tuned to meet the rigors of use in a processing plant. Detection algorithms for both the Vis/NIR spectrophotometric system and the multispectral imaging system need to be able to automatically compensate for changes in environmental conditions (seasonal, geographical, etc.) and feed that affect the poultry being processed, and an automated control system that improves the integration of ISL detection systems with commercial operations must be developed. Collaborating with Stork Gamco, Inc. (with which ISL has a CRADA) and poultry plant personnel operating under the HIMP system, ISL will further test inspection systems in this environment. Data will be gathered for preparation of Protocols that are needed for Stork Gamco to gain approval of the system by FSIS New Technology Staff. Finally, the ISL poultry inspection systems and a system being developed by ARS in Athens, Georgia, to detect fecal contamination on poultry carcasses need to be integrated.

There is a history of outbreaks of foodborne illnesses associated with fresh produce, including E. coli O157:H7 in lettuce; Cyclospora in raspberries; Salmonella spp. on sliced tomatoes, sprouts, watermelon and juice; and Shigella on lettuce, green onions and cilantro; this has raised concerns regarding potential problems in the microbial safety of fresh fruits and vegetables (Beuchat, 1996; IFPA, 1997; Anon, 1999; Hilborn et al., 1999; FDA, 2001). However, direct identification of the inoculum sources for contamination of fresh produce is usually difficult if not impossible, because sources can include soil, contaminated irrigation water, insects, inadequately composted manure, animal/human feces, and human handling (Buck et al., 2003; FDA, 2001).

In late 1990s, the apple-processing industry was alarmed by reports of E. coli O157:H7 contamination of unpasteurized apple juice and cider (CDC, 1996, 2000). Subsequently, a number of serious outbreaks of disease attributed to consumption of contaminated apple juice spurred the U.S. Food and Drug Administration (FDA) to ask ARS to develop instrumentation to detect apples contaminated with feces (FDA, 2001b). Animal manure and human fecal matter, which represent significant sources of human pathogens such as E. coli O157:H7, Salmonella and Cryptosporidium, remain the major sources of contamination for most produce-associated outbreaks of foodborne illness for which the source has been identified (IFPA, 1997; FDA, 1998; Bennett,1999; Brackett, 1999). In response to the FDA request in 2000, ISL started investigating methods for detection of fecal contamination on apples, with the specific goals of preventing contaminated apples from being processed into juice or cider, or from being consumed as fresh products. Over the last five years, we have successfully developed several cutting-edge sensing systems for detection of fecal contamination (Kim et al., 2004b, 2005; Lefcourt et al., 2005a, b).

More recently, FDA has raised concerns regarding contamination of cantaloupes and strawberries. Outbreaks of salmonellosis have been epidemiologically linked to consumption of fresh cantaloupes (FDA 2001); as a consequence, this produce has been targeted as a potentially hazardous food. Contaminated cantaloupes were found to be responsible for 2 deaths and 18 hospitalizations due to Salmonella between 2000 and 2002; subsequent investigations revealed unsanitary growing and packaging conditions (Anderson et al., 2002; FDA 2002). ISL initiated studies to determine whether methods developed to detect feces on apples could be used to detect feces on cantaloupes and strawberries, with encouraging results (Vargas, et al. 2005). An in-house survey of apple-sorting equipment manufacturers indicated that they would be interested in fecal detection only as part of a broader quality detection system, or otherwise if fecal detection was mandated by FDA. Thus, Objective 2 is to continue development, evaluation, validation and refinement of techniques for detecting feces and defects on fresh fruits and vegetables. Problems of implementing new systems using these techniques, or integrating these techniques with existing systems already in commercial use or under development in other ARS facilities, will be addressed. In this research, apples are used as a model to carry out initial studies, with subsequent studies to be performed on other fruit and vegetable crops such as cantaloupe. Efforts will be directed to developing robust detection technologies that can be applied to a broad range of produce.

Pathogen contamination can also occur through equipment contamination; consequently, food safety and sanitation of processing of plants is a major concern to U.S. consumers. Current procedures for sanitation inspection include visual inspection and the use of swabs. However, a swab-based point-source survey can be costly and time-consuming if large areas need to be tested. Also, one important issue for both FDA and FSIS is that a large amount of food is produced by small and very small processors. ARS is required to assist these small processing plants. The third objective therefore aims to develop portable devices that can be used in situ at processing plants for inspecting sanitation of processing lines. The cost of surveys will be reduced since the initial cost of the equipment will be less than $2K and there are no disposables. These low cost safety inspection devices will be economically viable not only for large processing plants but also for small and very-small plants. The devices will also allow regulators to keep automated historical records for regulatory purposes, which could also be used to determine if some aspect of processing routinely results in contamination of a specific location.

Food security has become an even greater issue since the terrorist acts of September 2001. Contaminants applied either deliberately or unintentionally to agricultural products or processing equipment must be made detectable. Optical sensing and imaging are advanced technologies showing promise in a variety of fields, including detection for bio-terror agents, water quality and safety, and fecal contamination on animal carcasses (e.g., News release: VerifEyeTM 2002, and U.S. Patent No. 5,914,247 by Casey et al., 1999). The proposed portable vision systems could readily be used for identifying such contaminating materials on agricultural products for food security. The ARS Administration recognized the importance of this research area by granting a Research Associateship to ISL for this area of work. Thus, Objective 3 will develop, evaluate, and validate portable low-cost optical and opto-electronic devices for in situ identification of contamination sites for use by producer/processing operations with goals of commercial implementation and expansion to include use in other areas, such as cleaning and sanitation, and military food security.

The goal of Objective 3 is to apply knowledge gained over time at ISL to the development of inexpensive portable optical and opto-electronic devices for detecting contamination areas where food is processed. The portable/wearable imaging devices will be designed to assist inspectors by highlighting areas with thin contaminants, much of which would not be easily spotted by the naked eye. Proposed work will establish optimal wavelengths for detection using visually enhanced direct viewing (e.g., binoculars), reflectance imaging techniques, or fluorescence imaging techniques. These results will be used to design, construct, and validate inexpensive portable/wearable devices. Initially, the devices will be designed to detect the presence of animal fecal matter and will be expanded to include detection of other organic materials that can harbor pathogen growth. Within this objective, strategic inspection models will also be developed in which data are shared among multiple inspectors using portable devices integrated with wireless image/voice communication capabilities.

2.List by year the currently approved milestones (indicators of research progress)
12-month milestones (3/23/2006 - 3/22/2007): Objective 1: - Upgrade multispectral imaging system with EMCCD camera, LED lighting and image processing algorithms. - Develop Fuzzy ARTMAP model for automated calibrations updating. - Develop control system for on-line operation of automated poultry inspection system. Objective 2: - Complete integration of apple prototype system with existing sorting machine. - Complete microbiological study of feces on apples. - Complete first detection studies for cantaloupes and strawberries. - Develop mathematical model of apple orientation. Objective 3: - Evaluation of on-site spectral measurements, sample collection for microbial analyses at FTSL. - Evaluation of fecal contamination on materials used for factory equipment. - Completion of prototype device design and development.

24-month milestones (3/23/2007 – 3/22/2008): Objective 1: - Complete in-plant validation of the multispectral imaging system on high-speed processing lines. - Validate automated system calibrations through in-plant testing conducted at multiple processing plant locations. - Implementation of the control system for in-plant testing. - Complete baseline data collection for commercial implementation of Vis/NIR inspection system. Objective 2: - Complete initial studies of feces on leafy vegetables. - Complete selection of candidate feces-detection algorithms for cantaloupes and strawberries. Objective 3: - Completion of on-site spectral and microbial analyses at FTSL and determine spectral band pairs to be used for color-mixing. - Completion of spectral and spatial analysis and determination of optimal spectral detection bands. - Integration of wireless communication capabilities to portable devices - Completion of color appearance computer simulation for optimizing two-band pairs for color-mixing. - Evaluation of fluorescent-dye and phage for detection of pathogens on apples.

36-month milestones (3/23/2008 – 3/22/2009): Objective 1: - Complete baseline data collection for commercial implementation of multispectral imaging system. - Complete development of the commercial high-speed Vis/NIR inspection system. Objective 2: - Complete tests of apple prototype system. - Complete construction of prototype imaging system for cantaloupes or strawberries. - Complete tests of orientation systems using different varieties of apples. Objective 3: - In-field/on-site evaluation of prototype imaging devices. - Development of an optically enhanced color-mixing device. - Development of a transportable LIF probe. - Development of strategic inspection management models.

48-month milestones (3/23/2009 – 3/22/2010): Objective 1: - Complete development of the commercial high-speed multispectral imaging inspection system. - Integrate multispectral imaging system with Vis/NIR inspection system. Objective 2: - Complete validation of prototype system using a number of apple varieties. - Complete tests of prototype imaging system for cantaloupes or strawberries. - Complete development of whole apple imaging system. Objective 3: - In-field/on-site evaluation of optically enhanced inspection device. - Evaluation of the techniques with other foods. - Refinement of strategic inspection management models via in-field trials.

60-month milestones (3/23/2010 – 3/22/2011): Objective 1: - Complete documentation for the ISL commercial poultry inspection systems. - Complete Objective 1. Objective 2: - Demonstrate commercial prototype apple system. Objective 3: - Demonstration of technology to commercial companies. - Demonstration of optically enhanced inspection device to commercial companies. - Demonstrate the concept/management model to commercial partners.

4a.List the single most significant research accomplishment during FY 2006.
We developed a fluorescence-based, inexpensive handheld imaging device for sanitation inspection of food processing plant equipment surfaces. We incorporated wireless image and voice communication capabilities to the portable imaging devices for wireless-based central inspection data management. Two prototype-handheld portable inspection devices equipped with head-mount displays and wireless image-voice communication capabilities were designed and built (U.S. patent application was submitted, May 2006). The potential applications for inspection of foods and processing plant sanitation, such as for fecal contamination on stainless steel plates that are typically used for manufacturing plant equipment, was demonstrated.

4b.List other significant research accomplishment(s), if any.
We sampled, acquired, and analyzed visible/near-infrared (NIR) spectra on-site at a poultry processing plant for the detection of organic material residues on equipment surfaces. Spectral features of fecal and ingesta residues and rubber belt and stainless steel backgrounds showed large differences in both visible and NIR regions, due to the diversity of their chemical compositions. Results showed that using simple ratio algorithms in the visible or NIR region could separate fecal matters and ingesta from rubber belt and stainless steel backgrounds with a success rate of over 97%. This research supports the development of rapid and low-cost sensing devices for in-situ inspection of fecal/ingesta contaminants at slaughter plants for sanitation verification and poultry safety.

We showed multispectral reflectance imaging techniques to be able to accurately detect diluted poultry residues, specifically feces and ingesta, and other product residues, such as blood, skin, meat, fat, and smeared fluids from poultry skin, on poultry processing equipment surfaces. We determined the optimal wavebands and classification algorithms to detect and discriminate between diluted residues in wet and dry conditions on the surface of poultry processing equipment. Results showed that low concentrations of wet and dry organic residues, diluted up to 1:100 by weight with double distilled water, could be detected with accuracies of 94.1% and 99.7%, respectively. These findings can be used for developing low-cost and wearable/portable multispectral imaging devices for sanitation monitoring of processing equipment in poultry processing plants. This information is very useful for researchers, engineers, regulatory agencies, and processors that are interested in inspection devices or systems for effectively monitoring poultry processing plant sanitation.

We used a laser-induced fluorescence imaging system (LIFIS) to demonstrate the potential use of fluorescence techniques for detection of a range of diluted poultry feces from various sections of the digestive tract, including gizzard, duodenum, small intestine, ceca, and colon, on processing plant equipment. The results showed that 1:5 and 1:10 diluted feces samples could be detected with 100% detection rates at the 580 and 630 nm emission bands regardless of feces type. Detection rates for 1:50 and 1:100 diluted samples at the 580-nm band were 96.0 and 89.3%, respectively, and those at the 630-nm band were 94.7 and 84.0%, respectively. Even minute amounts of fecal matter on processing plant equipment surfaces, not clearly visible to the human eye, could be detected. The findings for optimal spectral bands from this investigation were used in the development of the prototype portable imaging devices.

To determine if detection of fecal contamination on cantaloupes is possible using fluorescence imaging, we acquired hyperspectral images of cantaloupes artificially contaminated with a range of diluted bovine feces from 425 to 774 nm in responses to ultraviolet-A (320 - 400 nm) excitation. Evaluation of the resultant images indicated that 675 nm wavelength exhibited the greatest contrast between feces contaminated and untreated surface areas. Compared to the single-band images, two-band ratios were found to enhance the contrast between the feces contaminated spots and untreated cantaloupe surfaces. The 595/655, 655/520, and 555/655-nm ratio images provided relatively high detection rates ranging from 79% to 96% across all feces dilutions. However, both single-band and ratio methods showed a number of false positives caused by features such as surface scars on the cantaloupe rind. Principal component analysis (PCA) was performed using the entire spectrum of hyperspectral image data. Second and fifth principal component (PC) images exhibited differential responses between feces spots and false positives. The combined use of the two PC images demonstrated the detection of feces spots (e.g., minimum level of 16-microgram per ml dry fecal matter) with minimal false positives. Based on the PC weighing coefficients, the dominant wavelengths were 465, 487, 531, 607, 643, and 688 nm. This research demonstrated the potential of multispectral-based fluorescence imaging for online applications for detection of fecal contamination on cantaloupes.

In order to devise an inexpensive method for orienting apples for imaging, we developed a mathematical model using stability properties to explain the phenomenon in which, when rolling down a test track consisting of two parallel rails, apples tend to orient themselves so that the stem/calyx axis is parallel the plane of the test track and perpendicular to the direction of travel when the angular velocity crosses a threshold. A more complex model of the dynamics of the process is under development. Experimental protocols for actually testing the factors that might influence the orientation process are under development. Some of the factors to be examined were identified in the studies of stability.

4c.List significant activities that support special target populations.
None.

5.Describe the major accomplishments to date and their predicted or actual impact.
We developed a fluorescence-based, inexpensive handheld imaging device for sanitation inspection of food processing equipment surfaces. We incorporated wireless image and voice communication capabilities into the portable imaging devices for wireless-based central inspection data management. Two prototype-handheld portable inspection devices equipped with

head-mount displays and wireless image-voice communication capabilities (U.S. patent application was submitted, May 2006) were designed and built. The potential applications for inspection of foods and processing plant sanitation, such as fecal contamination on stainless steel plates that are typically used for manufacturing processing equipment, was demonstrated.

6.What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?

ISL continues to work with the U.S. Army Natick Soldier Center (NSC) to implement ISL multispectral laser-induced fluorescence imaging techniques for on-site safety evaluation of fresh foods for the U.S. Army.

ISL continues to work with Stork Gamco, Inc., of Gainesville, Georgia, to develop and manufacture commercial ISL on-line poultry inspection systems, under a Cooperative Research and Development Agreement.

ISL submitted a U.S. Patent application for the ISL portable imaging technology, entitled "Low-cost handheld active-lighting image devices and wireless networking for centralized inspection management for contamination scene inspection." (USDA/OTT Docket number: 0097.06)

Two ISL provisional patent applications were converted to utility (final) patents and submitted to the US Patent and Trades Office. (USDA/OTT Docket number:0017.04, Method to Detect Bone Fragments During the Processing of Meat or Fish; USDA/OTT Docket number 0018.04: Use of Nanosecond Scale, Time-Resolved, Imaging to Differentiate Contemporaneous Fluorescence Responses For Multiple Substances.)

An additional utility patent jointly invented with personnel at the University of Maryland, Baltimore County, was submitted: Title: Apparatus and method for orienting rotatable objects.

An ISL scientist was invited by the conference committee to present the ISL imaging technologies for food safety inspection at the PITTCON 2006 conference in Orlando, Florida.

An ISL scientist was invited by the International Fresh-Cut Produce Association to present the rapid ISL online fruit inspection method for detection of fecal contamination on apples at the Fresh-Cut Expo 2006 in Baltimore, Maryland.

An ISL scientist was invited by the ASABE conference committee to teach continuing professional development course entitled “Fluorescence imaging for agricultural uses” at the ASABE conference, Portland, Oregon 2006. Course was to introduce various fluorescence imaging technologies developed and implemented by ISL for food safety inspection.

ISL scientists demonstrated the potential use of stability analysis to explain ability to orient apples for imaging using inertial properties at the ASABE International Meeting in July 2006.

An ISL scientist was invited to give a presentation on using imaging and spectroscopy for non-destructive food safety inspection at the VI CONGRESO INTERNACIONAL DE INOCUIDAD ALIMENTARIA (6th International Conference on food safety), Guadalajara, Mexico, 2006.

ISL scientists demonstrated spectral and imaging techniques that can be used for identifying inoculated insect larvae that are reared for the production of biopharmaceutical compounds.

7.List your most important publications in the popular press and presentations to organizations and articles written about your work. (NOTE: List your peer reviewed publications below).

The May 2006 issue of Applied Optics featured ISL's binocular-based three-color mixing pictures on its cover page.

In August 2006, Newsroom of the International Society for Optical Engineers published an article entitled "Inspection poultry for safety and quality at high speed." This article is about ISL poultry inspection systems.

Review Publications

Liu, Y., Chen, Y.R., Wang, C.-Y., Chan, D.C., Kim, M.S. 2006. Development of hyperspectral imaging technique for the detection of chilling injury in cucumbers; spectral and image analysis. Applied Eng. in Agric. 22:(1):101-111.

Lefcourt, A.M. and Kim, M.S. 2006. Technique for normalizing intensity histograms of images when the approximate size of the target is known: Detection of feces on apples using fluorescence imaging. Computers and Electonics in Agriculture. 50:135-147.

Ding, F., Chen, Y.R., Chao, K., Chan, D.E. 2006. Two-color mixing for classifying agricultural products for safety and quality. Applied Optics. 45(4):668-677.

Yang, C.C., Chao, K., Chen, Y.R., dKim, M.S., Early, H.L. 2006. Simple region of interest analysis for systemically diseased chicken identification using multispectral imaging. Transactions of ASAE. 49(1):245-257.

Ding, F., Chen, Y.R., Chao, K., Kim, M.S. 2006. Three-color mixing for classifying agricultural products for safety and quality. Applied Optics. 45(15):3516-3526.