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Date of Submission of the report: 18th October 2004

BARD Project Number: US-3296-02

Project Title: Rapid detection of foodborne pathogens and non-pathogens in fresh produce using FT-IRS and Raman spectroscopy

Investigators

Principal Investigator (PI): Dr. Joseph Irudayaraj

Institutions

Penn State

Co-Principal Investigator (Co-PI): Dr. Ze'ev Schmilovitch A.R.O. The Volcani Center

Collaborating Investigators:

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Penn State

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A.R.O. The Volcani Center

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.
Agricultural products, food safety

Abbreviations commonly used in the report, in alphabetical order:

FTIR: Fourier-transform infrared, FT-Raman: Fourier-transform Raman,

DARPA: Defense Advanced Research Projects Agency, DOD: Department of Defense

Budget: IS: \$55,000

US: \$45,000

Total: \$100,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	1	1		2
Submitted, in review, in preparation	3	1		4
Invited review papers				
Book chapters				
Books				
Master theses	1			1
Ph.D. theses				
Abstracts		2		2
Not refereed (proceedings, reports, etc.)	2	2		4

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Dr. Chenxu Yu SS No. 398-17-1813

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1	2	3
Longer Visits (Sabbaticals)				

Description of Cooperation:

FTIR work was conducted by the PI's in the US while Raman work was conducted in Israel. Analysis methods were shared. Experimental design was also shared in the respective institutions in US and Israel. Dr. Irudayaraj was responsible for FTIR work while Dr. Debroy was responsible for microorganism handling and organization. Drs. Schmilovitch and Mizrach were responsible for Raman experiments and Dr. Kirtzman provided the microbiological expertise. Discussions were held by email (4-5 emails per month) during the project duration as well as by telephone. Review of the experiments as well as data analysis and exchange of data was conducted during the Israel PIs visit to the US and also during the ASAE meeting.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

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Abstract

Rapid detection of pathogens and hazardous elements in fresh fruits and vegetables after harvest requires the use of advanced sensor technology at each step in the farm-to-consumer or farm-to-processing sequence. Fourier-transform infrared (FTIR) spectroscopy and the complementary Raman spectroscopy, an advanced optical technique based on light scattering will be investigated for rapid and on-site assessment of produce safety. Paving the way toward the development of this innovative methodology, specific original objectives were to (1) identify and distinguish different serotypes of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* by FTIR and Raman spectroscopy, (2) develop spectroscopic fingerprint patterns and detection methodology for fungi such as *Aspergillus*, *Rhizopus*, *Fusarium*, and *Penicillium* (3) to validate a universal spectroscopic procedure to detect food-borne pathogens and non-pathogens in food systems.

The original objectives proposed were very ambitious hence modifications were necessary to fit with the funding. Elaborate experiments were conducted for sensitivity, additionally, testing a wide range of pathogens (more than selected list proposed) was also necessary to demonstrate the robustness of the instruments, most crucially, algorithms for differentiating a specific organism of interest in mixed cultures was conceptualized and validated, and finally neural network and chemometric models were tested on a variety of applications. Food systems tested were apple juice and buffer systems.

Pathogens tested include *Enterococcus faecium*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Bacillus cereus*, *Yersinia enterocolitis*, *Shigella boydii*, *Staphylococcus aureus*, *Serratia marcescens*, *Pseudomonas vulgaris*, *Vibrio cholerae*, *Hafnia alvei*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *E. coli* (O103, O55, O121, O30 and O26), *Aspergillus niger* (NRRL 326) and *Fusarium verticillioides* (NRRL 13586), *Saccharomyces cerevisiae* (ATCC 24859), *Lactobacillus casei* (ATCC 11443), *Erwinia carotovora* pv. *carotovora* and *Clavibacter michiganense*. Sensitivity of the FTIR detection was 10^3 CFU/ml and a clear differentiation was obtained between the different organisms both at the species as well as at the strain level for the tested pathogens. A very crucial step in the direction of analyzing mixed cultures was taken. The vector based algorithm was able to identify a target pathogen of interest in a mixture of up to three organisms. Efforts will be made to extend this to 10-12 key pathogens. The experience gained was very helpful in laying the foundations for extracting the true fingerprint of a specific pathogen irrespective of the background substrate. This is very crucial especially when experimenting with solid samples as well as complex food matrices.

Spectroscopic techniques, especially FTIR and Raman methods are being pursued by agencies such as DARPA and Department of Defense to combat homeland security. Through the BARD US-3296-02 feasibility grant, the foundations for detection, sample handling, and the needed algorithms and models were developed. Successive efforts will be made in transferring the methodology to fruit surfaces and to other complex food matrices which can be accomplished with creative sampling methods and experimentation. Even a marginal success in this direction will result in a very significant breakthrough because FTIR and Raman methods, in spite of their limitations are still one of most rapid and nondestructive methods available. Continued interest and efforts in improving the components as well as the refinement of the procedures is bound to result in a significant breakthrough in sensor technology for food safety and biosecurity.

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Achievements

Tasks accomplished through this feasibility study include the establishment of a detailed FTIR and Raman fingerprint of a range of pathogens and non pathogens. Key organisms examined include *E. coli*, *Enterococcus*, *Salmonella*, *Bacillus*, *Staphylococcus*, *Yersinia*, *Shigella*, *Pseudomonas*, *Vibrio cholerae*, *Enterobacter*, *Aspergillus*, and *Fusarium*. Calibration models were developed using multivariate statistics and independently validated. Alongside, probabilistic neural network models were also developed, trained, and validated. Sensitivity and specificity of detection was assessed in buffer systems and in simple food matrices. The most innovative achievement from this effort is the development of a vector-based algorithm for detection of a specific pathogen in mixed cultures. Other innovations include:

- ❖ Establishing the sensitivity limits of spectroscopic detection using the current protocol
- ❖ Detection and classification of microorganisms in food matrices
- ❖ Development of algorithms to extract the true fingerprint of microorganisms independent of the background

Experience gained through this feasibility study include

- ❖ Standardization of the neural network and chemometric models
- ❖ Sample handling and variability
- ❖ Effect of instrumental parameters in calibration model development

Reports from the Economic Research Service (ERS) indicate that the costs associated with six major pathogens: *S. aureus*, *Salmonella*, *E. coli* O157:H7, *C. Jenuni*, and *L. monocytogenes*, amount to at least \$6.9 billion annually (ERS, 2003). The Centers for Disease Control and Prevention (CDC) estimates that foodborne diseases cause approximately 76 million illnesses, 300,000 hospitalizations, and 5,000 deaths in the United States each year (CDC, 2000). According to the Office of the Regulatory Affairs (ORA), around 4563 food products have been recalled from the market in the year 2001 (ORA, 2003). The attempts set forth in the BARD grant US-3296-02 directly addresses the food safety as well as agro security both in the short and long-term. Unique features of spectroscopic detection are portability, nondestructive assessment, and rapid analysis as demonstrated in this work. The importance and relevance of FTIR and

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Raman spectroscopy is clear from the adoption of these technologies in addressing the sensing efforts for homeland security by DARPA and the DOD. With the advent of improved optics and more sensitive materials the field of spectroscopy is bound to take a significant leap in adapting these devices and new methods for real-time on-field detection in the long-term for food security as well as in homeland security.

The interdisciplinary research utilizes the expertise in spectroscopy, mathematical analysis, and food safety from the US team and the system design, modeling, and microbiology expertise from the team from Israel. The experience gained in sampling as well as the interpretation of the spectra was passed on to the team from Israel. The system based approach as well as the data analysis concepts was shared by the experts from Israel. The need to focus on detailed analysis strategies was found to be very critical in spectra interpretation, hence considerable amount of time was spent on developing tools for specific fingerprinting of pathogens when present in mixed cultures as well as in different backgrounds and in experimenting with dispersive Raman measurements. The design was consistent with the team's efforts. This may have resulted in not conducting a detailed experiment in examining the organisms on a solid surface because preliminary experiments indicated that the spectral quality needs to be given highest consideration. However, sample spectra from FT-Raman on direct examination of apple surfaces is included in the Appendix. Research proposed in the 2004-2005 submission has a major emphasis on direct detection on solid surfaces in addition to liquid systems.

A list of refereed publications that includes the acknowledgement to BARD are

- ❖ Yu, C., and Irudayaraj, J. 2004, Detection and identification of pathogenic microorganisms in mixed cultures by FTIR spectroscopy, *Vibrational Spectroscopy* (In Review).
- ❖ Gupta M.J., Irudayaraj, J., and Debroy, C. 2004. Spectroscopic quantification of bacteria using Artificial Neural Networks. *Journal of Food Protection* (**In Press**).
- ❖ Gupta M.J., Irudayaraj, J., Debroy, C., Schmilovitch, Z., Mizrach, A. 2004. Detection and differentiation of food pathogens using FTIR and Artificial Neural Networks *Transactions of ASAE* (In Review)
- ❖ Yu, C., Irudayaraj, J., Debroy, C., Schmilovitch, Z., Mizrach, A. 2004. Spectroscopic differentiation and quantification of microorganisms in apple juice. *Journal of Food Science: Vol 70* (**In Press**)
- ❖ Schmilovitch, Z., A. Mizrach, G. Kritzman, R. Korotic, J. Irudayaraj, and C. Debroy. (2003). Detection of Food Borne pathogens with Raman Spectroscopy. *Transactions of the ASAE*.
- ❖ Yu, C., Irudayaraj, J., Debroy, C., Schmilovitch, Z., Mizrach, A. 2004. Spectroscopic quantification and strain level differentiation of microorganisms. *Applied and Environmental Microbiology*: (In Review)

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<http://www.cdc.gov/maso/factbook/main.htm>. Accessed on 8 Oct. 2003.

Economic Research Service (ERS), USDA., <http://ers.usda.gov/Briefing/FoodborneDisease/>.

Accessed on 23 Sep. 2003.

Office of the Regulatory Affairs (ORA). Food and Drug Administration.

http://www.fda.gov/ora/about/enf_story/archive/2001/ch10/ch10_toc.htm.

Accessed on 23 Sep. 2003.

Appendix [DOUBLE SIDED]

i. Papers in Press

- ❖ Gupta M.J., Irudayaraj, J., and Debroy, C. 2004. Spectroscopic quantification of bacteria using Artificial Neural Networks. *Journal of Food Protection* (**In Press**).
- ❖ Yu, C., Irudayaraj, J., Debroy, C., Schmilovitch, Z., Mizrach, A. 2004. Spectroscopic differentiation and quantification of microorganisms in apple juice. *Journal of Food Science*: Vol 70 (**In Press**)

ii. Papers submitted to refereed journals

- ❖ Yu, C., and Irudayaraj, J. 2004, Detection and identification of pathogenic microorganisms in mixed cultures by FTIR spectroscopy, *Vibrational Spectroscopy* (In Review).
- ❖ Gupta M.J., Irudayaraj, J., Debroy, C., Schmilovitch, Z., Mizrach, A. 2004. Detection and differentiation of food pathogens using FTIR and Artificial Neural Networks *Transactions of ASAE* (In Review)
- ❖ Schmilovitch, Z., A. Mizrach, G. Kritzman, R. Korotic, J. Irudayaraj, and C. Debroy. (2003). Detection of Food Borne pathogens with Raman Spectroscopy. *Transactions of the ASAE*.
- ❖ Yu, C., Irudayaraj, J., Debroy, C., Schmilovitch, Z., Mizrach, A. 2004. Spectroscopic quantification and strain level differentiation of microorganisms. *Applied and Environmental Microbiology*: (In Review)

iii. Relevant data

- ❖ FT-Raman Spectra