WESTERN REGIONAL RESEARCH CENTER

USDA, ARS, PWA, Albany, California

WESTERN REGIONAL RESEARCH CENTER
USDA - AGRICULTURAL RESEARCH SERVICE
PACIFIC WEST AREA
ALBANY, CALIFORNIA

1997
RESEARCH ACCOMPLISHMENTS

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WRRC RESEARCH ACCOMPLISHMENTS FOR FISCAL YEAR 1997

Consumers, producers, processors and the environment all benefit from WRRC research. We are proud of the WRRC research staff, which through its program and facilities, has made significant contributions to science and technology. Benefits from our research include a more healthful and safer food supply, expanded markets for cereal grains, tree nuts, fruits and vegetables, and a better environment.

This summary of accomplishments presents our Center's research program. Individual project summaries include information on recent Accomplishments, Resources, Relevance to National Research Program, Impact, Interaction with Customers, Research Partners, Short-term Goals and Publications.

We bring our research to the marketplace through various partnerships including Cooperative Research and Development Agreements. If you would like further information, please contact the appropriate Research Leader, the Technology Transfer Coordinator or the Director. We look forward to hearing from you!

RESEARCH LEADERS

Pamela M. Keagy
PAMELA M. KEAGY
Cereal Product
Utilization

William J Hurkman
WILLIAM J. HURKMAN
Crop Improvement &
Utilization

WILLIAM F. HADDON
Food Safety & Health

BRUCE C. CAMPBELL
Plant Protection

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Process Chemistry &
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Technology Transfer
Coordinator

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ANTOINETTE A. BETSCHART
Director

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RESEARCH ACCOMPLISHMENTS FOR FISCAL YEAR 1997
WESTERN REGIONAL RESEARCH CENTER
ALBANY, CALIFORNIA

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
PACIFIC WEST AREA

Modecode: 5325-00-00
PACIFIC WEST AREA
WESTERN REGIONAL RESEARCH CENTER (ALBANY, CA)

Address: USDA, ARS, WRRC
800 BUCHANAN STREET
ALBANY CALIFORNIA 94710

Contact: ANTOINETTE A. BETSCHART
CENTER DIRECTOR

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MISSION STATEMENT

The Western Regional Research Center conducts mission-oriented research to enhance the healthfulness of foods; to develop new food and industrial products from renewable resources; and to protect and enhance the quality of the environment. The results of these research efforts are implemented through the effective transfer of new and innovative technologies to appropriate clients and users.

GOALS: A. To enhance healthfulness of foods by creating crop plants and food products that promote health, and by developing systems and methods that ensure the safety of the food supply. B. To develop new food and industrial products using biotechnology to develop improved, tailored agricultural crops and bioengineering to create new products from agricultural crops and processing coproducts. C. To protect and enhance the quality of the environment by developing ecologically sound methods for pest control and environmentally sound systems for efficient food and industrial processes. D. To ensure the effective transfer of new and innovative technologies by integrating technology transfer into program and project design and developing partnerships with private and public sector clients.

Total Scientists: 53 Total Personnel: 159 Total Allocation: 15,251,246

CEREAL PRODUCT UTILIZATION

Modecode: 5325-26-00
PACIFIC WEST AREA
WESTERN REGIONAL RESEARCH CENTER (ALBANY, CA)
CEREAL PRODUCT UTILIZATION RESEARCH
Facility: WESTERN REGIONAL RESEARCH CENTER

Address: USDA, ARS, WRRC, CPU
800 BUCHANAN STREET
ALBANY CALIFORNIA 94710

Contact: PAMELA M. KEAGY
RESEARCH LEADER

Telephone: (510) 559-5664

MISSION STATEMENT

The mission of this unit is to enhance marketability and healthfulness of agricultural commodities and processed products. Cereal grains, legumes and economically important fruits, nuts and vegetables are the focus of this research. Both fundamental and applied research approaches are used to solve problems and develop new value added products which will benefit the consumer, producer, economy and environment. Fundamental properties for food and industrial uses, nutritional attributes and consumer preferences are important considerations. Research approaches are multi disciplinary and are integrated with other WRRC units. Several aspects of the program integrate with and respond to the needs of Action Agencies.

Total Scientists: 12 Total Personnel: 39 Total Allocation: 3,372,110

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-22410-001-00D FY: 97 Mode Code: 5325-26-00
Title: Extrusion Processing of Insect Diets for Biological Control Programs
RL: P Keagy INVESTIGATORS: R. Edwards, J. O. Berrios, P. Keagy
SY Time: 1.05 NTL: $269,794
Start Date: 10/01/93 Term. Date: 9/30/98

ACCOMPLISHMENTS:
Experiments are underway with APHIS-Pink Bollworm Rearing Facility, Phoenix, to determine which elements in Wesson salts are actually nutritionally required in the extruded diet for the Pink Bollworm. Other efforts have concentrated on developing approaches for cost effective scale-up of laboratory procedures for mass rearing of augmentative biocontrol insects. Equipment to simultaneously fill 300 small rearing pockets with a gelling type artificial diet has been developed and evaluated. A second generation filler is now under construction. While intended for mass rearing of C. grandis ectoparasitoids at ARS-Weslaco, TX, the design should be applicable to other rearing systems as well. Development has begun on an inexpensive method for continuous sterilization of insect diets. The process, utilizing direct steam injection to temperatures to 150°C, is simple, inexpensive, and scalable over a wide range of capacities. The process is being developed in cooperation with ARS-Yakima, WA, for use with codling moth diet. Discussions are underway to establish a CRADA with a commercial firm for mass rearing of C. grandis. This would be cooperative with ARS-Weslaco, TX. A trust agreement has been established with the Calif. Dept. Food and Agriculture on behalf of the Cotton Pest Control Board to develop new technology to obtain egg pad segments with controlled numbers of eggs on each segment. Image analysis is being used to determine egg counts, and a $C0_2$ laser with automated controls will do the cutting operations.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
Plant Productivity/Plant Protection/Inserts and Mites/Insect Mite Biology and Biosystematics: Develop knowledge .. to facilitate the discovery of new and improved concepts, approaches and principles of control: Extrusion processing, which is now in operation at the Pink Bollworm Rearing Facility, APHIS, Phoenix, has been shown to be an efficient new technique for processing and sterilization of the Pink Bollworm diet. The diet is used in the sterile release program for control of the Pink Bollworm on cotton in the southern San Joaquin Valley of California. The process is usable at high production rates which will enable the facility to produce enough diet to operate at up to four times the original plant capacity. Current work

with the Pink Bollworm, Catolaccus grandis, and the codling moth is all directed toward development of new technology for insect control.

Commodity Conversion and Delivery/New Uses, New Products/Industrial Products (nonfood)/Industrial Processes and Products: Devise process improvements or alternative processing systems that increase the quality, safety, or value of feed, fiber, or industrial products: As an end product, the mass reared insects are new value added products with a value greater than the ingredient cost and at least equivalent to the cost of insect control by insecticide application. Production of sterile insect feeds, whether by extrusion processing or by live steam injection, are value-added processes, the processed diet being more valuable than the original ingredients. Additionally, the use of other ingredients, not formerly used in insect diets, is being examined as methods for changing the texture of the final product.

IMPACT:

Cost-effective pilot scale systems for the mass rearing of beneficial insects will be developed for transfer to the private sector. Commercial-scale mass rearing facilities will be constructed and operated by private companies. Insecticide use will decline and be replaced by the release of augmentative biocontrol insects (C. grandis and others) or sterile insects (Pink Bollworm, fruit flies).

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

Cotton Pest Control Board Annual Research Committee Meeting (Calif. Dept. Food & Ag, cotton producers, ARS, APHIS); ARS Augmentation Biological Control Workshop (ARS, APHIS); Workshop for the Economic Feasibility of Mass Rearing of C. Grandis (academia, ARS, APHIS, cotton producers, industrial biocontrol companies).

RESEARCH PARTNERS:

APHIS, PPQ, Pink Bollworm Rearing Facility.
Trust Agreement with Calif Dept of Food and Ag. (Cotton Pest Control Board).

GOALS FOR NEXT YEAR:

Complete extrusion experiments with the Pink Bollworm diet. Complete development of equipment for diet placement in individual rearing pockets. Begin work on process for picking and placing individual C. Grandis eggs, one to a pocket. Test and evaluate direct steam injection for sterilization of codling moth diet. Assess applicability of technology for other mass rearing systems. Complete development of system to cut segments of Pink Bollworm egg pads with constant numbers of eggs.

SUMMARY OF PUBLICATIONS /PATENTS:

Edwards, R. H., Miller, E., Becker, R., Mossman, A. P. and Irving, D. W. 1996. Twin screw extrusion processing of diet for mass rearing the pink bollworm. Trans. ASAE 39 (5): 1789-1797.

Ellis, J., Johnson, J., Chowdhury, M., Edwards, R., and Lacewell. 1997. Estimated economic feasibility of catolaccus grandis in control of the boll weevil. Team Tech. Rpt. 97-1, Texas A and M Univ., College Station, TX, 37 pp.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41000-029-00D FY: 97 Mode Code: 5325-26-00
Title: Control of Physicochemical and Nutritional Properties in Extruded Cereal Based Foods
RL: P. Keagy INVESTIGATORS: J. Berrios, R. Buttery, R. Edwards, P. Keagy, G. Takeoka, D. Wood
SY Time: 2.40 NTL: $577,478
Start Date: 11/08/93 Term. Date: 11/07/98

ACCOMPLISHMENTS:
Phytochemicals, particularly those possessing antioxidant and free radical scavenging activity, are currently of great scientific interest since epidemiological studies have indicated that their consumption is associated with a reduced risk of cancer and cardiovascular disease. We have investigated the type and amount of anthocyanins in black beans (*Phaseolus vulgaris* L.). These natural products are the most important group of water-soluble plant pigments visible to the human eye and have significant value as natural colorants as well as possessing important biological activity such as anti-inflammatory activity, antioxidant activity, and inhibition of larval growth in insects. Three major anthocyanins, delphinidin 3-glucoside (56% of the total anthocyanin content), petunidin 3-glucoside (26%) and malvidin 3-glucoside (18%) were identified in black beans and the monomeric anthocyanin content was 213 ± 2 mg/100 g of black beans (moisture content was 10.04 ± 0.02%). Anthocyanins are the primary colorants in black beans and we are optimizing extrusion parameters to preserve these constituents during processing.

We have produced precooked pinto and black bean flours on a continuous basis by twin screw extrusion in a much shorter time than the traditional kettle cooking and subsequent drum-drying process. Extruded pinto and black bean flours with a wide range of functional properties were developed by varying the flour pre-treatment, moisture level and extrusion parameters. The extruded pinto and black bean flours had good water absorption, good water holding capacity, and high viscosity making them well suited for instant soups, soup mixtures and other applications where the product is heated before serving. Texture-forming properties of high amylose starch-sucrose mixtures were determined as a function of treatment temperature, water/solids ratio and sugar/starch ratio.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):

4.1.2.1 (50 %) New Foods Develop technologies for producing new food products with increased nutrition, ease of preparation, and other quality traits consistent with consumer demands and export market efficiencies.

We have developed extrusion technologies that can be used to produce bean products such as expanded and non-expanded snacks possessing different shapes and textures (i.e., lighter and more crispy).

4.1.2.3 (50 %) Food Processes & Products
Devise process improvements or alternative processing systems--including bioengineering processes--that increase the quality, safety, nutritional quality, convenience, and value of food products; reduce processing wastes; and enhance environmental acceptability or reduce product costs.

We have developed a continuous extrusion process to produce precooked pinto and black bean flours in a much shorter time than the traditional kettle cooking and subsequent drum-drying process. Extrusion processing is a high-temperature/short-time process which can provide significant advantages in nutritional quality, processing time, energy savings and safety over traditional processing methods.

IMPACT:

This research will improve our understanding of the effects of value-added processing on the levels of important phytochemicals which may have protective effects against degenerative diseases. Technology for the production of nutritious, value-added food products, made by cost effective extrusion processing, will be made available to the private sector. Novel extrusion processing with the fortification of valuable nutrients and the preservation of phytochemicals will give the public access to more nutritious foods.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

Research results were presented at the following meetings: American Chemical Society, Scanning 97.

GOALS FOR NEXT YEAR:

Produce shelf-stable high-solids fruit products by extrusion. Determine heat transfer properties of high-solids fruit during extrusion. Examine methods for stabilizing bean colors during extrusion and for optimizing flavor and functional properties of extruded flours. Identify specific compounds or compound classes with high antioxidant activity in selected fruits and grains. Measure changes in

antioxidant fractions or in specific compound concentrations as a result of extrusion processing variables.

SUMMARY OF PUBLICATIONS/PATENTS:
Two Peer Reviewed Journals, Three Abstracts, One Book Chapter, Two Proceedings

Peer Reviewed Journal Articles:
Takeoka, G.R.; Full, G.H. and Dao, L.T. Effect of heating on the characteristics and chemical composition of selected oils and fats. *J. Agric. Food Chem.* (Accepted May 1997).

Takeoka, G.R., Dao, L.T.; Full, G.H.; Wong, R.Y.; Harden, L.; Edwards, R. and Berrios, J. Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. *J. Agric. Food Chem.* (Accepted June 1997).

Abstracts:
Berrios, J.; Swanson, B.G. and Cheong, W.A. Structural characteristics of stored black beans (*Phaseolus vulgaris* L.). Scanning 97, Monterey, CA, April 20-23, 1997.

Takeoka, G.R.; Dao, L.T.; Full, G.H.; Wong, R.Y.; Harden, L.; Edwards, R. and Berrios, J. Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. 214th National Meeting of the American Chemical Society (ACS), Las Vegas, NV, 7-11 September 1997.

Takeoka, G.R.; Buttery, R.G.; Ling, L.C.; Wong, R.Y.; Full, G.H. and Dao, L.T. Odor thresholds of various unsaturated branched esters. 214th National Meeting of the American Chemical Society (ACS), Las Vegas, NV, 7-11 September 1997.

Book Chapter:
Takeoka, G.R. and Full, G.H. Analysis of volatile constituents of fruit. In *Modern Methods of Plant Analysis*, Vol. 19, *Plant Volatile Analysis*; Linskens, H.F;. Jackson, J.F., Eds.; Springer-Verlag: Berlin, 1997; pp 23-46.

Proceedings:
Berrios, J.; Swanson, B.G. and Cheong, W.A. 1997. Structural characteristics of stored black beans (*Phaseolus vulgaris* L.). Scanning 19(3):215. Presented at Scanning 97, Monterey, CA, April 19-22, 1997.

Irving, D.W.; Venet, C. and Berrios, J. 1997. Microstructure of processed black beans (*Phaseolus vulgaris* L.): puree to drum-dried flakes. Scanning 19(3):214. Presented at Scanning 97, Monterey, CA, April 19-22, 1997.

PROJECT SUMMARY

Project Info Sheet

Prj Number: 5325-41000-034-00D FY: 97 Mode Code: 5325-26-00
Title: Development of Wheat Biopolymer Composites for Industrial and Food Applications
RL: P. Keagy INVESTIGATORS: G. Glenn, W. Orts, D. Irving
SY Time: 2.85 NTL: $682,234
Start Date: 03/06/96 Term. Date: 03/05/99

ACCOMPLISHMENTS:

Earlier results showed that aquagels could be used to make lightweight concrete. A patent was issued in January of this year to protect this invention. The most economical aquagel tested was made of wheat starch. It was determined that spherical beads of wheat starch rather than particles of angular shape were preferable in making lightweight concrete. The concrete products had lower density, lower thermal conductivity, and an attractive surface finish. Various starch companies are being approached about manufacturing starch beads for this new application.

Starch polymer formulations were tested as soil stabilizers in irrigation water. Some starch formulations were made that were up to 90% as effective as polyacrylamide (PAM) polymers in reducing soil erosion during irrigation. The starch polymers are degradable and may prove effective as replacements for PAM if costs can be held to less than $1/lb.

A new method of making starch-based foams with low density and a closed-cell structure was developed as a replacement for polystyrene in single-use, disposable food containers. The foams were made in 15 to 25 seconds using equipment commonly employed in the manufacture of plastic products. A patent application is being prepared to protect this invention. A second technology involving a 2 to 3 minute baking process has been used to make food containers to replace polystyrene. The ingredients of the foamed product include potato starch. A new formulation based on wheat starch has been developed which will reduce the cost of the foamed food containers substantially.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):

This project conforms with the fourth objective of ARS' six year implementation plan. More specifically, the project conforms with objective 4.1.1 which is to identify markets and develop technologies and process-engineering systems that lead to value-added industrial (nonfood) products from agricultural commodities or processing by-products and wastes.

IMPACT:

Some estimates on the market for lightweight concrete are as high as 0.5% of the concrete market which is more than 500 million tons per year. This new application for (starch-based) lightweight concrete has the potential to create an important new market for U.S. wheat. The use of wheat starch polymers in place of PAM could provide a new market for wheat. Last year, more than 150,000 acres of western irrigated farms used PAM to reduce erosion. Wheat starch polymers provide a degradable alternative. The commercial potential will be determine by the cost of the modified starch product. The potential impact of a starch-based foam product that has properties similar to polystyrene is enormous. The progress made thus far is encouraging as evidenced by the support from the private sector.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

More than 30 companies have contacted ARS regarding the availability of the wheat starch-based lightweight concrete. The research was presented at the Technology 2000 conference held in Los Angeles and was highlighted in recent television, radio, and telephone interviews. The work was recently featured in Popular Science and Agricultural Research magazine. The work on soil erosion was presented at the National Meeting of the American Chemical Society and at five separate meetings with potential industrial partners and cooperators. A presentation of the full research program was given to the Washington and Idaho wheat commissions. Three meetings were held with Wehah Inc., one of California's largest rice producers regarding wheat starch-based flavor encapsulation. Two meetings were held with Tenneco Packaging Inc. regarding the development of starch-based foams.

RESEARCH PARTNERS:

Trust - Washington and Idaho Wheat Commissions.
CRADA- Tenneco Packaging Inc.
CRADA - Wehah Inc.

GOALS FOR NEXT YEAR:

Complete the patent of the closed-cell starch foam. Further develop formulations using less expensive ingredients for making commercial foam packaging with the 2 to 3 minute baking process. Negotiate with starch manufacturers to produce commercial quantities of starch beads for commercialization of the lightweight concrete technology. Field test the most successful starch formulations for reducing irrigation induced soil erosion and investigate their market feasibility with market suppliers. Develop method of encapsulating flavors in puffed snack products.

SUMMARY OF PUBLICATIONS/PATENTS:

Peer Reviewed Publications:

Glenn, G. M., Miller, R. and Irving, R. W. 1990. Starch-based microcellular foams. In: Symposium Series 647, Agricultural Materials as Renewable Resources. Fuller, G., McKeon, T. A. and Bills, D. D., Eds. Chapter 7, pp.88-106.

Irving, D. W. and Jideani, A. I. 1997. Microstructure and composition of *Digitaria exilis* Stapf (acha): A potential crop. Cereal Chem. 74(3)224-228.

Kahlon, T., Chow, F., Irving, D. and Sayre, R. 1996. Cholesterol response and foam cell formation in hamsters fed two levels of saturated fat and various levels of cholesterol. Nutrition Research 16(8):1353-1368.

Glenn, G. M. and Hsu, J. 1997. Compression-formed starch-based plastic. Industrial Crops and Products. (Accepted 4/22/97).

Abstracts:

Orts, W. J. and Glenn, G. M. 1977. Use of natural polymer flocculating agents to control agricultural soil loss. National Meeting of the American Chemical Society. April conference.

Orts, W. J. and Glenn, G. M. 1977. Use of natural polymer flocculating agents to control agricultural soil loss. Fifth Chemical Congress of North America. November Conference.

Irving, D. W. and Cornish, K. 1997. Microstructure of rubber particles using cryo and conventional high-resolution scanning electron microscopy. Scanning 19(3):169. April meeting proceedings.

Irving, D. W., Venet, C. and De J. Berrios, J. 1997. Microstructure of processed black beans (*Phaseolus vulgaris* L.): puree to drum-dried flakes. Scanning 19(3):214. April meeting proceedings.

Patents:

Glenn, G. M. 1997. Aquagel-based lightweight concrete. U.S. Patent #5,595,595.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41430-006-00D FY: 97 Mode Code: 5325-26-00
Title: Flavor Optimization of Major Crops Through Control of Metabolic Processes
RL: P. Keagy INVESTIGATORS: R. Buttery, L. Ling, D.Stern, L. Hansen
SY Time: .95 NTL: $321,935
Start Date: 12-20-93 Term. Date: 12-19-98

ACCOMPLISHMENTS:
Practical methods to analyze important aroma and flavor compounds of popcorn were developed with the goal that these might eventually be used by the industry for quality control. This included discovery of new aroma compounds of popcorn that had not been previously suspected. A method (using sodium sulfate to absorb water) developed in the previous year under this CRIS, was applied to a number of different corn products to identify and determine the concentrations of the compound Furaneol (2,5-dimethyl-4-hydroxy-3(2*H*)-furanone). This compound possesses a "sweet aroma" which could effect the flavor of these products. This compound was identified in creamed and whole kernel canned sweet corn, in frozen and fresh sweet corn, in corn tortilla chips and in taco shells. The highest concentrations were found in tortilla chips and canned sweet corn. The method is much simpler than previous methods needed to isolate this water soluble compound which can not be isolated by conventional purge and trap, or steam distillation methods.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
The research has identified quality factors and developed methods to analyze them which will contribute to improved postharvest crop quality.

IMPACT:
Results of research will help control flavor quality in corn products.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
The results of the research was presented at the 212th American Chemical Society national meeting in Orlando, FL in Aug. 1996.

RESEARCH PARTNERS:
No CRADAs or other present written agreements but contact kept, advice given and some analyses carried out for Lauhoff Grain Co., Pacific Grain Products, Inc., Campbell Soup Co., Lipton Foods Inc. (formally Ragu).

GOALS FOR NEXT YEAR:

Explore the use of corn and wheat-based starch-based microcellular foams for encapsulating important organic chemicals such as flavor components and volatile insect attractants. Such microcellular foams are a modified dry form of starch which possess some pores in the 5-14 Angstrom range suitable for this type of encapsulation.

SUMMARY OF PUBLICATIONS/PATENTS:

Three papers in peer reviewed journals, one abstract.

(1) R. Buttery; L. Ling. 2-Ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine: Odor thresholds in water solution. Lebensm.-Wiss. u.-Technol., 30, 109-110 (1997).

(2) R. Buttery; L. Ling; D. Stern. Studies on popcorn aroma and flavor volatiles. J. Agric. Food Chem. 45, 837-843 (1997).

(3) R. Buttery; L. Ling. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone in corn products. J. Agric. Food Chem. 45, 1306-1308 (1997).

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41440-002-00D FY: 97 Mode Code: 5325-26-00
Title: Enhancing Functional and Physiological Properties of Cereals and Legumes Through Processing
RL: P. Keagy INVESTIGATORS: W. Yokoyama, B. Knuckles, T. Kahlon, P. Keagy, D. Wood
SY Time: 3.2 NTL: $737,910
Start Date: 11-20-95 Term. Date: 11-19-2000

ACCOMPLISHMENTS:

Cereal brans are an underutilized source of health-promoting nutrients. Vitamin E, a component of cereal oils, and catechin, a water soluble antioxidant, were shown to reduce atherogenic fatty streak formation and LDL in hamsters fed 30 IU/kg and 200 mg/kg, respectively. Other bran antioxidants are being evaluated. Beta glucan, a soluble fiber in oat and barley, and a plant glycoalkaloid that binds cholesterol were shown to reduce cholesterol in the hamster. Dietary cholesterol absorption was reduced as shown by GC/MS of deuterated sterols. Processes to enrich beta-glucan in flours are being tested. A process that improves bran flavor was discovered. A CRADA is in process. A more sensitive and precise method to characterize the processing behavior of cereal starches has been developed. This method may assist plant breeders to develop new rice varieties with desirable textural and processing characteristics necessary for competitiveness of U.S. grains in the world market. Cereal starches are extremely large polymers that have not been systematically characterized. Laser light scattering methods are being used to determine molecular characteristics that are related to digestibility and processing characteristics.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):

WRRC cereal nutrition and processing research adds value and improves the quality of postharvest products by enhancing nutrient availability and consumer acceptability. Research to characterize molecular properties of cereal starches is necessary to design new food processes which enhance nutrition and maintain the competitiveness of U.S. grains in the world market.

IMPACT:

Noninsulin diabetes affects 16 million Americans. Soluble fiber, found in barley and oat, has been shown to reduce the blood glucose after meals in human subjects and may be useful as a dietary aid for those predisposed to diabetes. Americans consume only half the recommended amount of dietary fiber. The development of

a process to modify the flavor of bran may increase consumption. Atherosclerotic lesions were reduced in test animals fed lipid and water soluble antioxidants from cereal brans.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
WRRC cereal nutrition research was presented at the following meetings: 212th Annual Meeting American Chemical Society, 81st Annual Meeting of the American Association of Cereal Chemists, 6th Int'l Congress on Cell Biology, Regional American Association of Cereal Chemists Bread Wheat Quality Meeting, California Rice Promotion Board Field Day, National Science Teachers Association Meeting, and Rice Technical Working Group Meeting.

RESEARCH PARTNERS:
CRADA w/Kellogg Co; SCA w/University of Calif, Davis; SCA w/California Rice Promotion Board; and SCA w/Brookside Pharmacal.

GOALS FOR NEXT YEAR:
Completion of research comparing and relating 1) biological effects of raw and processed wheat brans in lowering aberrant crypt colon cells in rats to 2) changes in bioavailability of fiber components. Modifying textural and physiological properties of cereal starches utilizing new knowledge gained from molecular structure by laser light scattering and more accurate rheological methods. Work with CRADA partner on improving palatability and extending food uses of cereal brans. Continue research on cereal bran, fibers and antioxidants, and their effects on dietary cholesterol uptake by GC/MS and antiatherogenic effects.

SUMMARY OF PUBLICATIONS/PATENTS:
Journals:
Yokoyama, W. H., Hudson, C. A., Knuckles, B. E., Chiu, M-C., Sayre, R. N., Turnlund, J. R. and Schneeman, B. O. 1997. Effect of barley B-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74:293-296.

Kahlon, T. S. and Chow, F. I. 1997. Hypocholesterolemic effects of oat, rice and barley dietary fibers and fractions. Cereal Foods World 42:86-92.

Kahlon, T. S. and Chow, F. I. 1996. Quantitative extraction of hamster liver lipid and cholesterol with supercritical carbon dioxide. JAOCS, 73(10)1341-1342.

Kahlon, T. S. and Chow, F. I. Cholesterol response and fatty streak formation in hamsters fed two levels of saturated fat and various levels of cholesterol. Nutr. Res. (submitted).

Knuckles, B. E., Hudson, C. A., Chiu, M. M. and Sayre, R. N. 1997. Effect of B-glucan barley fractions in high-fiber bread and pasta. Cereal Foods World 42:94-99.

Knuckles, B. E., Yokoyama, W. H. and Chiu, M-C. 1997. Molecular characterization of barley B-glucans by size-exclusion chromatography with multiple angle laser light scattering and other detectors. Cereal Chem (accepted).

Smith, J. G., Yokoyama, W. H. and German, J. B. 1997. Butyric acid from the diet - Action at the level of gene expression. Critical Rev. Food Nutr. (accepted).

Yokoyama, W. H., Knuckles, B. E., Stafford, A. E. and Inglett, G. E. Raw and processed oat ingredients lower plasma in the hamster. J. Food Science (submitted 4/97).

Abstracts:
Knuckles, B. E., Chiu, M. M., Yokoyama, W. H. and Sayre, R. N. Changes in physical characteristics of B-glucans during storage of barley and oat bran. Annual Meeting Amer. Assoc. Cereal Chemists, Baltimore, MD. Sep 9-15, 1996.

Kahlon, T. S., Chow, F. I. and Sayre, R. N. Hypocholesterolemic effects of rice, oat and barley dietary fibers. Annual Meeting Amer. Assoc. Cereal Chemists, Baltimore, MD. Sep 9-15, 1996.

Daudu, P. A., Yokoyama, W. H. and Pearson, D. A. B-glucan from barley inhibits soluble intercellular adhesion molecule-1 secretion by human umbilical cord vein endothelial cells in vitro. Amer. Soc. Cell Biol., San Francisco, CA. Dec 7-11, 1996.

Rein, D., Yokoyama, W. H., Xu, R., and German, J. B. Antioxidant effects in the atherogenic hamster model. Meeting Amer. Chem. Soc., San Francisco, CA. Apr 13-17, 1997.

Friedman, M., Fitch, T. E., Levin, C. E. and Yokoyama, W. H. Reduction of dietary cholesterol absorption and LDL plasma cholesterol in hamsters fed tomatine. Meeting Amer. Chem. Soc., San Francisco, CA. Apr 13-17, 1997.

Patents:
Knuckles, B. E. Separation of starch and cell wall components of barley and oats using a liquid (fluid) type sieve modified such that is used as the carrier fluid. (deferred pending further developmental work).

Yokoyama, W. H. Aldehyde-reduced, stabilized cereal bran (deferred, CRADA).

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-42000-030-00D FY: 97 Mode Code: 5325-26-00
Title: Detection of Aflatoxin in Tree Nuts Using Imaging Techniques
RL: P. Keagy INVESTIGATORS: T. Schatzki, P. Keagy
SY Time: 1.35 NTL: $394,080
Start Date: 12/20/96 Term. Date: 12/19/99

ACCOMPLISHMENTS:
Aflatoxin distribution in a single lot of pistachios (400 samples) has been measured and the distribution previously postulated has been verified. The real-time line-scanned, visible spectrum (image) sorter, previously reported, has been tested in two commercial pistachio plants. Successful selection of aflatoxin containing nuts has been shown, in back sorting reject streams, as well as in sorting main process streams. Algorithms for detecting insect damage in pistachios using x-ray film, previously tested for large nuts, has been extended to cover all nuts of commercial size. Improved decision algorithms, based on neural networks, have been developed. Algorithms to separate overlapping nut images and to establish nut orientation, needed for further decision algorithms, have been developed as well. New preprocessing algorithms for detecting insect damage in almonds, correlated to aflatoxin contamination, have been developed. A method for physically separating and orienting nuts has been postulated. If successful, this should simplify detection of defects and substantially reduce false positives. The feasibility of detecting embedded shell in almonds by x-ray has been shown, but not yet tested. The chemical basis for concealed damage in almonds has been established and a spectroscopic method for detecting such damage before roasting and loss of quality has been developed. A new sampling protocol using presorting which reduces required sample sizes from 740 lbs to 15 lbs has been developed.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
4.2.1. Identify and develop ways to eliminate toxic factors of natural origins from food.

IMPACT:
Development of improved product selection has already been accepted by the pistachio industry and has resulted, we are told, in increased U.S. market share in Europe. The newly developed testing method for the first time allows reliable testing of lots in commerce. Acceptance as a standard protocol is expected in short order. Application to other tree and ground nuts should solve a similar problem in those areas. Successful detection of insect infested almonds and pistachios is of high priority in the industry and will decrease the correlated aflatoxin levels in product-for-sale. Detection of concealed damage and embedded

shell was originally requested by the industry to improve quality and successful methods can be expected to be rapidly transferred.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
All nut research, presented at: Aflatoxin Elimination Meeting (AEM), 5th Int'l. Symposium on Fruit, Nut and Vegetable Production Engineering (5IS). Detection of concealed damage at: Almond Board of California. Image sorter at: AEM, 5IS, Paramount Farms, Valley Pistachio. Algorithms for insect damage in pistachios at: 5th, SPIE Conference on Optics in Agriculture (2 papers). Sampling protocol at: FAO, Rome, Italy (Oct 1997).

RESEARCH PARTNERS:
Almond Board of California (two SCA), and Carnegie Mellon University (two joint competitive grants with Prof. Casasent).

GOALS FOR NEXT YEAR:
Measure distribution of aflatoxin in insect damaged almonds. Test image sorter on other commodities. Extend algorithms for detecting insect damage in almonds to remove confounding by the germ region. Develop method for detecting concealed damage in real time. Extend algorithms for detecting insect damage in pistachios to use images obtained by image intensifier. Implement by use of real-time image analysis hardware. Test physical separation and orientation of nuts and, if successful, prepare patent application.

SUMMARY OF PUBLICATIONS/PATENTS:
Casasent, D., Sipe, M.A., Schatzki, T.F., Keagy, P.M. and Le, L.C. Neural net classification of x-ray pistachio nut data. SPIE Proc. 2907: 217-227, 1997.

Casasent, D., Sipe, M.A., Schatzki, T.F., Keagy, P.M. and Le, L.C. Neural net classification of x-ray pistachio nut data. Submitted to Lebensm.-Wissensch. u. Techn.

Casasent, D., Talukder, A., Keagy, P.M. and Schatzki, T.F. Detection and segmentation of items in x-ray imagery. Submitted to Trans. ASAE.

Casasent, D., Talukder, A., Keagy, P.M. and Schatzki, T.F. Detection and segmentation of items in x-ray imagery. 5th Int'l Symposium for Fruit, Nut and Vegetable Processing, Sept, 1997, Davis, CA.

Keagy, P.M., Schatzki, T.F., Le, L., Casasent, D. and Weber, D. Expanded image data base of pistachio x-ray images and classification by conventional methods. SPIE Proc. 2907: 196-204, 1997.

Keagy, P.M., Schatzki, T.F., Le, L., Casasent, D. and Weber, D. Expanded image data base of pistachio x-ray images and classification by conventional methods. 5th Int'l Symposium for Fruit, Nut and Vegetable Processing, Sept, 1997, Davis, CA.

Pearson, T.C. Machine vision apparatus and method for sorting objects. U.S. Patent, granted June 16, 1997.

Pearson, T.C. and Schatzki, T.F. Use of a machine system for sorting pistachios for aflatoxin. 5th Int'l Symposium for Fruit, Nut and Vegetable Processing, Sept, 1997, Davis, CA.

Pearson, T.C. and Schatzki, T.F. Distribution of aflatoxin in pistachios 5. Use of a machine system for sorting. Submitted to J. Agric. Food Chem.

Schatzki, T.F., The distribution of aflatoxin in pistachios. Western Pistachio News: 11-12, Winter, 1996.

Schatzki, T.F. Distribution of aflatoxin in almonds. J. Agric. Food Chem. 44: 3595-3597, 1996.

Schatzki, T.F. and Pan, J. Distribution of aflatoxin in pistachios. 4. Distribution in small pistachios. J. Agric. Food Chem. 45: 205-207, 1997.

Schatzki, T.F. Sampling and testing pistachios for aflatoxin. 5th Int'l Symposium for Fruit, Nut and Vegetable Processing, Sept, 1997, Davis, CA.

Schatzki, T.F. Elimination of aflatoxin in tree nuts through post-harvest sorting. Aflatoxin Elimination Conference, Fresno, CA, October 28-29, 1996.

Schatzki, T.F. Distribution of aflatoxin in pistachios. 6. Effect of pre-sorting on sampling. Submitted to J. Agric. Food Chem.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-44000-003-00D FY: 97 Mode Code: 5325-26-00
Title: Image Analysis and Other Physical Methods for the Detection of Inclusions and Structure in Materials of Agricultural Interest
RL: P. Keagy INVESTIGATORS: T. Schatzki, P. Keagy
SY Time: 1.2 NTL: $389,189
Start Date: 10/01/93 Term. Date: 09/30/98

ACCOMPLISHMENTS:
Efficacy of internal defect detection in apples by x-ray was tested in two modes, visual recognition in still images and visual recognition on a simulated process line display. Five cultivars and five types of defect were tested in combination. For still images acceptable recognition (>50% recognition, <5% false positives) was obtained for senescence browning of Red Delicious, for watercore and stem rot in Fuji (requiring apple orientation) and possibly watercore in Red Delicious, and for codling moth damage in all cultivars 8-19 days after larval entry, depending on cultivar. When images were scrolled across the screen at increasing rates, recognition fell off to unacceptable levels at rates one half that corresponding to a commercial sorting line. A program has been developed to correct five types of errors introduced into x-ray images by photodiode arrays. Work has commenced to develop a next-generation x-ray system (based on image intensifiers and real-time image processing hardware) for on-line food inspection, in particular for detecting small inclusions and insects. Installation is complete and testing has started.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
4.1.2. Identify market and develop technologies that lead to value-added foods and food products.

IMPACT:
A successful x-ray inspection system will allow for the first time non-destructive detection of many interior defects in apples. This will produce a higher quality and higher value in domestic and especially in export markets subject to foreign (New Zealand, European) competition. An on-line high-resolution x-ray system is a major need in the food industry. Availability will allow inspection for insects, among others, with particular application to insects in grain. Further development of equipment in that area is presently held up for lack of such capability. Removal of hidden insects will greatly improve U.S. export position with Canada and other countries where cold weather prevents many insect problems.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

Detection of defects in apples by x-ray imaging was presented at: Beltsville conference on status of apple research at ARS, Washington Tree Fruit Research Commission, SPIE conference on Optics in Agriculture, Northeast Regional Agric. Eng. Service. Image restoration of line-scanned x-ray images was presented at: ASAE national meeting, 5th Int'l. Symposium on Fruit, Nut and Vegetable Production Engineering.

RESEARCH PARTNERS:

Washington Tree Fruit Research Commission (Trust).

GOALS FOR NEXT YEAR:

Develop machine recognition algorithms which detect internal defects in apples at high process line speeds. Complete testing of image intensifier system and test x-ray spectrum of the source. Commence project to prove out system by detecting hidden insects in wheat by machine methods.

SUMMARY OF PUBLICATIONS/PATENTS:

Haff, R.P. and Schatzki, T.F., Image restoration of line-scanned x-ray images. Optical Engineering, accepted July, 1997.

Haff, R.P. and Schatzki, T.F., Image restoration of line-scanned x-ray images. 5th Int'l Symposium for Fruit, Nut and Vegetable Processing, Sept, 1997, Davis, CA.

Haff, R.P. and Schatzki, T.F., Image restoration of line-scanned x-ray images. ASAE paper 97-6043.

Schatzki, T.F., Haff, R.P., Young, R., Can, I., Le, L.-C. and Toyofuku, N. Defect detection in apples by means of x-ray imaging. 1. Human recognition of still images, for Trans. ASAE.

Schatzki, T.F., Haff, R.P., Young, R., Can, I., Le, L.-C. and Toyofuku, N. Defect detection in apples by means of x-ray imaging. 2. Recognition of moving images, for Trans. ASAE.

Schatzki, T.F., Haff, R.P., Young, R., Can, I., Le, L.-C. and Toyofuku, N. Defect detection in apples by means of x-ray imaging. SPIE Proc. 2907: 176-185, 1997.

Schatzki, T.F., Haff, R.P., Young, R., Can, I., Le, L.-C. and Toyofuku, N. Defect detection in apples by means of x-ray imaging, in Sensors for Nondestructive Testing, Northeast Regional Agricultural Engineering Service, Ithaca, NY, 1997.

CROP IMPROVEMENT AND UTILIZATION

Modecode: 5325-32-00
PACIFIC WEST AREA
WESTERN REGIONAL RESEARCH CENTER (ALBANY, CA)
CROP IMPROVEMENT & UTILIZATION RESEARCH
Facility: WESTERN REGIONAL RESEARCH CENTER

Address: USDA, ARS, WRRC, CIU
800 BUCHANAN ST.
ALBANY CALIFORNIA 94710

Contact: WILLIAM J. HURKMAN
RESEARCH LEADER

Telephone: (510) 559-5750

MISSION STATEMENT

The Crop Improvement and Utilization Research Unit conducts research to enhance agronomic properties and end-use quality of crop plants. Research goals are accomplished by integrating biochemical and molecular approaches with biotechnological strategies. Research priorities focus on improving wheat quality through identification of structure/function relationships of proteins (glutenins) related to quality, developing transgenic wheat lines with improved quality and agronomic traits, decreasing the deleterious effects of elevated temperature on wheat quality, and expansion of the wheat genetics database. Additional research goals focus on development of soybean lines containing modified oils with improved properties for industrial uses, development of transgenic potato cultivars with reduced glycoalkaloid levels and increased resistance to the postharvest problems of blackspot bruise and bacterial soft rot transfer of technology for production of hypoallergenic guayule latex to private industry, and development of new crops for domestic rubber production.

Total Scientists: 13 Total Personnel: 40 Total Allocation: 3,324,831

PROJECT SUMMARY

Project Info Sheet

Prj. Number: 5325-44000-025-00D FY: 97 Mode Code: 5325-32-00
Title: Molecular Structures in Wheat Grain that Determine Functionality for Food and Non Food Uses
RL: William Hurkman INVESTIGATORS: D. Kasarda and W. Vensel
SY Time: 2.0 NTL $478,988
Start Date: 05/23/96 Term. Date: 5/22/99

ACCOMPLISHMENTS:
Methods have been evaluated for recovering recombinant proteins similar to wheat glutenin subunits from *E. coli* and characterization of molecules that correspond to variations on the high-molecular-weight glutenin subunits (HMW-GS) has been carried out by circular dichroism and FT-infra red spectroscopy. With the availability of transgenic wheat lines from the complementary molecular biology project at this location, emphasis has been shifted to determining the effects of hybrid HMW-GS on the structure of glutenin polymers in transgenic lines. Preliminary evidence for a particular disulfide linkage being favored in the hybrid subunit (and in glutenin of normal bread wheat in consideration of the design of the hybrid HMW-GS) has been obtained and is being substantiated. This work is focused on defining the way in which monomeric glutenin proteins arrange themselves into polymeric forms through combinations of inter- and intra-molecular disulfide bond formation during protein biosynthesis in the endosperm of the developing grain. The structure of these polymers, which is determined by intrinsic genetic factors interacting with environmental factors, is the single most important determinant of the mixing and baking character of wheat flour doughs in both bread and durum wheat lines.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
This research is directed toward defining the basis of wheat grain quality at the molecular level in order to guide genetic engineering approaches to control of quality and variation with environment. Wheat is, of course, a major US crop, with almost half exported. Uniformity of quality is of great concern to both domestic and overseas users.

IMPACT:
Gluten proteins are the single most important component of wheat flour in relation to the bread making quality, pasta making quality, and the quality of many other products made from wheat flour (products worth several billion dollars each year). Quality uniformity is of vital importance to processors. Research in this project is aimed at providing an understanding at the molecular level of the basis for this quality and its variation with wheat genetic background and the environment. The information will be used especially in the genetic engineering of new wheat cultivars with improved quality and stability.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Project wheat protein research was presented at the International Cereal Chemistry Symposium on New Trends in Analytical Methods, The German Chemical Society Meeting (Section on grain chemistry), and at ABRF `97: Techniques at the Genome/Proteome Interface.

RESEARCH PARTNERS:
Although no CRADA's, Trusts, or SCA's are in effect, active cooperation and exchange of scientists continues with the Food Research Institute, Technical University of Munich, Garching, Germany; the Department of Agrobiology and Agrochemistry of the University of Tuscia, Viterbo, Italy: and with the Department of Agronomy, University of California, Davis. The exchanges with the Technical University of Munich are funded by a grant from the German Ministry of Agriculture.

GOALS FOR NEXT YEAR:
Determine the effects of recombinant proteins in transgenic wheats on the structure of glutenin polymers in relation to wheat quality improvement. Evaluate the importance of subunit protein conformation relative to subunit ability to form intermolecular disulfide crosslinkages in determining dough viscosity and elasticity. Use information obtained to investigate the possibility of genetically engineering glutenin subunit structure to enhance characteristics for fiber formation and other nonfood polymer uses. This latter effort is related to the possibility of producing recombinant peptides in transgenic wheat grain by overexpression of the desired gene while suppressing expression of the genes for native gluten protein.

SUMMARY OF PUBLICATIONS/PATENTS:
Köhler, P., Keck-Gassenmeier, B., Wieser, H., and Kasarda, D. D. 1997. Molecular modeling of the N-terminal regions of high molecular weight glutenin subunits 7 and 5 in relation to intramolecular disulfide bond formation. Cereal Chem. 74:154-158.

Vensel, W. H., Adalsteins, A. E., and Kasarda, D. D. 1997. Purification and characterization of the glutenin subunits of Triticum tauschii, progenitor of the D genome in hexaploid bread wheat. Cereal Chem. 74:108-114.

Srinivasan, U., Leonard, N., Jones, E., Kasarda, D. D., Weir, D. G., O'Farrelly, C., and Feighery, C. 1997. Absence of oats toxicity in adult celiac disease. Brit. Med. J. 313:1300-1301.

Zhang, N., Jones, B. L., and Tao, H. P. 1997. Purification and characterization of a new class of insect a-amylase inhibitors from barley. Cereal Chem. 74:119-122.

Kasarda, D. D. *In press*. Gluten and gliadin: precipitating factors in coeliac disease. Proceedings of the 7th International Symposium on Coeliac Disease, September 5-7, 1996, Tampere, Finland.

D'Ovidio, R., Masci, S., Porceddu, E., and Kasarda, D. D. *Accepted*. Active duplication of the 1Bx7 high-molecular-weight glutenin subunit gene in the bread wheat (Triticum aestivum L.) cultivar 'Red River 68.' Plant Breeding.

Müller, S., Vensel, W. H., Kasarda, D. D., Köhler, P., and Wieser, H. *Accepted*. Disulphide bonds of adjacent cysteine residues in the LMW subunits of glutenin. J. Cereal Science.

Kasarda, D. D., Woodard, K. M., and Adalsteins, A. E. *Submitted*. Resolution of high-molecular-weight glutenin subunits by sodium dodecylsulfate polyacrylamide gel electrophoresis incorporating a neutral pH buffer system. Cereal Chem.

Vensel. W. H., and Harden, L. 1997. Determination of sulfhydryl content of wheat storage proteins by electrospray ionization mass spectrometry. Presented at Association of Biomolecular Resource Facilities Meeting: Techniques at the Genome/Protenome Interface Symposium. Abstract.

PROJECT SUMMARY
Project Info Sheet

Prj. Number: 5325-21430-002-00D FY: 97 Mode Code: 5325-32-00
Title: Application of Biotechnology to Wheat Improvement
RL: W.F. Hurkman INVESTIGATORS: O. Anderson, A. Blackall
SY Time: 1.9 NTL $: 620,287
Start Date: 07/15/96 Term. Date: 07/14/99

ACCOMPLISHMENTS:
1) First demonstration that altering a single parameter of a single wheat seed protein can change dough processing characteristics. HMW-glutenin subunits with engineered different lengths show a linear effect on dough mixing time. 2) First demonstration that altering levels of a single protein can affect dough mixing properties. Transgenic wheat with different levels of added HMW-glutenin show a dosage effect on dough properties. 3) Demonstrated a wheat seed gene promoter supporting high levels of protein synthesis sufficient for modifying quality characteristics or be used in industrial/pharmaceutical materials in cereal seeds. 4) Contributed to the construction of a synthetic wheat HMW-glutenin gene for use in molecular dissection of dough characteristics. 5) Isolated genes for starch elongation and branching. 6) First demonstration of the phenomenon of transgene "sense-suppression" in a monocot species. Can be used to down-regulate specific gene expression such as in weakening doughs for noodle/biscuits. 7) Established that both transgene over-expression and sense-suppression were stable effects sufficient for use in cultivar development. 8) Initiated transfer of transgenes from laboratory wheat lines into elite lines of the mid-plains and California. 9) Developed improved transformation vectors and strategies to address regulatory issues in transgenic crops. Included construction of transformation vectors without the ampicillin gene causing regulatory concerns, and progress in a collaborative project to eliminate the marker gene from a transgenic crop. 10) First isolation of wheat ω-gliadin genes (laboratories world-wide have attempted this for ten years, and this is the last of the major wheat storage protein gene families to be isolated). 11) Transformed barley and oats with wheat HMW-glutenin genes and demonstrated high levels of expression. Will be used to study gluten structure/function relationships and may yield novel products from these crops.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
This project utilizes advanced molecular biological and transformation technologies to modify grain for enhanced quality and production and novel utilizations. This contributes to Objective 2, Plant Productivity, particularly 2.1.2, Plant Genetics and Breeding: Improving genetic material for production efficiency and product quality. The work also provides gene controlling elements useable in creating novel products in the grain with potential for new, value-added utilizations. This contributes to Objective 4, Commodity Conversion and Delivery. In addition, the

project aspects dealing with researching fungal resistance and providing gene promoters for use in anti-pathogen strategies in the seed contribute to section 2.2 Plant Protection.

IMPACT:

This research is expected to provide enough molecular understanding of wheat dough processing characteristics to design rational approaches to bioengineering new cultivars with both improved and novel characteristics. This ability will both impact cultivar development for existing domestic uses of wheat and will allow targeted wheat development for export and niche markets. The demonstration that the wheat quality-related genes can be synthesized at high levels in other cereals opens the potential for novel food products from all the other major cereals (barley, oats, maize, rice). Newer projects in starch modification and fungal resistance have similar potentials to impact both economic and agronomic wheat traits.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

Research results were presented at the following meetings/sites: Cornell University, Plant Molecular Biology Research Unit, ARS-USDA, Beltsville; International Wheat Quality Meeting (3 presentations and posters), 7th International Seed Protein Conference, Gatersleben, Germany; 6th International Gluten Workshop, Sydney, Australia (3 presentations, 1 poster); American Association of Cereal Chemists, Baltimore, Maryland; American Bakers Association Annual Meetings, Coeur-d'Alene, Idaho, National Association of Wheat Growers, Portland, Oregon; International Wheat Quality Conference, Manhattan, Kansas; Wheat Crop Germplasm Committee; University of Hamburg, Germany; Purdue University; Washington State University; Fusarium Meeting, Peoria, Illinois. On-site (WRRC) representations to representatives from Cargill, Danone, Zeneca, Midwest Grain Products, Con Agra, Dalgety (member of BRDC), Washington-Idaho Wheat Commissions, Resource Seeds.

RESEARCH PARTNERS:

Research collaborations include: CSIRO, Australia, on dough micro-mixing experiments investigating the molecular basis of dough functionality; University of Minnesota and Alabama A&M on oat transformation; Plant Gene Expression Center (ARS) on target gene transformation and marker excision; National Center for Agricultural Utilization Research (ARS) for fungal resistance; breeders and germplasm researchers at University of California-Davis, University of Nebraska, Washington State University; Weizmann Institute on seed protein translation, modification, and protein body formation; North Dakota State (ARS) on durum wheat transformation for improve quality; IRRI and CIMMYT international rice and wheat/corn centers, on glutenin transformation; University of Missouri on glutenin gene chromatin organization.

GOALS FOR NEXT YEAR:
Protein quality modifications: Carry out initial testing of wheat transformed with the two high quality associated HMW-glutenin genes. Begin field tests of transgenicwheat with altered dough properties. Continue studies of transgene stability in both greenhouse and field conditions. Continue micro-dough-mixing experiments to include alteration of HMW-glutenin disulfide bonds patterns, size of glutenin molecule's effect on mixing times, and altering patterns of glutenin polymerization as it relates to dough properties. Finish gene constructions to allow detail subcellular examination of seed protein processing and protein body formation. Further definition of the DNA sequences controlling developmental and quantitative aspects of seed storage protein gene expression. Starch modifications: Transform wheat with gene constructions to alter starch properties. Isolate additional starch enzyme pathway genes. Gene isolations: Finish sequencing 1-2 ω-gliadin genes (never before isolated). Assess exploratory wheat endosperm EST project and analyze DNA sequences for clones of relevance to ARS research. Fungal resistance: Develop gene constructs of use in conferring fungal resistance in wheat. Initiate transformation of wheat with potential fungal resistance gene constructs. Study the stability of wheat HMW-glutenin gene expression in barley and oats, and begin quality testing once sufficient material is available. Transformation efficiency: Collaborative experiments to determine if marker genes can be removed from transgenic wheat, and other experiments to attempt to increase the efficiency of wheat transformation.

SUMMARY OF PUBLICATIONS/PATENTS:
5 Peer Reviewed Papers, 7 Abstracts, 3 Proceedings, 1 Patent issued, 1 Patent Continuation-in-kind filed, 1 Patent filed.

Anderson, O.D., Litts, J.C., Greene, F.C. The α-gliadin gene family: I. Characterization of ten new wheat α-gliadin genomic clones, evidence for limited sequence conservation of flanking DNA, and southern analysis of the gene family. Theor. Appl. Genet. 1997 (in press).

Anderson, O.D. and Greene, F.C. The α-gliadin gene family: II. DNA and protein sequence variation, subfamily structure, and origins of pseudogenes. Theor. Appl Genet. 1997 (in press).

Anderson, O.D., Bekes, F., Gras, P., Kuhl, J.C., Tam, A. Use of a bacterial expression system to study wheat high-molecular-weight (HMW) glutenins and the construction of synthetic HMW-glutenin genes. Sixth International Gluten Workshop, Sydney, Australia, pp. 195-198. 1997.

Anderson, O.D., Litts, J.C., Greene, F.C. Update on analysis of the alpha-gliadin gene family: Listing all reported DNA clones, sequence variation in different domains, cysteine conservation and generation of pseudogenes. Sixth International Gluten Workshop, Sydney, Australia, pp. 203-206. 1997.

Blackall, A.E. and Anderson, O.D. Patent Serial No. 5,650,558, "Glutenin genes and their uses." Issued July 22, 1997.

Blackall, A.E., Le, H.Q., Bekes, F., Gras, P.W., Shimoni, Y., Galili, G., and Anderson, O.D. Applications of molecular biology in understanding and improving wheat quality. Proceedings of the International Wheat Quality Conference. Manhattan, KS. 1997. (accepted May, 1997).

D'Ovidio, R., and Anderson, O.D. Construction of novel wheat high-M_r glutenin subunit gene variability: modification of the repetitive domain and expression in *E. coli.* J. Cereal Sci. 25:1-8. 1997.

Shimoni, Y., Blechl, A.E., Anderson, O.D., and Galili, G. A recombinant protein of two high molecular weight glutenins alters gluten polymer formation in transgenic wheat. J. Biol. Chem. 272:15466-15495. 1997.

Vasil, I.K. and Anderson, O.D. Genetic engineering of wheat gluten. Trends in Plant Sciences 2:292-297. 1997.

Anderson, O.D., Blechl, A.E. Engineering and deployment of glutenin genes for enhanced gluten strength in wheat. Proceedings of the Annual Meeting of the American Society of Agronomy. Anaheim, CA. (accepted July, 1997). (abstract)

Anderson, O.D., Blechl, A.E. The Relationships Between the Structural Features and the Functional Properties of Wheat High-molecular-weight Glutenin Subunits. Cereal Foods World (accepted April, 1997). (abstract)

Blackall, A.E., Anderson, O.D., and Bekes, F. Wheat quality improvement via biotechnology. Proceedings of the International Wheat Quality Conference. Manhattan, KS. (accepted May, 1997). (abstract)

Blackall, A.E., Le, H.Q., Anderson, O.D., McCue, K., Gras, P.W., Bekes, F. Flour improvement by genetic engineering of wheat. Proceedings of 7th International Symposium on Seed Proteins of Plants. J. Plant Physiology. (accepted June, 1997). (abstract)

Blackall, A.E., Le, H.Q., Anderson, O.D., Gras, P., Bekes, F. Effecting changes in high-molecular-weight glutenin composition. Cereal Foods World. (accepted April, 1997). (abstract)

McCue, K. and Anderson, O.D. Cloning and characterization of starch branching enzymes from wheat. Proceedings of the International Wheat Quality Conference. Manhattan, KS. (accepted May, 1997). (abstract)

McCue, K.F. and Anderson, O.D. Starch branching enzymes I and II from what endosperm. Proceedings of the 5th International Congress of Plant Molecular Biology. Singapore. (accepted April, 1997). (abstract)

PROJECT SUMMARY

Project Info Sheet

Prj Number: 5325-42430-001-00D FY: 97 ModeCode: 5325-32-00
Title: Practical Application Of Molecular Genetics For Improved Potato Cultivars
RL: William J. Hurkman INVESTIGATORS: W. Belknap and M. Brown
SY Time: 2.0 NTL $: 682,435
Start Date: 6/19/92 Term. Date: 6/18/97 (Project Statement in review)

ACCOMPLISHMENTS:
Postharvest disorders of the potato result in annual losses in the hundreds of millions of dollars in the US. Over the past year we have developed a series of transgenes (chimeric genes constructed in the laboratory) to address several sources of these postharvest losses, as well as to decrease levels of undesirable glycoalkaloids in potato tubers. A total of 142 transgenic potato clones constructed in the past year are currently being evaluated under field the field in Idaho and Maine. These clones express one of 8 new transgenes designed to confer resistance to blackspot bruise, fungal and bacterial pathogens as well as decrease glycoalkaloids. In the past year we have demonstrated the effectiveness of these transgenes in down-regulating glycoalkaloid biosynthesis, or conferring resistance to bacterial soft rot (*Erwinia carotovora*) or blackspot bruise in green house experiments. In addition, we have further characterized the contributions of mobile DNA elements to the evolutionary architecture of plant genes, and are using this knowledge to design improved transgenes.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
The program is directed toward the development of new knowledge and technology required for more productive, higher quality, potato cultivars. The transgenic varieties produced will proved safer, more marketable product per unit land. These benefits will result from decreases in both postharvest losses and the levels of natural toxicants in the potato tubers. The research also addresses plant productivity via enhancement of germplasm by facilitating introduction of desirable traits from wild Solanum species with prohibitive levels of steroidal glycoalkaloid toxicants. Finally, the research produces new molecular tools, such as transcriptional control elements, for the construction of improved transgenes for other crop species.

IMPACT:
In many growing seasons blackspot bruise and bacterial soft rot (causal agent *Erwinia carotovora*) represent the major sources of postharvest loss in potato. The development of transgenes which confer resistance to these disorders will result in significant savings to both growers and processors. As *Erwinia* species also inflict losses on a wide variety of other commodities, the effective antibacterial transgenes we have developed are currently being introduced into tomato and apple by private sector cooperators. We have recently filed a patent on critical sequences for down-regulation of glycoalkaloids in potato, and negotiations are currently

underway for both licensing of the technology and the development of a CRADA to move these transgenes into the marketplace. Finally, our research into genomic architecture and promoter structure has led to the development of useful transcriptional control elements which have been requested by, and sent to, dozens of private and public sector laboratories for construction new transgenes for dicotyledonous crops.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

(1) Pathogen resistance-conferring transgenes were requested by Harris Moran Seeds, San Juan Bautista, CA, and have been introduced into several crop species. Transgenic plants are currently being evaluated.

(2) Pathogen resistance-conferring transgenes were requested by Canadian Forest Service, Sainte-Foy, Quebec, and have been introduced into poplar and pine. Transgenic plants are currently being evaluated.

(3) Transcriptional control elements for construction of plant transgenes, isolated and characterized in this lab, have been sent to two corporate (Planet Biotechnology, Mountainview, CA and Calwest Seeds, West Salem, WI) and six public sector laboratories since 10/1/97.

(4) DowElanco has requested several pathogen-resistant transgenic potato clones for evaluation of commercial potential, we are currently awaiting approval from USDA/APHIS to send these clones to their Indianapolis, IN facility.

(5) We are currently developing a CRADA and SBIR funding proposal with Small Potatoes, Inc. (Madison, WI) for commercialization of the glycoalkaloid-reducing transgenes.

RESEARCH PARTNERS:

(1) CRADA w/Dry Creek Laboratories, Modesto, CA. (CRADA No. 58-3k95-M-434 "Improvement of Fungus Resistance in Potatoes and other Crop Species")

(2) CRADA w/Demeter Biotechnologies, Research Triangle Park, NC. (CRADA No. 58-3K95-3-183 "Improved Solanaceous Crops")

GOALS FOR NEXT YEAR:

Complete first year field evaluation of current transgenic clones for identification of transgenes with potential for commercialization. Transgenes conferring qualitative resistance to pathogens or bruising, or demonstrated ability to decrease glycoalkaloids, will be moved toward commercialization with either existing or new CRADA partners. Transgenes with more limited phenotypic effects will be modified by changing transcriptional properties (altered profile using different transcriptional control elements or increased transcription using scaffold attachment regions) and or functional sequences (new anti microbial coding domains and antisense sequences). In addition, novel transcriptional control elements with potential broad applications in applied agricultural biotechnology will be characterized in transgenic plants.

SUMMARY OF PUBLICATIONS/PATENTS:

Moehs, C.P., Allen P.V., Friedman, M. and Belknap, W.R. (1997) Cloning and Expression of Solanidine UDP-glucose Glucosyltransferase from Potato. The Plant Journal **11**: 227-236.

Oosumi, T. and Belknap W.R. (1997) Characterization of the *Sol3* family of nonautonomous transposable elements in tomato and potato. Journal of Molecular Evolution, In Press.

Moehs, C.P., Allen P.V., Rockhold, D.R., Stapleton, A., Friedman, M. and Belknap, W.R. DNA Sequences encoding solanidine UDP-glucose glucosyltransferase and use to reduce glycoalkaloids in solanaceous plants. U.S. Patent Dockett No: 0011.97. 1997.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41430-005-00D FY: 97 ModeCode: 5325-32-00
Title: Modification Of Vegetable Oils As Raw Materials For Industrial Uses
RL: W. Hurkman INVESTIGATORS: T. McKeon 1.0, J. Lin 1.0,
A. Stafford 1.0
SY Time: 3.0 NTL: $613,846
Start Date: 10/31/93 Term. Date: 10/30/98 or 9/30/97

ACCOMPLISHMENTS:
Castor oil is a strategic material because it serves as a source of vital lubricants, greases and engineering plastics. There is no domestic source of castor oil because of castor's hyperallergenicity and castor meal toxicity, and all industrial nations use and import castor oil. There is thus an important effort to develop transgenic crops that will provide a domestic source of high-ricinoleate oil. The gene that synthesizes the major component of castor oil (ricinoleate), has been expressed in transgenic plants, but the ricinoleate content is low. We have developed a castor-based in vitro system that duplicates castor oil biosynthesis and have used this system to identify the enzymatic steps that cause high ricinoleate production.

One of the key chemical uses of castor oil is as a source of 11-amino-1-undecanoic acid, the monomer used for production of Nylon 11, an engineering plastic. The fatty acid cis-vaccenate can be used to produce this same monomer using cheaper and "greener" chemistry. Milkweed (*Asclepias*) is a potential source of cis-vaccenate (octacec-11-enoate), but its production is low because the plant still makes considerable amounts of oleate (octacec-9-enoate). We have demonstrated the key role of a desaturase and elongation enzyme system from *Asclepias currassavica* in producing cis-vaccenate instead of oleate, and are initiating biochemical and DNA sequence comparisons to identify desaturases that will lead to high cis-vaccenate production. These results will be useful in developing a domestic source of oil that can provide the Nylon 11 monomer. This research thus enhances the use of *Asclepias*, a crop long recognized as having great potential for industrial-use, but which is only recently starting to be developed.

We have developed the filamentous fungus *Neurospora crassa* as a model for elucidating the complexity of lipid biogenesis and oil body formation in oilseeds. The advantage of using the fungus lies in the time saving (three days for transgenic *Neurospora* vs. several months for transgenic plants), reduced complexity, and the ability to use liquid culture to evaluate and influence lipid metabolism. While the *Neurospora* system does not perfectly compare to the processes occuring in developing oilseeds, we have delineated numerous common aspects of fatty acid and lipid biosynthesis. We have also identified products of potential interest to industry: a *Neurospora* mutant that produces very long chain w-3 polyunsaturates for the food and food supplement industry. We have also determined feeding

conditions that make *Neurospora* a good source of 2-hydroxy-palmitate, 2-hydroxy-stearate and sphingolipids. These products are important to the cosmetic industry and their current source is animal neural tissue, which European processors have expressed concern about as a result of Bovine spongiform encephalopathy (BSE).

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
The goal of this project is to develop a domestic source of vegetable oils that provide fatty acids for industrial uses. We use biochemical approaches to identify key metabolic steps that lead to high levels of the target fatty acids and we provide unique research capability in this area. Our product will provide new genetic stock that serve as renewable resources for industrial chemicals. As such, this project meets the following National Research Priorities: identification of plant genetic resources and improvement of plant germplasm; development of renewable resources to replace petroleum-derived products; development of chemical feedstock for environmentally-benign chemical processes; development of value-added products for the export market.

IMPACT:
The outcome of this research will have significant effects at the national and international level by providing technology that will be broadly applicable to improvement of crops by genetic modification. The immediate applications of our research will provide vegetable oils that replace imported castor oil, a critical strategic material, and oils that can be converted to engineering and specialty plastics by "green chemistry". Such plastics are expected to have the highest growth rate of all polymer materials. The technology we develop to maximize production of desired fatty acids in oils will be broadly applicable and lead to a wide range of vegetable oils that replace petroleum-derived chemicals. The benefits derived from this research include use of oilseeds such as soybean for replacement of moderate to high value petroleum-based chemicals, oilseed crops with value-added products "built-in", reduction of imports, and expanded uses for oilseed crops. Successful development of inhibitor-based technology for genetic manipulation will provide an alternative to anti-sense inhibition and as a complementary technology, it could prevent the inherent "leakiness" of anti-sense. This technology would have major impact on genetic engineering of all crops.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
American Association of Industrial Crops 9/21-9/25/1996; Unilever, 12/96; Calgene, 4/97; Neugenesis, Inc. 4/30-5/8/97; National Plant Lipid Cooperative, 6/4-6/8/97; International Society for Fat Research, 9/8-9/12/97

RESEARCH PARTNERS:
Research collaboration w/New Mexico State University; Research collaboration w/LC Resources, Inc.; Research collaboration w/Neugenesis; MTA w/Rutgers University;

GOALS FOR NEXT YEAR:

Our major goals focus on increasing production of specific fatty acids in plants: In order to aid development of a castor oil substitute in transgenic plants, we will isolate the enzymes that drive high production of ricinoleate and clone the corresponding cDNA's for use in enhancing ricinoleate production.

One complication of introducing new enzymes transgenically is that endogenous competing enzymes of differing specificity remain, reducing yield of desired product. We are developing peptide inhibitors to specific fatty acid desaturases. These inhibitors can be delivered transgenically and will allow substitution of alternate desaturases- this will provide an effective alternative for anti-sense inhibition of expression. Our initial application will be to enhance cis-vaccenate production in transgenic plants. We expect this technology to be broadly applicable for shifting metabolic pathways to produce desired products.

SUMMARY OF PUBLICATIONS:

Peer-Reviewed Journals, published or in press:

McKeon, T.A., Goodrich-Tanrikulu, M., Lin, J.T. and Stafford, A.E. 1997. Pathways for fatty acid elongation and desaturation in Neurospora crassa. Lipids 32, 1-5.

McKeon, T. A., J.-T. Lin, M. G. Tanrikulu and Stafford, A. E. 1997. Ricinoleate biosynthesis in castor microsomes. Industrial Crops and Products: accepted 10/28/96.

Goodrich-Tanrikulu, M., Stafford, A.E., Lin, J.T. and McKeon, T.A. 1996. Metabolism of ricinoleate by *Neurospora crassa*. Applied Microbiology and Biotechnology, 46: 382-387.

Zhu, P.L., Dolan, J.W., Snyder, L.R., Djordjevic, N.M., Hill, D.W., Lin, J.T., Sander, L.C. and VanHeukelem, L. 1996. Combined use of temperature and solvent strength in reversed-phase gradient elution. IV. Selectivity for neutral (non-ionized) samples as a function of sample type and other separation conditions. Journal of Chromatography 756, 63-72.

Lin, J.T., Woodruff, C.L. and McKeon, T.A. 1997. Non-aqueous reversed-phase high-performance liquid chromatography of synthetic triacylglycerols and diacylglycerols. Journal of Chromatography, accepted 4/22/1997

Siler, D.J., Goodrich-Tanrikulu, M., Cornish, K., Stafford, A.E. and McKeon, T.A. 1997. Composition of rubber particles of Hevea brasiliensis, Parthenium argentatum, Ficus elastica and Euphorbia lactiflua indicates unusual surface structure. Plant Physiology and Biochemistry, accepted 4/4/97

Peer-Reviewed Journals, Submitted to RMIS:

Stafford, A.E., McKeon, T.A. and Goodrich-Tanrikulu, M. 1997. Conversion of palmitate to unsaturated fatty acids differs in a Neurospora crassa mutant with impaired fatty acid synthase activity. Lipids. accepted with minor revision, 7/14/97

Lin, J.T., Woodruff, C.L., Lagouche, O.J., McKeon, T.A., Stafford, A.E., Goodrich-Tanrikulu, M., Singleton, J.A. and Haney, C.S. 1997. Biosynthesis of triacylglycerols containing ricinoleate in castor microsomes using 1-acyl-2-oleoyl-3-phosphocholine as the substrate of oleoyl-12-hydroxylase. Lipids.

Tanrikulu, M.G., Stafford, A.E. and McKeon, T.A. 1997. In vivo evidence for isotope discrimination by the delta-9 fatty acyl desaturase in Neurospora crassa. Lipids.

Tanrikulu, V.M., Howe, K., Oldrup, L., Stafford, A.E. and Nelson, M.A. 1997. Changes in fatty acid composition of Neurospora crassa accompany sexual development and ascospore germination. Microbiology.

Lin, J.T., Snyder, L.R. and McKeon, T.A. 1997. Prediction of relative retention times of triacylglycerols in a non-aqueous reversed-phase high-performance liquid chromatography. Journal of Chromatography.

Proceedings published:

Goodrich-Tanrikulu, M., Stafford, A.E. and McKeon, T.A. 1997. Metabolism of palmitate differs in Neurospora crassa mutants with impaired fatty acid synthase. *in*: John P. Williams, Mobashsher U. Khan and Nora W. Lem (eds.) Physiology, Biochemistry and Molecular Biology of Plant Lipids. Kluwer Academic Publishers, Dordrecht. pp. 60-62

Lin, J.T., Woodruff, C.L., Lagouche, O.J. and McKeon, T.A. 1997. Phospholipid metabolism by castor microsomes. *in*: John P. Williams, Mobashsher U. Khan and Nora W. Lem (eds.) Physiology, Biochemistry and Molecular Biology of Plant Lipids. Kluwer Academic Publishers, Dordrecht. pp. 113-115

Book Chapters published:

McKeon, T.A., Lin, J.T., Goodrich-Tanrikulu, M. and Stafford, A.E. 1996. Genetic modification of oilseed crops to produce industrial chemicals. *in*: Fuller, G., McKeon, T.A. and Bills, D. (eds.) Agricultural Materials as Renewable Resources: Non-food and Industrial Applications. ACS Books. pp. 158-178

Fuller, G., McKeon, T.A. and Bills, D. 1996. Nonfood Products from Agricultural Sources. *in*: Fuller, G., McKeon, T.A. and Bills, D. (eds.) Agricultural Materials as Renewable Resources: Non-food and Industrial Applications. ACS Books. pp. 1-10

Abstracts:

Lin, J.T., Lagouche, O.J., Woodruff, C.L., McKeon, T.A., Stafford, A.E., Goodrich-Tanrikulu, M., Singleton, J.A. and Haney, C.S. 1997. Biosynthetic pathway of triricinolein in castor microsomes. International Society for Fat Research, September 8-12, 1997, Kuala Lumpur.

McKeon, T.A., Rittig, F.T., Tanrikulu, M.G., Woodruff, C.L., Stafford, A.E. and Lin, J.T. 1997. Fatty acid production in Asclepias species. International Society for Fat Research, September 8-12, 1997, Kuala Lumpur.

Goodrich-Tanrikulu, M. and McKeon, T.A. 1997. Neurospora crassa mutants with reduced fatty acid synthase activity. 1997 Symposium on the Biochemistry and Molecular Biology of Plant Fatty Acids and Glycerolipids. June 4-8, 1997.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41000-018-00 D FY: 97 Mode Code: 5325-32-00
Title: Domestic Production of Natural Rubber
RL: W. Hurkman INVESTIGATOR: K. Cornish
SY Time: 1.0 NTL $: 370,672
Start Date: 05/21/93 Term. Date: 05/20/98

ACCOMPLISHMENTS:
Natural rubber is a strategic raw material used in huge quantities to support our medical, commercial, transportation and defense industries. All natural rubber currently used is obtained from the single, genetically-narrow, tropical species *Hevea brasiliensis.* We are developing new rubber-producing crops to reduce the United States' dependence on rubber imports and to protect the natural rubber supply. The first of our crops has just entered the commercialization phase: we have obtained a U.S. patent on the process for producing hypoallergenic rubber latex from the desert shrub guayule (*Parthenium argentatum*) and have exclusively licensed this patent to the Philadelphia-based Yulex Corporation. In support of the commercialization effort we have developed a new, simple quantitative procedure to analyze latex content in different ages and lines of guayule. We have demonstrated the effectiveness of flocculating reagents in the reduction of fine solids in guayule homogenates, thus streamlining the latex purification procedure.

Our research has also been aimed at the improvement of guayule as a domestic crop and towards the development of annual rubber-producing crops for the United States. This biotechnological approach requires a thorough biochemical understanding of the key steps regulating rubber yield and quality as well as proven tissue culture and transformation techniques. We have developed tissue culture protocols taking guayule from callus to regenerated plants and our first control transformants have been achieved. Also, we have biochemically characterized *in vitro* rubber biosynthetic activity in three contrasting rubber-producing species and have now developed a model of rubber transferase activity. This model includes two distinct binding sites for allylic and non-allylic substrates in a probably tubular enzyme positioned in the rubber particle membrane. We have shown rubber transferase to be an indeterminant length enzyme with an unusual degree of stereochemical and size tolerance which we plan to exploit in targeting substrate pools to rubber biosynthesis, away from the usually competing isoprenoid pathway. We have also shown that polymer length, a prime determinant of rubber quality, is regulated by the identity and concentration of substrate, with the allylic pyrophosphate initiator substrate having the predominant effect. We have also characterized the structure of the rubber particle surface, and through an interdisciplinary approach including electron microscopy, electron paramagnetic resonance and qualitative and quantitative lipid and protein analyses, have shown that rubber particles are surrounded by intact monolayer membranes.

These studies may led to an understanding of the ontogeny of rubber particles, a worthwhile research goal since the number of rubber particles also affects overall rubber yield.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
This research project relates to several areas of the NRP as described in the 1992-1998 ARS Implementation Plan:

Objective 1 - Soil, water, air. Guayule is a native plant, which will be grown commercially in the southwestern states even on relatively poor soils, and which uses less water than conventional crops (cotton, alfalfa) and does not require pesticide use.

Objective 2 - Plant productivity. Guayule production and future annual rubber-producing crops will greatly increase U.S. competitiveness in the enormous natural rubber global market place (approx 4.5 million tons/year, over 40,000 different products). Guayule latex will provide rubber primarily for the hypoallergenic medical products market (over 300 medical devices) a market in which *Hevea* latex cannot safely be used (due to life-threatening allergic reactions that they cause) and synthetic products are both more expensive and lack the desired performance characteristics of natural rubber. products. The U.S. market for latex gloves alone is approx $3.5 billion/year. The medical products market is avidly awaiting the production of guayule latex and U.S. farmers are very interested in the crop. The granting of the patent license to enable the production of the latex of the shrub was accomplished in early 1997 making guayule commercialization a reality. Cost analyses indicate that guayule latex can be produced for approximately the same as *Hevea* latex although, of course, a premium should be levied for its hypoallergenicity.

Objective 4 - Commodity conversion and delivery. Hypoallergenic guayule latex provides a classic example of a value-added commodity, as well as a large and expanding global market for this non-food crop.

IMPACT:
WRRC rubber research has led to two patents related to the hypoallergenic guayule latex discovered and developed by WRRC researchers. The patent for the process by which the latex is produced was issued in December, 1996, whilst the patent on the hypoallergenic latex itself was allowed in July 1997. The exclusive license to both patents was awarded to Yulex Corporation in January 1997. Hypoallergenic latex products, safe for the millions of hypersensitive people, provide an enormous market representing the first commercially viable application for guayule in many decades. Commercialization should lead to wide-scale (at least 15 million acres), nonsubsidized guayule farming throughout the southwestern United States. Future research should lead to higher-yielding guayule lines, as well as annual rubber-producing crops.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
WRRC rubber research was presented at the following meetings: First Asia-Pacific EPR/ESR Symposium, Hong Kong; Scanning 97, Monterey, CA; 16th Ann Symp, Current Topics in Plant Biochem, Physiol. and Mol. Biol., Columbia, MO; 5th US-Japan Seminar on Natural Products Biosynthesis; Int. meeting of Association for the Advancement of Industrial Crops, San Antonio, TX. Also, extensive discussions with representatives of industry, and of the Food and Drug Administration, Office of Compliance.

RESEARCH PARTNERS:
SCA w/Greece; SCA w/South Africa; Patent license to Yulex Inc.
Also, collaborations with: USDA Water Conservation Lab., University of Arizona, University of Akron, Johns Hopkins University, Cleveland Clinic Foundation, and FDA.

GOALS FOR NEXT YEAR:
Perform first transformations of guayule germplasm with genes for substrate biosynthesis, regenerate and evaluate transformants. Isolate and sequence rubber particle proteins from guayule and *Ficus*. Prepare cDNA libraries from different rubber-producing species. Continue studies of polymer length regulation, including molecular weight profile analyses of newly synthesized rubber by gel permeation chromatography. Develop micro-analytical methods for rubber quantification in seedlings and transformants (existing Sohxlet methods require many grams of material).

SUMMARY OF PUBLICATIONS/PATENTS:
(4 Peer Reviewed Journals, 1 Chapter, 1 Patent, 9 Abstracts)

CORNISH, K., D.L. BARTLETT Stabilization of particle integrity and particle-bound *cis*-prenyl transferase activity in stored, purified rubber particles. Phytochemical Analysis. 8: 130-134. 1997.

CORNISH, K., D.J. SILER Alternative natural rubber. Chemtech. 26: 38-44. 1996.

SILER, D.J., M. GOODRICH-TANRIKULU, K. CORNISH, A.E. STAFFORD, T.A. McKEON Composition of rubber particles of *Hevea brasiliensis*, *Parthenium argentatum*, *Ficus elastica* and *Euphorbia lactiflua* indicates unconventional surface structure. Plant Physiology and Biochemistry. 1997. In press

SILER, D.J., K. CORNISH, R.G. HAMILTON Absence of cross-reactivity of IgE antibodies from *Hevea brasiliensis* latex allergic subjects with a new source of natural rubber latex from guayule (*Parthenium argentatum*). J. Allergy and Clinical Immunology. 98: 895-902. 1996.

CORNISH, K., D.J. SILER Hypoallergenic latex: a gateway to guayule commercialization. The Plant Genetics News Letter. 1997. in press

CORNISH, K. Hypoallergenic Natural Rubber Products from *Parthenium argentatum* (Gray) and other non-*Hevea brasiliensis* species, U.S. Patent No. 5580942. 1996.

CORNISH, K., D. J. SILER, J. CASTILLON, M.H. CHAPMAN Regulation of *cis*-1,4-polyisoprene biosynthesis (natural rubber) in plants. Int. Symp. on Isoprenoid Biochemistry, Japan, 1996

CORNISH, K., H.F. BADER, C.D. LYTLE Manufacture and testing of guayule latex products. Int. Meeting AAIC, San Antonio, TX, 1996.

CORNISH, K., M.H. CHAPMAN Effect of *cis-* and *trans*-allylic diphosphates on rubber biosynthesis by isolated rubber particles of *Parthenium argentatum* (Gray). Int. Meeting AAIC, San Antonio, TX, 1996.

CASTILLON, J., K. CORNISH *In vitro* regulation of polymer length and rubber molecule initiation in *Parthenium argentatum*. Int. Meeting AAIC, San Antonio, TX, 1996.

SILER, D.J., K. CORNISH, R.G. HAMILTON Immunological studies demonstrate absence of cross-reactivity between guayule latex proteins and serum IgE antibodies from more than 300 latex-allergic adults and children, but that guayule latex proteins are immunogenic. Int. Meeting AAIC, San Antonio, TX, 1996.

SILER, D.J., M. GOODRICH-TANRIKULU, K. CORNISH, A.E. STAFFORD, T.A. McKEON Composition of rubber particles of *Hevea brasiliensis, Parthenium argentatum, Ficus elastica,* and *Euphorbia lactiflua* indicates unusual particle structure. Int. Meeting AAIC, San Antonio, TX, 1996.

CORNISH, K., D.J. SILER, J.J. WINDLE An EPR spin probe analysis of *Hevea, Euphorbia, Ficus* and *Parthenium* rubber particles. First Asia-Pacific EPR/ESR Symposium, Hong Kong, 1997.

IRVING, D.W., K. CORNISH Microstructure of rubber particles using cryo and conventional high resolution scanning electron microscopy. Scanning 97, Monterey, CA, 1997.

CASTILLÓN, J., K. CORNISH Effect of substrate concentrations on *in vitro* rubber biosynthesis using enzymatically-active rubber particles from different species. 16th Ann Symp, Current Topics in Plant Biochem, Physiol. and Mol. Biol., Columbia, MO, 1997.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-43000-023-00D FY: 97 Mode Code: 5325-32-00
Title: Effect of Environment on Protein Quality in Wheat
RL: W. Hurkman INVESTIGATORS: S. Altenbach, F. DuPont, W. Hurkman
SY Time: 2.75 NTL $: 683,705
Start Date: 3/11/94 Term. Date: 3/10/99

ACCOMPLISHMENTS:
In the rapidly changing world marketplace customers are placing greater demands on the wheat industry for grain of higher and more consistent quality. Environmental effects account for as much as 60% of the variation observed in end-use quality. This CRIS project is aimed at increasing the competitiveness of US wheat in the global market by developing wheat that produces grain with consistent quality despite changing environmental conditions. The initial goal of the project was to identify changes in gene expression and protein accumulation that occur when developing grain is exposed to high temperatures and determine the mechanism by which these changes affect flour quality. Wheat plants were grown under defined temperature regimes in greenhouse and growth chambers. Using sensitive techniques, we demonstrated that there was surprisingly little effect of heat on the expression of individual gluten seed storage protein genes or on the accumulation of the gluten proteins, despite reports in the literature to the contrary. RT-PCR techniques were developed to quantify levels of transcripts for a sampling of individual seed storage protein genes within the complex glutenin and gliadin gene families. Proteins were fractionated based on solubility and analyzed by HPLC and SDS-polyacrylamide gel electrophoresis. In addition, antibodies and DNA probes were used to evaluate effects of heat on several non-storage proteins during grain development. One significant effect of heat was an increase in the accumulation of a heat shock protein, HSP70, in the endosperm. A cDNA for the HSP70 was isolated and sequenced. Other specific changes in accumulation of proteins in an aqueous fraction are now being evaluated, as is the effect of heat on the polymerization of the storage proteins. Studies using the 2 gram mixograph to evaluate changes in quality parameters are in progress in collaboration with the Western Wheat Quality Laboratory in Pullman, WA.

RELEVANCE TO NATIONAL RESEARCH PROGRAM:
The US produces about 2.4 billion bushels of wheat per year with a value of about $7.7 billion. Specifications from buyers for wheat of certain quality are becoming more commonplace with the increased sophistication of customers in both the domestic and foreign marketplace, privatization of the wheat market, and increased mechanization of the processing industry both here and abroad. The environmental conditions under which wheat is grown are known to have substantial effects on the end-use quality of even the best wheat genotypes. While the environmental conditions under which wheat is grown are impossible to control, an understanding

of the effects of environmental conditions on end-use quality provides new approaches for improving the stability and consistency of US wheat. The production of grain with consistent quality is important with respect to the strategic trade position of the United States relative to other exporters, especially Canada, and was a major factor in initiating development of a quality-oriented marketing system as defined by the Grain Quality Acts of 1986 and 1990. Research to improve market quality and US competitiveness in the global market is identified as a high priority under Objective 2, Plant Productivity, in the 1992-1998 Agricultural Research Service Program Plan.

IMPACT:
This project will identify genes that can be selected by wheat breeders or modified in transgenic plants in order to provide wheat with consistent flour quality. Development of such improved wheat lines will increase profitability for growers and bakers, provide the US baking industry with flour of consistent quality, and increase competitiveness of US grown wheat in the global market.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Research accomplishments were presented at the International Wheat Quality Conference, Manhattan, KS, the Annual Meeting of the American Association of Cereal Chemists, Baltimore, MD, the Seed Biology Conference, Fort Collins, CO, and the Washington/Idaho Wheat Commission/WRRC Annual Meeting, Albany, CA. Project members also attended the American Bakers Association/ARS Annual Meeting, Coeur d'Alene, ID and the Meeting of the Northern California Section of the American Association of Cereal Chemists, Sacramento, CA and interacted with visitors from ConAgra, Inc, Omaha, NE and Dalgety Food Technology Center, Cambridge, England.

RESEARCH PARTNERS:
Western Wheat Quality Laboratory, Pullman, WA.

GOALS FOR NEXT YEAR:
Identify effects of heat on protein patterns in aqueous fractions. Purify proteins that show significant changes in accumulation and isolate corresponding cDNAs. Determine the effects of heat on glutenin polymer size classes. If the main effect of heat is on size class distribution rather than on the amount of specific components, studies will be carried out to evaluate the effect of heat on polymer formation during grain development. Continue cooperation with the Western Wheat Quality Laboratory to evaluate effects of heat treatments on flour quality. Establish relationships between the effects of heat on individual proteins/genes and flour quality.

SUMMARY OF PUBLICATIONS/PATENTS:

(3 Peer Reviewed Journals, 3 Abstracts, 2 Proceedings)

Altenbach, SB. Quantification of individual low molecular weight glutenin subunit transcripts in developing wheat grains by competitive RT-PCR. (Submitted to Theoretical and Applied Genetics)

Altenbach, SB, Kitisakkul, S. Transcript levels of LMW and HMW glutenin subunit genes and gliadin genes in wheat grains subjected to high temperature stress. Cereal Foods World 1997. (In Press)

Altenbach, SB, Suyenaga, K. Analysis of transcript levels for individual LMW and HMW glutenin genes in developing wheat seeds by competitive RT-PCR. Gluten '96, Proceedings of the Sixth International Gluten Conference. pp 94-98. 1997.

DuPont, FM, Chan, R. Effects of high growing temperature on protein accumulation and polymerization during development of wheat endosperm. Cereal Foods World 1997. (In Press)

DuPont, FM, Hurkman, WJ, Tanaka, CK, Chan, R. BiP, HSP70, NDK, and PDI in wheat endosperm: I. Accumulation of mRNA and protein during grain development. (Submitted to Physiologia Plantarum)

Hurkman, WJ, Bernardin, JE, Tanaka, CK, Combs, A, and DuPont FM. Characterization of HSP70 gene expression in endosperm of developing wheat grains. Gluten '96, Proceedings of the Sixth International Gluten Conference. pp 454-456. 1997.

Hurkman, WJ, DuPont, FM, Altenbach, SB, Combs, A, Chan, R, Tanaka, CK, Reuveni, M, Bernardin, JE. BiP, HSP70, NDK, and PDI in wheat endosperm: II. Effects of high temperature on mRNA and protein accumulation. (Submitted to Physiologia Plantarum)

Hurkman, WJ, Tanaka, CK. Expression of two chaperones and a foldase is regulated by high temperature in the endosperm of developing wheat grains. Cereal Foods World 1997. (In Press)

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-21000-005-00D FY: 97 Mode Code: 5325-32-00
Title: Development of Resources for Genetic Improvement of Small Grains and Sugarcane
RL: W. Hurkman INVESTIGATORS: O. Anderson
SY Time: 0.1 NTL $: 196,631
Start Date: 10/01/95 Term. Date: 09/30/98

ACCOMPLISHMENTS:
Added DNA probe information to the database (the GrainGenes database) world-wide-web interface. Initiated the design and implementation, in collaboration with computer staff at the National Agriculture Library, for user-interactive forms for data entry and correction. Initiated sequencing of DNA probes within the probe repository. Initiated coordination with the Stanford Center for DNA Sequencing to sequence Triticeae probes. Established three mirror sites for the database in Europe. First instance of U.S. Triticeae mapping laboratories committing to direct submission of new mapping data to the database instead of publishing. Continued increasing the database sections on genetic mapping, DNA probes, and traits.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
This project is a major component of Objective 6, Systems Integration Special program on Plant Genomes. The project provides the main genetic/molecular information collation and analysis site for data on wheat, barley, rye, oats, and sugarcane. The project also supports Objective 6 by the utilization, maintenance, and development of DNA probes for genetic mapping, breeding, and gene isolation for the crops of its responsibility. The fundamental nature of the resources provided also support Objectives 2 (Plant Productivity) and 4 (Commodity Conversion Delivery).

IMPACT:
The database is accessed approximately 1000 times per day by an average of 100 different sites in the U.S. and worldwide. The GrainGenes database remains the most complete collection of computer accessible data for the crops of its responsibility (wheat, barley, rye, triticale, oats, and sugarcane). In addition, the GrainGenes Probe Repository continues characterization, maintenance, and distribution of mapped clones for the same crops. For example, in the last month the Repository has received 11 requests for 350 DNA clones.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Project results and updates were presented at: International Wheat Quality Meeting, International Triticeae Mapping Initiative Workshop, Plant and Animal Genome V Meeting. Personnel contacts included: Researchers from Canada, France, Japan, University of California-Davis, Danone Inc., Washington-Idaho Wheat Commission.

RESEARCH PARTNERS:
SCAs with University of California-Davis, and University of Missouri. Coordination with ARS database projects at Cornell University, University of Missouri, Iowa State University, and the National Agricultural Library.

GOALS FOR NEXT YEAR:
Continue increasing the data volume within the database. Enhance the world-wide-web interface for easier user access and utilization. Put in place a systematic procedure for maintaining the most up-to-date genome mapping data from both the literature and directly from mapping laboratories world-wide. Finish sequencing the DNA probes within the probe collection. Interact with other database projects to implement the new JAVA interface for the ACEDB database software. Enhance and expand the use of user-interactive forms for data entry and correction. Establish the software to efficiently process DNA sequence information for DNA probes and ESTs.

SUMMARY OF PUBLICATIONS/PATENTS: Database is accessible at http://wheat.pw.usda.gov/ and http://probe.nalusda.gov/.

Lazo, G.R., Anderson, O.D., and Matthews, D.E. Expanding data collection for the GrainGenes Genome Database. Plant and Animal Genome Conference V, San Diego, CA Jan., 1997. (Abstract)

Lazo, G.R. The GrainGenes genome database as a resource for quality information._ Proceedings of the International Wheat Quality Conference. Manhattan, KS. (accepted May, 1997). (Abstract)

Lazo, G.R., Matthews, D.E., Anderson, O.D. GrainGenes: An update on the genome database for small grains and sugarcane. Proceedings of the International Triticeae Mapping Initiative 1997 Workshop, Clermont-Ferrand, France. (accepted June, 1997). (Abstract)

FOOD SAFETY AND HEALTH RESEARCH

Modecode: 5325-21-00
PACIFIC WEST AREA
WESTERN REGIONAL RESEARCH CENTER (ALBANY, CA)
FOOD SAFETY AND HEALTH RESEARCH
Facility: WESTERN REGIONAL RESEARCH CENTER

Address: USDA, ARS, WRRC, FSH
800 BUCHANAN STREET
ALBANY CALIFORNIA 94710

Contact: WILLIAM F. HADDON
RESEARCH LEADER

Telephone: (510) 559-5610

MISSION STATEMENT

The Food Safety and Health unit conducts research to enhance the safety of the nation's food supply. Basic and applied food safety research in such areas as intervention strategies for control of pathogenic bacteria and minimization of naturally occurring or process-induced toxicants are pursued in an interdisciplinary format embracing the specialties of chemistry, microbiology, biochemistry, immunology, molecular biology and nutritional toxicology. To expedite the transfer of science and technology results from its research, the group forms active partnerships with the food industry, sister regulatory agencies, and the scientific community. The research activities of the Food Safety and Health Unit promote a safe, affordable food supply for the consumer, satisfy research and regulatory needs of other government agencies, and lead to important new food safety processes for industry.

Total Scientists: 9 Total Personnel: 22 Total Allocation: 2,611,745

PROJECT SUMMARY

Project Info Sheet

Prj Number: 5325-42000-021-00D FY: 97 Mode Code: 5325-21-00FY: 97
Title: Control of Pathogens on Surfaces of Poultry and of Fruits and Vegetables
RL: W. F. Haddon INVESTIGATORS: W. F. Haddon, R. Binder, W. Gaffied, R. Wong, S. Kint, M. Friedman
SY Time: 5.05 NTL: $1,424,147
Start Date: 08/01/96 Term. Date: 07/31/99

ACCOMPLISHMENTS:
The challenges of increased national concern over pathogen-caused disease in the US and the threat new emerging strains of pathogenic bacteria with increased virulence are being met with a new interdisciplinary program to address bacterial attachment to food surfaces at the molecular level and to devise new measurement strategies to provide rapid confirmation of bacterial type in support of FSIS regulatory needs. Preliminary data indicating the ability to distinguish *Salmonella* and *Campylobacter* based on protein and phospholipid profiles led to the purchase and installation of a new laser-mass spectrometer for rapid, precise bacterial analysis. The project contributed funds toward purchase of a Confocal microscope jointly with CRIS 5325-42000-022-00D. This new instrument will allow observation of live bacteria on surfaces with better than 1 micron planar resolution, and sufficient depth profiling to observe biofilm communities directly. The project statement is in review.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
In the 1992-1998 Six Year implementation plan for the Agricultural Research Service, food safety research is designated as a "crosscutting" program with the general goal to "provide a means to ensure that the food supply is safe for consumers and that food and feed meet foreign and domestic regulatory requirements". The Six Year Plan addresses pathogen control research specifically with a mandate that "the research will also emphasize new approaches to methods of detection, alternative processing, and definition of microenvironmental conditions in food that are conducive or unfavorable to pathogen growth."

IMPACT:
Pathogen control research will lead to improved understanding of adhesion of pathogens to food surfaces, improved methods of pathogen control for industry, reduced incidence of human disease caused by bacterial pathogens, and adoption of specific, sensitive measurement technologies by FSIS, FDA, and other agencies.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
The research program on pathogen control was presented to the 1996 ARS/FSIS Food Safety Planning Workshop, the 8th California Institute of Food and Agriculture Research (CIFAR) conference "Ensuring Safe Foods into the 21st Century", and to the California Department of Food and Agriculture (CDFA). Research presentations were made to the American Chemical Society and to the American Society for Mass Spectrometry.

RESEARCH PARTNERS:
CRADA with EnviroLogix Inc., Westbrook Me on development of monoclonal antibody test for glycoalkaloids.

GOALS FOR NEXT YEAR:
Evaluate the efficacy of known antimicrobial compounds, including natural products, on disinfection of surface-bound pathogens.

Using spectroscopic, physical, and computational methods, begin to identify and describe mechanisms for the attachment of pathogens to surfaces.

Develop mass spectrometric methods for rapidly characterizing purified pathogenic strains of *Salmonella, Campylobacter* and *E. coli.*, including *E. coli.* O157:H7.

Apply mathematical techniques including principal component analysis and artificial neural network analysis methods to extend mass spectral sensitivity for pathogen analysis and to improve performance for mixtures of pathogens.

Develop industry partnership for mass spectrometric sample introduction methods for bacteria.

SUMMARY OF PUBLICATIONS/PATENTS:
Haddon, W. F., Binder, R. G., Harden, L. A., Wong, R. Y., Freeman, B. A. and Wilson, R. E. "*Risk Assessment of Chlorination in Poultry Processing*". 45th Conf. on Mass Spectrometry, ASMS, Palm Spring, CA, June 1-5, 1997.

Friedman, M "*Potato Polyphenols: Role in the Plant and in the Diet*" In Antinutrients and Phytochemicals in food Shahidi, F. (Ed.) American Chemical Society Symposium Series. P. 61-93. 1997.

Gaffield, W. "*Recent Studies of Plant Teratogens*" American Chemical Society Abstracts. Accepted. June 1996.

Friedman, M., Kozukue, N., Harden, L. A., "*Structure of the Tomato Glycoalkaloid Dehydrotomatine*" Journal of Agricultural and Food Chemistry. 45, 1541-1547. 1997.

Friedman, M., "*Folic Acid Protects Against Alpha-Chaconine-Induced Disruption of the Integrity of Frog Embryo Cell Membranes*" J. Agric. Food Chem. 45, No. 10, 1997. in press.

Gaffield, W., "*Induction of Terata in Hamsters by Solanidane Alkaloids Derived from Solanum Tuberosum*" Chemical Research in Toxicology. 9:426-433. 1996.

PROJECT SUMMARY

Project Info Sheet

Prj Number: 5325-42000-022-00D FY: 97 ModeCode: 5325-21-00
Title: Adhesion of Pathogens to Surfaces of Poultry and to Fruits and Vegetables
RL: W. F. Haddon INVESTIGATORS: R. E. Mandrell, D. Brandon,
L. Crawford, W. F. Haddon, R. Wong
SY Time: 4.80 NTL:$1,287,463
Start Date: 12/01/96 Term. Date: 12/31/99

ACCOMPLISHMENTS:
The increasing number of food-borne outbreaks due to bacteria is of major concern to consumers, food processors, food safety researchers and regulatory agencies. We are in the initial stages of developing a new project to study the molecular mechanisms of adhesion of human pathogens to food surfaces. Methods have been established for isolating pathogens from foods, identifying strain similarities, and preparing outer membranes and purifying outer membrane factors that may be important in adhesion of pathogens to food surfaces. We have produced mouse monoclonal antibodies (MAbs) that recognize common or specific epitopes of C. jejuni. These will be characterized to determine the specific molecules recognized. We have begun studies to develop a relevant model of attachment of pathogens to chicken skin.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
Part of the ARS mission is to "...ensure adequate availability of high-quality, safe food and other agricultural products to meet the nutritional needs of the American consumer, to sustain a viable and competitive food and agricultural economy, and to maintain a quality environment and natural resource base". The ARS has responsibilities to "Conduct research on broad regional and national agricultural and related problems" and to "Provide technical expertise to meet national food, food safety, and environmental emergencies". The research proposed will address issues related directly to the ARS Mission to maintain and increase the safety of the food supply. The ARS is the major federal agency for supporting this type of work.

IMPACT:
The objectives of this project will help in understanding the ecology, and the mechanisms of adhesion, of pathogens in foods and will lead to development of new strategies for controlling the numbers and/or the virulence of pathogens in foods. Reagents will be produced for development of sensitive and specific assays for identifying pathogens present in foods.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Attended the 1996 Annual Meeting of the American Society of Microbiology and the ARS Poultry Research Workshop (attended by ARS, FSIS and representatives of the poultry industry).

RESEARCH PARTNERS: None.

GOALS FOR NEXT YEAR:

Develop better methods for isolating multiple strains of C. jejuni from chicken and establish methods for analyzing the similarity of strains (i.e., pulsed field gel electrophoresis patterns vs. antigen expression by immunochemical assays with MAbs). Anti-pathogen MAbs will be characterized and used to (I) develop methods for capturing pathogens from foods, (ii) develop assays for identifying pathogens in situ, (iii) identify potential attachment factors and (iv) to study adhesion and environmental regulation of attachment factors. We will develop an assay for studying attachment of C. jejuni to chicken skin, and begin studies with this model system to identify molecules involved in attachment. Additional MAbs will be produced to screen for inhibitors of attachment. Multiple strains of C. jejuni other species of Campylobacter and other poultry pathogens will be screened to determine whether they attach by a similar mechanism. We will study infected chicken tissue to determine the relevance of the model system to attachment due to processing conditions. Begin studies of the ecology of enterohemorrhagic E. coli (e.g., O157, other shiga toxin producing strains) related to a fruit or vegetable. Develop methods for observing specific pathogens in biofilms on foods and for identifying low numbers of C. jejuni or Salmonella species present in a naturally contaminated food (i.e., not contaminated artificially). Begin studies to screen libraries of compounds for inhibitors of attachment.

SUMMARY OF PUBLICATIONS/PATENTS:

Brandon, D. L., Holland, K. P., Dreas, J. P., Henry, A. C., and Kishore, R. "*Study of ELISA Screening Methods for Benzimidazoles: Applicability to a Regulatory Program*" 111 th AOAC International Meeting. September 7-11, 1997. San Diego, CA. Abstract No. G-315. 1997.

Brandon, D. L., Bates, A. H., Montague, W.C., Binder, R. G., and Barker, S. A. "*ELISA of Fenbendazole Residues in Bovine Milk*" Association Official Analytical Chemists Annual Intrl Meeting & Exposition. September 7-11, 1997. San Diego, CA. Abstract No. G-312. 1997.

Gulati, S., McQuillen, D. P., Mandrell, R. E., Jani, D. B., and Rice, P. A. "*Immunogenicity of Neisseria gonorrhoeae Lipooligosaccharide Epitope 2C7, Widely Expressed in Vivo with No Immunochemical Similarity to Human Glycosphingolipid*" The Journal of Infectious Diseases. 174:1223-37. 1996.

Preston, A., Mandrell, R. E., Gibson, B. W. And Apicella, M. A. "*The Lipooligosaccharides of Pathogenic Gram-Negative Bacteria*" Critical Reviews in Microbiology, 22(3):139-180. 1996.

Flounders, A.W., Brandon, D.L., and Bates, A.H., "*Patterning of immobilized antibody layers via photolithography and oxygen plasma exposure*". Biosensors Bioelectron. 12. 447-456. 1997.

Crawford, L. And Myhr, B.C. A preliminary assessment of the toxic and mutagenic potential of steroidal alkaloids in transgenic mice. Fd. Chem. Toxicol.33, 191-194. 1996.

PLANT PROTECTION RESEARCH

Modecode: 5325-41-00
PACIFIC WEST AREA
WESTERN REGIONAL RESEARCH CENTER
PLANT PROTECTION RESEARCH
Facility: WESTERN REGIONAL RESEARCH CENTER

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Contact: BRUCE C. CAMPBELL
RESEARCH LEADER

Telephone: (510) 559-5846

MISSION STATEMENT

The mission of this Unit is to reduce or eliminate insects, weeds and microbes which impact agricultural horticultural and recreational areas of the Pacific West Region. Goals are to develop new pest control methods which are efficient, ecologically benign and improve quality and safety of food and work and rural environments. Control of insects which attack tree nuts exploits natural chemicals used in insect communication and host finding. Feeding damage by these insects leads to secondary infection by aflatoxigenic fungi affecting the quality and exportability of tree nut products. Natural products of tree nuts and microbes, and microbiological organisms are identified which prevent production of aflatoxins or growth of aflatoxigenic fungi. New searches are made for biological control agents of noxious weeds, such as yellow starthistle, which have significant impact on range, farm and recreational land use and are not amenable for control using chemical herbicides. The efforts of the Unit entail a strong, multi-disciplinary approach involving chemistry, genetics, microbiology, entomology and ecology including collaborative efforts with other USDA, industry, university, county and state action agencies.

Total Scientists: 7 Total Personnel: 17 Total Allocation: 1,749,374

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-22000-012-00D FY: 97 Mode Code: 5325-41-00
Title: Biological Control of Yellow Starthistle and other Non-indigenous Plant Pests in the Western USA
RL: B. C. Campbell INVESTIGATORS: J. K. Balciunas
SY Time: 1.0 NTL: $220,808
Start Date: 10-1-95 Term. Date: 9/29/00

ACCOMPLISHMENTS:
Despite the release and establishment of 5 insect species from Greece which attack yellow star thistle (YST), this exotic weed continues to expand its range in western ranges and wildlands at an exponential rate. It has now become the most widespread weed in the USA, with many tens of millions of acres infested -- up from 8 million acres in 1985 -- in California alone. Agents which attack the vegetative portions of YST are desperately needed, but previous overseas searches had not located any suitable insects. Last year, the project leader found a promising apionid weevil, *Ceratapion basicorne*, boring into root crowns of YST rosettes in Turkey and Greece. This year, he studied the field host range of this insect in Georgia (former USSR), Turkey, and Greece -- as well as initiating laboratory host range evaluations at our quarantine facility in Albany. We are also continuing our quarantine and field evaluations of the safety and host range of an accidentally introduced seed head fly, *Chaetorellia succinea*. This fly may prove to be a very good agent for YST. This FY, we initiated, in cooperation with Hong Kong University, a isozyme study of YST from selected locations in Europe, Asia, and Africa. The origin(s) of the YST in the USA is still not known. This research should help answer this question and guide us in our searches to locate potential biological control agents for YST. With the cooperation of CA Dept. of Food and Agriculture, we are in the third year of a 5+ yr study of the impact of previously released YST agents at 3 study sites in Central CA. A sub-project targeting scotch thistle, funded by the Departments of Agriculture in Oregon and California, entered it's second year. This summer we are evaluating in quarantine the host range of two strains (species?) of Lixus weevils which damage scotch thistle in both the adult and larval stages.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
This project is highly relevant to numerous national initiatives and programs including: control of exotic pests, reduced pesticide use, increased reliance on IPM, grazing land management, biodiversity, and improvement of rural life.

IMPACT:
Our impact studies should demonstrate the effect which our insect bioagents are having on YST. This should enhance support not only for our YST project, but for other biological control projects as well. Ultimately, the new agents we are developing for YST and Scotch thistle should provide some measure of control for these two serious weeds. Even a slight decrease in infestations would make

millions of acres of rangeland productive, help maintain biodiversity of natural ecosystems, decrease the use of chemical herbicides, and improve the quality of life not only for rural residents, but all citizens, since their welfare also depends on healthy natural ecosystems and productive agriculture.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Since Oct. 1, research conducted by the Albany Quarantine has been presented at the following workshops and meetings: Annual Calif. & Oregon Depts. of Agricul. Joint Weed Biocontrol Meeting; Calif. Exotic Pest Plant Council annual meeting; Univ. of Calif. Statewide Biological Control Planning Meeting; W-185 Project Annual Meeting; U.C.-Berkeley Biological Control Center meeting (3 times); U.C.-Davis Weed Group meeting (2 times); Cal EPPC Board Meeting (4 times); CA Interagency Weed MOU committee (3 times); CA Cattlemen's Association Annual Meeting; annual planning meeting for joint CDFA-ARS biocontrol of weeds research; met with representatives of The Nature Conservancy (Diane Vosick & John Randall); met with Dr. Baldo Villegas to discuss joint publications and future research on *Chaetorellia succinea*; met Napa Co. Ag Commissioner (Mike Dannenberg) and his sci. staff; CA Agricultural Commissioners and Sealers Association, Annual Biological Control and IPM Conference; Beef & Range Field Day at UC Sierra Foothill Research Center. The Albany Quarantine also hosted meetings by Cal EPPC and CA Interagency Weed MOU committees, as well as visits by CA Cattlemen's Association officials.

RESEARCH PARTNERS:
SCA w/Univ of Idaho; Trust Agreement w/ Oregon Dept. of Agriculture; Agreement (in-kind) w/Ca Dept. Food & Agric.; research partnership w/Hong Kong Univ.; research partnership w/Biological Control Group - CDFA; research partnership w/Napa Co. Agric. Commissioner

GOALS FOR NEXT YEAR:
Continue quarantine evaluations of current YST and scotch thistle agents; complete field evaluation of *C. succinea*; complete collections of YST seeds for isozyme analysis in Hong Kong; initiate cooperative agreement with scientists in Turkey; build up resources and capabilities of the Albany Quarantine.

PUBLICATIONS:

Manuscripts published during FY-97

Balciunas, J.K. and D.W. Burrows. **1996**. Distribution, abundance and field host-range of *Hydrellia balciunasi* Bock (Diptera: Ephydridae), a biological control agent for the aquatic weed, *Hydrilla verticillata* (L.f.) Royle. **Australian Journal of Entomology**. 35:125-130

Balciunas, Joseph K., D.W. Burrows and M.F. Purcell. **1996**. **Australian Surveys (1985-1992) for Insect Biological Control Agents of *Hydrilla verticillata***. Tech. Report A-96-5, U.S. Army Engineer Waterways Experiment Station, Vicksburg, Mississippi. 281 pp.

Balciunas, J.K., D.W. Burrows and M.F. Purcell. **1996**. Comparison of the physiological and realized host-ranges of a biological control agent from Australia for the control of the aquatic weed, *Hydrilla verticillata*. **Biological Control** 7:148-158.

Burrows, D.W., Balciunas, J.K. and E.D. Edwards. **1996**. Herbivorous insects associated with the paperbark *Melaleuca quinquenervia* and its allies: V. Pyralidae and Other Lepidoptera. **Australian Entomologist**. 23:7-16.

Gagne, Raymond J., Joseph K. Balciunas, and Damien W. Burrows. **1997**. Six new species of gall midges (Diptera: Cecidomyiidae) from *Melaleuca* (Myrtaceae) in Australia. **Proceedings Entomological Society of Washington** 99(2):312-334.

Purcell, Matthew F., Joe K. Balciunas, and Peter Jones. **1997**. Biology and host-range of *Boreioglycaspis melaleucae* (Hemiptera: Psyllidae), potential biological control agent for *Melaleuca quinquenervia* (Myrtaceae). **Environmental Entomology** 26:366-372.

Naumann, I.D., and J.K. Balciunas. **1997**. A sawfly larvae feeding on a aquatic fern (Hymenoptera: Symphyta: Pergidae). **Australian Entomologist** 24(1):39-47.

Approved, awaiting publication

Burrows, D.W. and J.K. Balciunas. (in press). Biology, distribution, and host-range of the sawfly, *Lophyrotoma zonalis* (Hym., Pergidae), a potential biological control agent for the paperbark tree, *Melaleuca quinquenervia*. **Entomophaga.**

Burrows, Damien W. and Joe K. Balciunas. (in press). Biology and host-range of *Pomponatius typicus* (Heteroptera: Coreidae), a potential biological control agent for the paperbark tree, *Melaleuca quinquenervia* in south Florida. **Journal of Australian Entomology**.

Burrows, Damien W. and Joe K. Balciunas. (submitted). Distribution, biology, and host-range of the melaleuca leaf-blotching bug, *Eucerocoris suspectus* (Hemiptera: Miridae), a potential biological control agent for the paperbark tree, *Melaleuca quinquenervia* (Myrtaceae). **Bulletin of Entomological Research.**

Chan, Kathleen L. and Charles E. Turner. (submitted). Discovery of the gall mite *Aceria genista* (Nalepla) (Acarina: Eriophyidae) on Gorse and French Broom in the United States. **Pan-Pacific Entomologist.**

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-42000-019-00D FY: 97 Mode Code: 5325-41-00
Title: Reduction of Aflatoxin in Tree Nuts and Figs through Control of Major Insect Pests
RL: B. C. Campbell INVESTIGATORS: B. Campbell, D. Light, J. Roitman, R. Buttery, R. Wong
SY time: 3.55 NTL: $867,778
Start Date: 10/01/94 Term. Date: 06/30/99

ACCOMPLISHMENTS:
Tree nuts (almonds, walnuts & pistachios) are among the top 20 of all Californian farm products and in the top ten of exported agricultural products. Over 70% of these nuts are exported and detection of aflatoxins in these nuts are food-safety and trade issues because of extremely low thresholds (<5 ppb) set by importing nations. Aflatoxigenic fungi infect tree nuts as a result of insect-feeding damage. We significantly improved traps (two to three-fold) to capture egg-laying females of major insect pests invading tree nut orchards. We identified blends of host-plant odors and insect pheromones that synergize insect attractancy. We identified insect antifeedant natural products in hulls of nuts to be used to deter insect feeding. We isolated natural volatiles in tree nut orchards on a time basis to identify odors emitted by ripening nuts that attract insect pests. Insect pests were collected from a wide-range of geographic locations (Southern California to Washington) and host-plants (nut and fruit trees) for genetic tests on dispersion and host-plant race formation. Techniques developed have fundamental implications to integrated pest-control methodology.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
Commodity, Conversion and Delivery- Aflatoxins are recognized human mutagens and teratogens and their occurrence in foods is a major food-safety issue. Reduction of aflatoxin in tree nuts improves acceptability of this highly exported commodity. Soil, Water, and Air- Methods developed to control insect pests of tree nuts reduces or eliminates use of chemical pesticides, a food safety and human health concern to field-workers, residents and consumers, and improves overall quality of rural environments by reducing chemical toxicants in aerial, aquatic and subterranean ecosystems. Plant Productivity- Identification of natural chemical constituents to affect insect behaviors has potential for augmentation through breeding or genetic engineering. This project falls directly within the High Priority ARS Crosscutting Programs of Food Safety, and Environmentally Compatible Pest Control and secondarily within Water Quality Protection.

IMPACT:

Reductions of aflatoxins in tree nut crops to levels below those imposed by regulatory agencies and importing governments will improve human health and safety and increase exportability and competitiveness of US tree nuts in foreign markets. Developed genetic identification techniques are applicable to identification of a wide-range of population and introduced exotic pests.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

Aflatoxin Elimination Workshop; Federal Interagency Conference on Mycotoxin Research; Codling Moth Meeting for Walnuts; Pomology Extension Continuing Conference; California Conference on Biological Control; International Insect Chemoreception Conference; Annual Meeting of the Entomological Society of America; International Congress of Entomology; CSIRO Entomology, Canberra, Australia; New South Wales Agriculture, Orange, NSW, Australia

RESEARCH PARTNERS:

SCA w/ California Dept. of Food and Agric.; RAs w/ University of California, Davis and Parlier; MOU w/ TrÄcÄ; Field testing plots in a number of private, commercial nut orchards

GOALS FOR NEXT YEAR:

Isolate and identify natural products in a variety of nut tissues which deter insect feeding. Examine and identify constituents of fungal sclerotia that have potent anti-insect feeding activity. Isolate and identify tree nut volatiles associated with "hull split" which appear to acts as cues for insect migrations into orchards. Further test combinations of plant volatiles and insect pheromones to improve trap captures. Test blends of plant volatiles and insect pheromones as potential confusants when they are broadcasted in an orchard environment (a larger scale of dissemination of the volatiles than in the trapping protocols). Test methods of disseminating these blends in an orchard either through mechanical emitters, encapsulating membranes, or slow-release capillary-like tubing. Develop genetic methods for distinguishing insect pest populations. Initiate electrophysiological studies on insect neurophysiological responses to plant volatiles and pheromones. Conduct flight tunnel bioassays of test compounds.

SUMMARY OF PUBLICATIONS/ PATENTS:

5 Peer Reviewed Journals, 4 Book Chapters, 5 Proceedings, 2 Abstracts, 1 Govern. Pub.

Chen, D.-Q., Campbell, B. C., and Purcell, A. H. A new rickettsia from an herbivorous insect, the pea aphid Acyrthosiphon pisum (Harris). Current Microbiol. 33: 123-128. 1996.

Raguso, R.A., Light, D.M., and Pichersky, E. Electroantennogram responses of Hyles lineata (Sphingidae: Lepidoptera) to volatile compounds from Clarkia breweri (Onagraceae) and other moth-pollinated flowers. J. Chem. Ecol. 22: 1735-1766. 1996.

Raguso, R. And Light, D. M. Electroantennogram Responses of Male Sphinx Perelegans (H. Edwards) hawkmoths to floral and "green-leaf volatiles". Entomol. Exp. Appl. 1997.

Campbell, B.C., Bourgoin, T., and Steffen-Campbell. Molecular phylogeny of planthoppers (Insecta, Hemiptera, Archaeorrhyncha): Evolutionary affiliations and historical biogeography of the enigmatic Tettigometridae. Cladistics (in press 2/97).

Andres, N. G., and Campbell, B. C. A phylogeny of the phylum Cnidaria based on 18S rDNA nucleotide sequences. Biological Bulletin. (submitted 12/96).

Campbell, B.C., Steffen-Campbell, J.D., and Gill, R.J. Origin and radiation of whiteflies: an initial molecular phylogenetic assessment. pp. 26-51. In. Bemisia 1995: Taxonomy, Biology, Damage Control and Management (D. Gerling and R. T. Mayer, eds.). Intercept, Andover, UK. 1996.

Light, D.M., and Jang, E.B. Plant volatiles evoke and modulate tephritid behavior. pp. 123-133. In. Fruit Fly Pests: A World Assessment of Their Biology and Management (B.A. McPheron and G.J. Steck, eds.). St. Lucie Press, Delray Beach, FL. 1996.

Jang, E.B., and Light, D.M. Attraction of female Mediterranean fruit flies to identified components of the male-produced pheromone: qualitative aspects of mahor, intermediate, and minor components. pp. 115-121. In. Fruit Fly Pests: A World Assessment of Their Biology and Management (B.A. McPheron and G.J. Steck, eds.). St. Lucie Press, Delray Beach, FL. 1996.

Jang, E.B., and Light, D.M. Olfactory semiochemicals of tephritids. pp. 73-90. In. Fruit Fly Pests: A World Assessment of Their Biology and Management (B.A. McPheron and G.J. Steck, eds.). St. Lucie Press, Delray Beach, FL. 1996.

Campbell, B.C., Heraty, J., and Steffen-Campbell, J.D. Molecular phylogenies and their application to biological control. Proceedings XXth International Congress of Entomology. Florence, Italy. 1996.

Campbell, B.C., Bourgoin, T., Steffen-Campbell, J.D., Sorensen, J.T., and Gill, R.J. Phylogenetic affiliations of Fulgoromorpha (Hemiptera: Archaeorrhyncha) inferred from 18S rDNA nucleotide sequences. Proceedings XXth International Congress of Entomology. Florence, Italy. 1996.

Light, D.M., and Flath, R.A. Host plant volatiles synergistically enhance the capture of males in commercial sex pheromone traps for various moth species. p 472. Proceedings XXth International Congress of Entomology. Florence, Italy. 1996.

Campbell, B.C., Sorensen, J.T., Gill, R.J., and Steffen-Campbell, J.D. Evolutionary affiliations among hemipteran (s.l.) suborders: paraphyly of Homoptera and non-monophyly of Auchenorrhyncha. Proceedings of the 9th International Auchenorrhyncha Congress. Sydney, Australia. 1997.

Light, D.M., and Flath, R.A. Host plant volatiles synergistically enhance the capture of males in commercial sex pheromone traps for various moth species. p. 197. Proceedings International Symposium on Olfaction and Taste XII and Achem XIX. 1997.

Campbell, B.C., Steffen-Campbell, J.D., Pickett, C., and Hoelmer, K. A. Phylogenetic affiliations and genetic identification of cryptic species of Eretmocerus. Abstr. Annual Meeting Entomological Society of America. 1996.

Campbell, B.C. Approaches towards controlling aflatoxins in tree nuts and figs. Abstr. Federal Interagency Mycotoxin Conference. Wash. DC. 1997.

Campbell, B.C. Use of natural products to control infection and growth of aflatoxigenic aspergilli in tree nuts. Aflatoxin Elimination Workshop. Fresno, CA. Govern. Pub. 1996.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-22000-020-00D FY: 97 Mode Code: 5325-41-00
Title: Control and Prevention of Aflatoxin Formation in Tree Nuts
RL: B. C. Campbell INVESTIGATORS: R. J. Molyneux, S. S. T. Hua
N. Goodman
SY Time: 3.00 NTL:$669,540
Start Date: 10/01/94 Term. Date: 09/30/99

ACCOMPLISHMENTS:
Pistachio hulls prevent *Aspergillus flavus* colonization and germination, and aflatoxin production. Anacardic acids isolated from the hulls have been shown to completely suppress aflatoxin formation *in vitro*, probably through sequestration of essential metal ions such as copper and zinc, but have no effect on germination of the fungus. Post-harvest fresh and rehydrated dried closed-shell pistachios have been found to rapidly develop high levels of aflatoxin (up to 100,000 ppb) through colonization of the stem-end, demonstrating that early splits or insect attack are not essential for aflatoxin contamination. Walnut hull components, especially the naphthoquinone, juglone, inhibit *A. flavus* colonization and prevent aflatoxin production while developing embryos support fungal colonization but do not accumulate aflatoxins, indicating the presence of endogenous natural resistance factors. A visual bioassay system has been developed to screen for yeast strains that suppress growth of aflatoxigenic fungi and a number of strains have been isolated which inhibit germination, colony expansion and sporulation.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
Commodity, Conversion and Delivery- Aflatoxins are recognized human mutagens and teratogens and their occurrence in foods is a major food safety issue. Reduction of aflatoxin in tree nuts improves acceptability of this high value export commodity. *Plant Productivity-* Identification of natural chemical resistance factors to suppress aflatoxin formation and their augmentation through breeding or genetic engineering has potential to increase productivity of high value nut crops. This project falls directly under the *High Priority ARS Crosscutting Programs of Food Safety* and *Environmentally Compatible Pest Control.*

IMPACT:
Reduction of aflatoxins in tree nut crops to levels below those imposed by regulatory agencies and importing governments will improve human health and safety and increase exportability and competitiveness of US tree nuts in foreign markets.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Research on prevention of aflatoxin formation was presented at the following meetings:
10th Aflatoxin Elimination Workshop;
96th American Society of Microbiology General Meeting;
American Phytopathological Society Annual Meeting.

RESEARCH PARTNERS:
University of California, Davis (Profs. Gale McGranahan (walnuts) and Tom Gradziel (almonds)).

GOALS FOR NEXT YEAR:
Establish the mechanism of inhibition of aflatoxin production by anacardic acids from pistachio and investigate structure-activity relationships. Evaluate the effect of juglone and related naphthoquinones *in vitro* on aflatoxin production and suppression of *A. flavus* growth. Isolate, identify and analyze for changes in levels of aflatoxin-suppression factors in developing walnuts. Investigate susceptibility and/or resistance of almond varietals and breeding crosses to aflatoxin production (in collaboration with T. Gradziel, UC Davis). Select yeast strains with greatest potential for suppression of aflatoxigenic Aspergilli and establish optimum growth conditions.

SUMMARY OF PUBLICATIONS/PATENTS:
8 Peer Reviewed Journals; 5 Abstracts, 1 Book Chapter.

Mahoney, N. E. and Rodriguez, S. B. Aflatoxin Variability in Pistachios. Appl. Environ. Microbiol. 62:1197-1202. 1996.

Gardner, D. R., Panter, K. E., Molyneux, R. J., James, L. F., and Stegelmeier, B. L. Abortifacient Activity in Beef Cattle of Acetyl- and Succinyl-Isocupressic Acid from Ponderosa Pine. J. Agric. Food Chem. 44: 3257-3261. 1996.

Pfister, J. A., Stegelmeier, B. L., Cheney, C. D., James, L. F., and Molyneux, R. J. Operant Analysis of Chronic Locoweed Intoxication in Sheep. J. Anim. Sci. 74: 2622-2632. 1996.

Molyneux, R. J., Pan, Y.T., and Elbein, A. D. Polyhydroxy Alkaloid Glycosidase Inhibitors from Plants: Structure, Distribution, and Function. Glycobiology 6:724. 1996.

Goldmann, A., Message, B., Tepfer, D. A., Molyneux, R. J., Duclos, O., Boyer, F.-D., and Elbein, A. D. Biological Functions of the *Nor*Tropane Alkaloid Calystegine B_2 and Analogs: Structure-Function Relationships. J. Nat. Prod. 59:1137-1142. 1996.

WGardner, D. R., Panter, K. E., Molyneux, R. J., James, L. F., Stegelmeier, B. L., and Pfister, J. A. Isocupressic Acid and Related Diterpene Acids from *Pinus ponderosa* as Abortifacient Compounds in Cattle. J. Nat. Toxins 6:1-10. 1997.

Asano, N., Kato, A., Miyauchi, M., Kizu, H., Tomimori, T., Matsui, K., Nash, R. and Molyneux, R.J. Specific Alpha Galactosidase Inhibitors, N-Methyl Calyssteginės. Structure/Activity Relationships of Calystegines from Lycium Chinense. Eur. J. Biochem. 1997.

Asano, N., Kato, A., Matsui, K., Molyneux, R.J. Watson, A. Calystegines in Edible Fruits and Vegetables on Mammalian Liver Glycobiology. Glycobiology 1997.

Mahoney, N. E. and Rodriguez, S. B. Sources of Aflatoxin Variability in Pistachios. Abstr. Aflatoxin Elimination Workshop, Fresno, CA., 56. 1996.

Mahoney, N. E. and Molyneux, R. J. The Stem End: A Potential Entry Point for *A. Flavus* in Pistachios. Abstr. Aflatoxin Elimination Workshop, Fresno, CA., 57. 1996.

Hua, S.-S., Grosjean, O.-K. and Baker, J. Phenolic Signal Molecules Inhibit Aflatoxin Biosynthesis by *Aspergillus flavus*.

Hua, S.-S., Baker, J. and Grosjean, O.-K. Biological Control of *Aspergillus flavus* by Saprophytic Yeasts in Pistachios. Abstr. 96th Am. Soc. Microbiol. Gen. Mtg., 395. 1996.

Hua, S.-S., Baker, J. and Grosjean, O.-K. A Visual Assay to Study the Inhibitory Mechanisms of Antagonistic Yeasts on Aflatoxin Production by *Aspergillus* spp. Abstr. Am. Phytopathological Soc. Ann. Mtg., 83. 1996.

Molyneux, R. J., Nash, R. J., and Asano, N. The Chemistry and Biological Activity of Calystegines and Related *Nor*-tropane Alkaloids, pp. 303-343. In "Alkaloids: Chemical and Biological Perspectives," vol. 11, Ed. S. W. Pelletier, Elsevier Science (Pergamon), Oxford, UK. 1996.

PROCESS CHEMISTRY AND ENGINEERING

Modecode: 5325-38-00
PACIFIC WEST AREA
WESTERN REGIONAL RESEARCH CENTER (ALBANY, CA)
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MISSION STATEMENT

The mission is to expand utilization and improve quality of fruits, vegetables and cereal grains for food and industrial uses. The unit research objectives are: 1) to create new industrial uses for agricultural products, 2) to create processes for producing new or improved fruits, vegetables, and cereal products and for improving their marketing efficiency and 3) to improve the quality and extend the shelf-life of fruit and vegetable products. The unit includes chemical and agricultural engineers, food technologists, chemists, plant physiologists and biologists.

Total Scientists: 12 Total Personnel: 32 Total Allocation: 3,466,719

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-21430-004-00D FY: 97 Mode Code: 5325-38-00
Title: In vitro creation & commercialization of high solids tomatoes & high-solids tomatoes
RL: George H. Robertson INVESTIGATORS: B. Ishida and G. Robertson
SY Time: 1.05 NTL $359,101
Start Date: 10/29/92 Term Date: 10/28/97

ACCOMPLISHMENTS:

Recently the importance of lycopene, the compound primarily responsible for the red color in tomato, has been recognized for its role in cancer risk reduction. Epidemiological studies show decreased risk in prostate cancer correlated to ingestion of tomato products. In addition, in in-vitro studies on human endometrial, mammary, and lung cancer cells, lycopene was much more effective in inhibiting cell proliferation than both a- and b-carotene. Previously, lycopene was valued highly only because of its attractive color, imparted to tomato products such as tomato paste and sauces; hence, much effort was placed on producing high solids, high lycopene tomatoes for the processing industry. We have developed a high lycopene tomato (average lycopene concentration, 391.3 mg g^{-1} fresh weight) by culturing VFNT Cherry tomato in vitro. This is approximately eight times the lycopene concentration in standard tomato fruit (43.6 - 59.6 $^{-1}$ fresh weight). Efforts are underway to translate the production of high lycopene tomatoes from in-vitro culture to the greenhouse and field.

Our experiments on in-vitro-cultured tomato fruit and calyces show that the bioregulator CPTA induces an increase in lycopene synthesis to even higher concentrations (an average of 697.7 mg g^{-1} fresh weight) and increases in a number of flavor volatiles, some of which are derived from carotenoids (e.g., 6-methyl-5-hepten-2-one, b-cyclocitral, b-damascenone, and pseudoionone) as well as those not related to carotenoids (e.g., geranial, 3-methylbutanal, 1 pentanol, (Z)3-hexenol, and methylsalicylate). Similar changes occurred in in-vitro-cultured tomato calyces grown at 16°C, which previously were shown to develop into fruit tissue at this temperature, in the presence of CPTA. This increased production cannot be explained solely by the inhibition of cyclases that prevent the formation of b-carotene. Stimulation of synthesis of enzymes in the lycopene biosynthetic pathway to probable.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):

The activity within this project is consistent with a number of the goals of the ARS: an agricultural system that is highly competitive in the global economy, a safe and secure food and fiber system, and a healthy, well-nourished population.

STP 4.3.3.1 Crops: Increase knowledge of those mechanisms in maturation, ripening, and senescence that govern postharvest quality factors and shelf life of agronomic/horticultural crops.

STP 2.1.2.5 Plant Germplasm Evaluation: Evaluate germplasm for useful characteristics that can be utilized in enhancement and breeding programs.

IMPACT:

Consumers are now becoming aware of the protective effect of tomatoes against cancer and are expected to respond positively to tomato fruit having a high lycopene content. If the decrease in cancer risk that has been correlated to the consumption of tomato products is confirmed to be a result of its lycopene content, high lycopene tomatoes will have a great impact on improving the health of the world population. In addition to health benefits, increased lycopene in tomatoes will increase the economic potential of the crop for tomato growers and processors because of this added health benefit. Increase in red pigment in the tomato will greatly benefit tomato processors in the production of tomato pastes, tomato sauces, etc., which will have more intense color, which has always been a desirable factor in tomato products. High lycopene tomatoes will therefore have an immediate major economic impact on the tomato industry because of the resulting increase in value of the crop and its many products. This will also benefit the product in international markets. A recent news release from Great Britain cites the production of a genetically engineered (Agrobacterium-mediated) tomato that could help beat cancer. Its lycopene content is twice (compared to ours having eight times) normal levels of lycopene and four times the normal level of b-carotene.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:

Informal discussions have taken place with members of the California Tomato Growers Association 50th Annual Meeting in Sacramento, CA, informing them of our recent findings. They have indicated much interest in the results of this research.

RESEARCH PARTNERS: No formal partnership.

GOALS FOR NEXT YEAR:

Translate high lycopene tomatoes from in-vitro culture to greenhouse production. This will require isolating factors that cause the increase in lycopene concentration. Evaluate a series of bioregulators obtained from Henry Yokoyama for their ability to increase concentrations of lycopene (both cis- and trans) and flavor compounds. (CPTA is not appropriate for use in food because of the concentration required--75 ppm.) A number of these bioregulators, which are available through Yokoyama, are effective in increasing important plant constituents at concentrations less than 1 ppm.

SUMMARY OF PUBLICATIONS/PATENTS:
Ishida, Betty K., Say, Brian Ripening of in vitro cultured tomato calyx is caused by overexpression of the AGAMOUS gene., World Congress on In Vitro Biology. San Francisco, CA 6/22-27, 1996. In Vitro 32(3):ii p 84A, 1996.

Ishida, B. K. Expression of the AGAMOUS gene results in ripening of in vitro-cultured tomato calyx. Molecular Biology of the Tomato, University of California, Davis August 1-4, 1996.

Ishida, B.K., Mahoney, N.E. CPTA induces increased lycopene and flavor volatile production in in-vitro-cultured tomato calyces and fruit. Physiologia Plantarum. 1997.

Approved:
Ishida, B. K.; Say, Brian Induction of agamous gene expression plays a key role in in vitro-grown tomato calyx ripening. Plant Molecular Biology.

PROJECT SUMMARY

Project Info Sheet

Prj. Number: 5325-41000-028-00D FY: 97 Mode Code: 5325-38-00
Title: New Process Operations and Systems for Refining and Converting Grains to Value Added Products
RL: G. Robertson INVESTIGATORS: D. Wong, G. Robertson
SY Time: 2.50 NTL: $ 570,083
Start Date: 5/1/93 Term Date: 4/30/98

ACCOMPLISHMENTS:
This pioneering effort seeks to define new enzyme-like catalysts through directed evolution applied to the enzyme itself. In this way enzyme activity is tailored to the function desired rather than the consequence of a mutation or evolutionary change of an entire organism. The research is applied to the barley alpha-amylase and is focussed on tailoring the catalytic domain of the molecule. The target is an enzyme capable of high rates of saccharification on wheat starch that is solid and not heated to gelatinization temperatures. This will impact not only wheat to ethanol processes but any fermentation relying on wheat starch as a fermentation source. The positive result will be reduction in energy for the saccharification step and for reduced capital cost. To date we have isolated from a cDNA library nearly complete amylase genes and have synthesized the remaining portions. Work to express this gene in suitable expression system is underway and will be followed by the development of a combinatorial library based on random mutation of the gene.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
STP 4.1.1.1 New Uses, Products, & Materials: Develop technologies foe converting plant- and animal-derived commodities to value-added industrial raw materials and products.

STP 4.1.1.2 Energy from Renewable Resources: Develop agriculturally based bioenergy production systems to generate alternatives to fossil fuels and their marketable byproducts.

This project is formally a part of the National Biofuels Program with focus on wheat to ethanol endeavors. Although focusses on wheat starch, once the combinatorial library is constructed, this approach can be relatively quickly applied to other carbohydrates such as corn starch and cellulose with broader impact on national energy programs.

IMPACT:
Immediate beneficiaries of the research will include wheat gluten-starch manufacturers since they produce the starch substrate for ethanol and other fermentations, wheat growers because the market for wheat to ethanol will be more attractive, and consumers because of the benefits of the use of renewable fuel additives and because of a reduction in the need for subsidy.

These benefits arise because of the project's immediate practical impact on the conversion of wheat to fermentation substrate-ethanol. Successful development of a cold-starch degrading enzyme(s) will produce greater energy efficiency and yield as well as reduced capital and operating cost in what is now known as the liquefaction and saccharification or cooking step. This arises because of the elimination of the need for liquefaction at high temperature.

The technologies and methodologies pioneered here not only will have direct impact on the reactive technology described above but also has the potential for pioneering and leading the development for tailoring of enzymes to be applied in processing of agricultural materials and for expanding our understanding o how enzymes work. For instance, the library produced for wheat and starting from alpha-amylase can be screened against carbohydrates from other grains such as corn and even against cellulose although more iterations may be necessary to direct the evolutionary process to a successful result. Other enzymes that are subject to optimization include enzymes effecting crosslinking of polymers, enzymes providing specific functional properties in food systems, and enzymes for analytical or test purposes. A large segment of food and agriculture could benefit from this research.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Subject technology presented in overview to the 1996 ASAE Liquid Biofuels meeting in Nashville, TN October 1996. This research will be reported to the Enzyme Engineering Conference in Beijing (Engineering Foundation) and to the American Association of Cereal Chemists.

RESEARCH PARTNERS: No formal partners at present.

GOALS FOR NEXT YEAR:
The research of this project will expand to include directed molecular evolution against the binding domain of the starch enzyme (ARS Research Associate). Efforts will also be made to develop rapid instrumental screening methods so that we can screen for relative catalytic activity against cold starch.

SUMMARY OF PUBLICATIONS/PATENTS:
Robertson, G. H., Wong, D., Kurtzman, Jr. Ralph H., Cao, T., Wong, C. Novel systems for refining and conversion of wheat to value-added products and ethanol. Liquid fuels from renewable resources conference proceedings. Nashville, TN 9/15/96.

Wong, D., Robertson, G. H., Tillin, S. J., Wong, C., Identifying peptide ligands for barley alpha-amylase. American Association of Cereal Chemists, Annual Meeting, San Diego 10/13/97.

Wong, D., Robertson, G. H., Tillin, S. J., Wong, C. Creating high affinity ligands for pancreatic and barley alpha-amylases by phage display. Enzyme Engineering XIV, Engineering Foundation Conference, Beijing, China 10/13/97.

Wong, D., Robertson, G. H., Tillin, S. J., Wong, C. Creating peptide ligands for barley alpha-amylase using combinatorial phage display libraries. Cereal Chemistry.

Wong, D., Pavlath, A. E., Robertson, G. H. Combinatorial approach in generating RNA and DNA enzymes.. Proceedings US-Japan natural resources protein resources protein panel.

PROJECT SUMMARY
Project Info Sheet

Prj. Number: 5325-41000-030-00D FY: 97 Mode Code: 5325-38-00
Title: New Technologies for Separation of Wheat Starch and Protein
RL: G. Robertson INVESTIGATORS: G. Robertson
SY Time: 1.20 NTL: $ 353,436
Start Date: 5/12/94 Term Date: 5/11/99

ACCOMPLISHMENTS:

This project seeks to define solutions for the separation of wheat into value-added protein and starch components. We have recently presented this information for the first time to the International Wheat Gluten Association and have a manuscript and patent application approved enabling full public disclosure. During FY 97 we discovered and defined methods by which ethanol can be employed as a washing fluid for hydrated dough of hard wheats. This requires attention to the level of hydration, mixing, temperature of the wash and to the water content of the washing fluid. We have found that we can produce gluten concentrates that are substantially equivalent in yield and concentration to those produced by the conventional Martin Process in less than half the time. We have found that the gluten produced in ethanol washing dries more rapidly than that produced by water-washing. We have also found functionality improvements as measured by standard tests. In addition, in unreported data, we have discovered significant changes to flour by non-extractive contact of flour with ethanol. Because the system of processing envisioned here is closed, no liquid wastes should be produced and a relatively easy recovery of all fractions, including oils, is within reach. The findings of this research effort could significantly impact the energy economy, the cost (lowered capital, smaller equipment) and product quality for wheat fractionation and significantly improve the overall energy economy and cost for wheat to ethanol conversion plants.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):

STP 4.1.1.6 Industrial Processes and Products: Devise process improvements of alternative processing systems--including bioengineering processes-- that increase the quality, safety, and value of feed, fiber, and industrial products; reduce processing wastes; and enhance environmental acceptability or reduce product costs.

STP 4.1.2.3 Food Processes and Products: Devise process improvements of alternative processing systems--including bioengineering processes--that increase the quality, safety, nutritional quality, convenience, and value of food products; reduce processing wastes; and enhance environmental acceptability or reduce product costs. This project is not formally a part of the National Biofuels Program, but impacts biofuels issues directly because all US and Australian wheat to ethanol endeavors make use of Martin-like protein from starch separations at the beginning of the ethanol process.

IMPACT:

Immediate beneficiaries of the research will include gluten manufacturers since they currently conduct wheat protein from starch separation, wheat growers because the

market for wheat to refined fractions will be more attractive, and consumers because of the benefits of the use of agriculturally derived products. Manufacturers of products from refined fractions of wheat will benefit from the reduced costs of operation. These products include ethanol, cosmetics, corrugated starch, starch based plastics, and foods which employ wheat gluten or starch in their formulations. The research can also impact the success of other research activities at WRRC to the extent that these are dependent on the cost of the refined fractions.

The potential benefits will include cost reductions for the refined fractions that stem from anticipated greater energy efficiency and reduced capital investment. Additional market impact will arise as the gluten fractions produced by the technology are applied in new ways such as a packaging films. To the extent the research reduces costs, it will improve US competitiveness with foreign manufacture of gluten.

Scientific or knowledge-based impact will arise as changes in functionality that arise as a result of exposure of flours to ethanol are explored. The new perspective can lead to improved understanding of the interactions of dough constituents.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Interaction has included members of the ABA at annual meeting in Idaho, Spring 1997 and as an invited speaker and meeting participant with the International Wheat Gluten Manufacturers Association. Subject technology presented in overview to the 1996 ASAE Liquid Biofuels Meeting in Nashville, TN October 1997. A presentation also will be made to the American Association of Cereal Chemists in October 1997. Discussions have been initiated with Craig Morris, Pullman Wheat Quality Lab to identify the ability of these processes to handle soft wheat types.

RESEARCH PARTNERS: No formal partners at present.

GOALS FOR NEXT YEAR:
The research of the project will focus on developing a mechanical device to more effectively conduct the separation described above. Effectiveness will be assessed by rate and by completeness of separation. We will also assess the capability of the method for separation of gluten from soft wheats, changes to gluten functionality by virtue of ethanol exposure and the fundamental causes of the changes, and the fate of lipids in the ethanol washing. We look also to develop systems of technologies for the subfractionation of the gluten making use of unreported temperature/ethanol concentration solubility relations. The project will expand its efforts into the definition of new uses for these fractions through addition of a materials scientist.

SUMMARY OF PUBLICATIONS/PATENTS:
Robertson, G. H., Wong, D., Kurtzman, Jr. R. H., Cao, T., Wong, C. Novel systems for refining and conversion of wheat to value-added products and ethanol. Liquid fuels from renewable resources conference proceedings, Nashville, TN 9/15/96.

Robertson, G. H., Cao, T. K. New production methods for wheat gluten and starch. International Wheat Gluten Association, St. Michaels, MD. 6/24/97.

Robertson, G. H., Trung K. Substitution of concentrated ethanol for water in the washing fractionation of hydrated wheat. Cereal Chemistry (submitted).

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41000-031-00D FY: 97 Mode Code: 5325-38-00
Title: Novel Biopolymers Based on Agricultural Sources
RL: G. Robertson INVESTIGATORS: A. E. Pavlath and G. H. Robertson
SY Time: 1.05 NTL: $254,311
Start Date: 10/29/95 Term Date: 10/28/98

ACCOMPLISHMENTS:
Based on the crosslinking hypothesis which lead to last year's success of obtaining biodegradable films from alginic acid and wool keratin, additional flexible, transparent and water-insoluble films were obtained from pectin and chicken feather keratin. Tensile strength studies showed that crosslinking with calcium made alginic acid films 4-5 times stronger. In case of pectin, the strengthening was less. Chicken feather keratin, waste product of large quantities, gave strong films both by wet-pressing and casting from solution. Similar methods with commercial gluten, a wheat protein, also resulted in excellent films. It was also found that gluten with glycerin provides a very strong adhesive for wood, paper and even metal when pressed at 130°C and 5000 p.s.i.

RELEVANCE TO NATIONAL RESEARCH PROGRAM:
STP 4.1.1.1 New uses, Products & Materials: Develop technologies for converting plant- and animal-derived commodities to value-added industrial raw materials and products.

The developers of this project provide not only potential substitutes for non-biodegradable synthetic wrapping materials, but also possible new uses for surplus agricultural products.

IMPACT:
This research to develop new conversion and reaction technologies for biopolymers will impact consumers through the development of environmentally benign, biodegradable materials that reduce the need to import foreign petroleum, the need for landfill, and the persistence of carelessly discarded and unsightly packaging materials. The research also impacts producers of agricultural materials who provide the substrate for the technologies being researched. Specifically impacted are wheat growers and gluten manufacturers through conversion of gluten into new products and chicken processors through the development of uses for previously discarded feathers.

New knowledge about the crosslinking chemistries of complex polymers and their subfractions (especially gluten), will arise as these are put to use in new ways employing the technologies under investigation.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Some of the results were presented at the '96 Analytical Pyrrolysis Meeting and on a lecture tour to various universities. Attended the Fresh Cut Fruits and Vegetable Processors Meeting where the edible film process was promoted and received with great interest requesting licensing opportunities.

GOALS FOR NEXT YEAR:
Efforts will be concentrated on the use of gluten for biodegradable wrapping materials and new type of adhesives. In order to strengthen the forming films the deamidation of the gluten will be carried out to provide more potential crosslinking points with multivalent ions. Mixing with other natural polymers, such as alginic acid, pectin, chitosan and keratin will also be investigated to obtain synergetic effects. Initial results on the use of gluten as adhesives will be followed up by investigating other crosslinking agents.

SUMMARY OF PUBLICATIONS/PATENTS:
Pavlath, Attila E; Camirand, Wayne M; Robertson, George H. Agricultural materials to help the environment. Environmental Chemistry Workshop for the Baltic Countries, Palanga, Lithuania 6/11/97.

Pavlath, A. E., Wong, D. S. W., Hudson, J., Robertson, G. H. Edible films for the extension of shelf life of lightly processed agricultural products. Chapter 8 in Agricultural materials as renewable resources. American Chemical Society pp 107-119. 1996.

Pavlath, A. E., Wong, D. S. W. And Robertson, G. H., Chitosan (Preparation, Structure and Properties in Polymeric Materials Enc. Ed. Salamone, J. C., CRC Press, Boca Raton, 1996 vol. 2C, 1231-34.

Wong, Dominic W. A., Camirand, W. M., Pavlath, A. E. Structures and functionalities of milk proteins. Critical Reviews in Food Science and Nutrition, 36 (8): 807-844 (1996).

Wong, Dominic W. S., Gregorski, Kay S., Hudson, Joyce S., Pavlath, A. E. Calcium alginate films: thermal properties and permeability to sorbate and ascorbate. J. Food Science, 61(2) 337-341 (1996).

Approved:
Pavlath, Attila E; Houssard, Catherine; Camirand, Wayne M; Robertson, George H. Keratin Films from Wool. North American Chemical Congress, Cancun, Mexico.

Pavlath, Attila E; Houssard, Catherine; Gossett, C.; Camirand, W. M.; Robertson, G. H. Biodegradable films from proteins and polysaccharides. Japan Natural Resources Protein Resources Protein Panel.

Pavlath, Attila E; Houssard, Catherine; Camirand, Wayne M.; Robertson, G. H. Clarity of films from wool keratin. J. Textile Chemistry.

Pavlath, Attila E; Wong, Dominic, Pallos, Ferenc, Hemling, Thomas C. Patent disclosure 0190.96.

Pavlath, Attila E., Gossett, Cyrille, Camirand, Wayne M., Robertson, George H. Ionomeric films from alginic acid. Jornal of Polymer Chemistry. 1997.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41000-032-00D FY: 1997 Mode Code: 5325-38-00
Title: New Technologies to Increase Utilization of Fruits and Vegetables as Restructured Products
RL: George Robertson INVESTIGATORS: C. Huxsoll, T. McHugh
SY Time: 2.35 NTL: $628,936
Start Date: 11/05/95 Term Date: 11/04/98

ACCOMPLISHMENTS:
We applied extrusion and molding technologies to form value-added, restructured fruit products. Concentrated purees were used as the primary starting material for these processes. Starch was added to the purees to modify the structure of the final product. We developed a line of apricot, strawberry, grape, orange and peach fruit pieces containing up to 80% fruit using starch molding. We discovered that drum drying the fruit puree prior to extrusion enabled the formation of 100% fruit products and filed a patent on this process. All of these fruit pieces can be used as healthy snacks for children and adults. Products were presented at the 1998 Congressional Budget Hearings as well as the Celebration of America's Bounty. We also applied fruit-based edible wraps to fresh-cut apples in order to extend their shelf-life and filed a patent on the concept.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
This research addresses several national goals:

1) It has direct impact on the utilization of U.S. grown fruit and vegetable crops, especially seasonal crops, by extending the processing season to the entire year.

2) It addresses the goal of improved nutrition by providing alternative food choices for attaining the USDA recommended guidelines for fruit and vegetable consumption.

3) Because many of the fruit and vegetable processing plants are located in rural areas, this research leads to enhancement of the economics of these areas by offering the potential for more year-around employment and increased value of the products that are manufactured and sold.

STP 4.1.2.3 Food Processes & Products: Devise process improvements or alternative processing systems--including bioengineering processes--that increase the quality, safety, nutritional quality, convenience, and value of food products; reduce processing wastes; and enhance environmental acceptability or reduce product costs.

IMPACT:
The potential scientific impact of this research will result from the new methods that are being developed to evaluate the ingredients that are being used and the final products that are being made, as well as from the study of mechanisms of ingredient/process interactions. For example, new dynamic mechanical analysis (DMA) methods will be

developed to monitor textural and rheological properties in addition to more conventional universal testing machine analyses. New "electronic nose" technology will be applied to sensory analyses of aromas, in addition to GC-mass-spectrometry. Advanced methods of multivariate analysis, such as artificial neural networks (ANN) will be applied to predict product quality and properties.

The project has a high potential for impact on several sectors of the economy. Growers will benefit from increased outlets and increased value for items made from their products. Losses incurred as a result of production in excess of demand should be diminished. Processors will benefit from being able to pack to market demands and from increased revenues resulting from added value of the products that are made. Extending the total processing season will also permit processors to distribute capital and overhead costs over a longer period.

Social impacts of the research will result from a potential improvement in nutrition, by providing fruit and vegetable items in alternate forms that will increase the total consumption of these nutritionally desirable materials. Social benefits will also accrue to the communities in which the resulting products are made, by increasing the potential for year-around employment. Because many of the processing facilities are located in small rural communities, the impact of increased year-around employment may be very significant.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Restructured fruit research was formally presented to the following groups: American Association of Cereal Chemists, Apricot Producers of California, Celebration of America's Bounty, Congressional Appropriations Committee, Institute of Food Technologists, Agricultural Research Service Apple Program Review and the Agricultural Research Service ERIC and WRAC. We also participated in the California Canning Peach and the California Pear Growers Associations Annual Meetings.

RESEARCH PARTNERS:
CRADA with Chosen Foods, LC entitled Development of Value-Added, Molded Restructured Fruit Products.

3) CRADA with Alpha Food Ingredients Entitled Modified Polyactic Acid Polymers to Improve Properties of Restructured Fruit and Vegetable Products (terminated March 1997).

GOALS FOR NEXT YEAR:

1) Continue our research on the development of novel molded and extruded restructured fruit products. Evaluate product texture, flavor, aroma, color, shelf-life and sensory attributes.

2) Initiate development of vegetable-based and fruit/vegetable combination restructured foods.

3) Develop alternative methods for preserving fruits and vegetables to be used for restructured foods. Initial efforts are focused on modified approaches for dehydrofreezing and dehydrocanning that avoid the use of sulfur dioxide.

SUMMARY OF PUBLICATIONS/PATENTS:

McHugh, Tara M. Career Opportunities in Food Chemistry. American Chemical Society (mtg).

McHugh, T. M., Huxsoll, C. C., Krochta, J. M. Permeability properties of fruit puree edible films. J. Food Sci. 61(1):88-91 (1996).

McHugh, T. M., Huxsoll, C. C., Robertson, G. H. Fruit puree-based edible films and coatings. Chapter 13 in Chemistry of Novel Foods, Spanier, Ed. pp 167-176 1996.

Approved publications and patents (Performance Year: 9/1/96...7/30/97:
McHugh, Tara M. Career Opportunities in Food Chemistry. American Chemical Society (mtg).

McHugh, Tara M. New technologies to increase utilization of fruits as restructured products.. American Association of Cereal Chemists.

McHugh, Tara M., Huxsoll, Charles C. Restructured Fruit and Vegetable Products and Processing Methods.. US Patent (docket no. 0222.96).

Huxsoll, Charles C. Evaluation of Near Infrared Spectroscopy for grading raisins. Journal of Food Processing and Preservation. 1997.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-41000-033-00D FY: 97 Mode Code: 5325-38-00
Title: New Bacterial Polysaccharides for Food and Industry
RL: George H. Robertson INVESTIGATORS: M. Smith and J. Zahnley
SY Time: 2.05 NTL: $291,886
Start Date: 04/24/97 Term Date: 10/23/97

ACCOMPLISHMENTS:
Bacteria of the genus *Leuconostoc* produce a variety of dextrans which differ in structure and properties. Many of these dextrans are potentially useful as industrial polysaccharides or food additives, and as a domestic source of gums. The enzymes that make dextrans might be useful for engineering carbohydrates to possess specific properties. We have evidence that the number of different types of dextrans made has been grossly underestimated because past research focused on the structures of crude alcohol fractions instead of on the different types of dextran-synthesizing enzymes. The small size of the field of dextran research and domination of the field by two laboratories has resulted in the establishment of untrue or partly true dogmas which have limited the possibilities for useful applications and slowed progress. This last year, we have been overthrowing these dogmas and our research is now progressing rapidly and is having a major impact. Some of our discoveries are not yet published. We discovered a new polysaccharide (and a new enzyme which synthesizes it), in strain B-1355, which no one outside WRRC believed existed. We created a double mutant, the first of its kind, which is highly enriched in constitutive synthesis of the new enzyme. We predicted (and NCAUR confirmed) the presence of 1,2-glucosidic linkages in the mutant polysaccharide, a type of linkage not previously seen in this strain. We submitted a paper for publication on the new enzyme and mutant. We have been collaborating over the last three months with NCAUR in Peoria, Illinois to work out the full structure, and the results so far show that it is different from the other dextrans. NCAUR will be publishing a paper on the structure as a result of our work. We created more different types of mutants (many are completely novel) than anyone else and have begun patenting our strains (three patent proceedings started). We submitted nearly a dozen new mutant strains to the NCAUR stock culture collection. We published an article overthrowing a theory which said that enzymes produced by sucrose cultures could not be characterized by electrophoresis because dextrose produced in sucrose cultures interfered with electrophoresis. This theory was an attempt to explain why everyone's electrophoresis results were different and why ours were wrong; it caused much confusion and delays in our publications. We proved that our results are correct and everyone else's was wrong. We collaborated with NCAUR to find out why our electrophoresis results were different from theirs and traced the problem to an unnecessary sample pretreatment step which degraded the enzymes that NCAUR was using in the mistaken belief that you had to get rid of dextrans before electrophoresis. We proved that the pretreatment is unnecessary and NCAUR now gets the same results that we get; they are writing a paper about it. We exchanged mutant strains. We discovered for the first time, that some of the enzymes (including alternansucrase) are cell-associated, while others are extracellular. This is a

completely novel concept in this field. These insoluble enzymes are routinely overlooked because of the routine practice of throwing away the cells without looking at them and using the supernatant fractions of cultures as sources of the enzymes. This discovery will be helpful to NCAUR in their attempt to purify the alternansucrase. We discovered that the insoluble enzymes can be made soluble by mutation; we and NCAUR have alternansucrase-enriched mutants that make a soluble form of alternansucrase (NCAUR was unaware of this, but we alerted them to it). We showed NCAUR that their alternansucrase mutants produce alternansucrase constitutively (they said in their publication that it was not), a fact they were unaware of because they did not check the cells. We discovered a way to make dextran-synthesizing enzymes without adding sucrose to the cultures or isolating constitutive mutants; a method useful for all the strains of *Leuconostoc.* We submitted a manuscript for publication on it; it will have an enormous impact when it is published. We know our published research is already having a large impact because we have been contacted by the leading European researchers (in France) and asked for our opinions and advice on a problem they are having. They wanted some of our mutants and offered the prospects of joint efforts and publications. Our continued collaboration with NCAUR should stimulate rapid progress in the field and will lead to the development of new polysaccharides and new applications for dextrans and dextran-like polymers. Our research is already increasing the productivity of NCAUR. We are delivering the goods like no one else.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
STP 4.1.1.1 New Uses, Products & Materials: Develop technologies for converting plant- and animal-derived commodities to value-added industrial raw materials and products.

STP 4.1.2.2 New Food Ingredients: Devise technologies for converting plan- and animal-derived components to food ingredients that contribute to nutritional enhancement, sensory properties (flavor/pigments/texture), and functional performance, or to serve as caloric replacers in formulated foods.

IMPACT AND POTENTIAL:
Our research is having a major scientific impact on the field of dextran production by *Leuconostoc mesenteroides*. The most important scientific impact of our research is the overthrow of incorrect dogmas and theories about the dextran-synthesizing enzymes of *L. mesenteroides*. Because of these incorrrect beliefs, there was no reliable way to develop new strains of dextran-producing bacteria and no reliable way to identify what kind of mutant you had, once made. As a result of our research new strains can be reliably screened and identified. We used our methods to discover a new dextransucrase from strain B-1355 which no one knew existed, because methods available before we developed our method were inadequate to detect the enzymes. Our methods can be applied to all strains of *L. Mesenteroides* to develop or discover new dextrans and dextran-producing enzymes and our method will certainly lead to the development of many new strains and dextrans. Our work will increase the available number and kinds of dextrans and enzymes which can be screened for commercial uses, because the ability to detect novel enzymes has been greatly extended. Our unpublished results show that some strains produce multiple enzymes, although only a

single dextran fraction has been reported. Each enzyme produced by a strain of *L. mesenteroides* is likely to be different from other enzymes produced, so the number of different kinds of dextrans produced by *L. mesenteroides* strains has probably been grossly underestimated. Our methods allow us to obtain a truer picture of dextran production than was previously possible.

The primary use for *L. mesenteroides* strains and enzymes is for the production of dextran from strain B-512F, which has an annual consumption (1983 to 1985) in the U.S. of approximately 2,600 metric tons at prices ranging from $35000 - $390,000 per metric ton. Dextran consumption represents about 0.11% of total industrial polysaccharide consumption, while the combined consumption of gums for industrial purposes represents approximately 5% of total annual consumption (for industrial purposes) with an estimated value of more than $200 million. Dextrans are increasingly of interest to the food industry because of their ability to control texture and bind water. To the extent to which dextrans replace gums, the economic impact of dextran research could be considerable. The enzymes which synthesize dextrans are also valuable, because they often synthesize novel linkages which are resistant to hydrolytic enzymes. Enzymes from strain B-1299, which synthesize carbohydrates containing $\alpha(1\rightarrow2)$ linkages, are now being produced commercially because the carbohydrates are useful to the cosmetics industry and are useful for synthesizing low calorie sweetners. Our mutant strain R1510, one of our mutant strains, is one of the few strains of *L. mesenteroides* which synthesizes carbohydrates containing $\alpha(1\rightarrow2)$ linkages and produces nearly the same amount of enzyme per unit cell mass as strain B-1299. Strain R1510 has two advantages over strain B-1299: (1) it produces as much or more enzyme when grown on glucose as the growth substrate it does when grown on sucrose and (2) it produces a single enzyme rather than a mixture of enzymes. This means that strain R1510 might be more useful than strain B-1299 for constructing enzyme reactors and obtaining purified enzymes free of unwanted activities. It will also be easier to control the process of synthesizing sweetners using R1510 than with B-1299. The R1510 polysaccharide will probably be useful as a matrix for attaching chemical groups and perhaps as an encapsulating agent.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
213th National Meeting of the American Chemical Society (carbohydrate division, April 15, 1997). 17th International Congress of Biochemistry and Molecular Biology (American Chemical Society, August 28, 1997), collaboration with NCAUR.

RESEARCH PARTNERS: NCAUR

GOAL FOR NEXT YEAR:
Make mutants of strain B-523 and B-1118 (makes mutant-like polymers and three to six enzymes whose products are not known). Finish research on a possible bacteriocin (and possible plasmid which can be used for genetic transfers) produced by a dextran-producing *Leuconostoc* strain. Begin research on sequencing dextransucrase and alternansucrase genes from strain B-1355 and mutansucrase genes from B-523 (the sequences are patentable). We want to compare the sequences of the alternansucrase and dextransucrase genes in the parent B-1355 strains to the sequences of our mutant strains to find out why the mutant strains overproduce enzymes. Complete research on regulation of dextransucrase/alternansucrase/GTE-1 gene expression. Identify the cellular location of the insoluble enzymes and find out why mutant enzymes become soluble. Find out why dextransucrase/alternansucrase/GTF-1 and other enzymes tend to degrade in the absence of detectable proteases when attempts are made to purify them.

SUMMARY OF PUBLICATIONS/PATENTS:
Smith, Michael R. Evidence for a GTE from L. Mesenteroides B-1355 synthesizing a(1-2) glucosidic linkages. 213th National Mtg. American Chemical Society, San Francisco, CA 4/13/97.

Smith, Michael and Zahnley, James C. Leuconostoc mesenteroides B-1355 mutants producing alternansucrases exhibiting decreases in apparent molecular mass. Applied and Environmental Microbiology, 1977, p 581-586. 1977.

Approved:
Smith, Michael R. Evidence for a GTF from L. Mesenteroides B-1355 synthesizing a(1-2) glucosidic linkages..213th National Mtg. American Chemical Society, San Francisco, CA.

Zahnley, James C.; Smith, Michael R. Glycosyltransferase profiles of representative strains of leuconostoc mesenteroides. 17th International Congress of Biochemistry and Molecular Biology, San Francisco, CA.

Smith, Michael R.; Zahnley, J. A glucosyltransferase from leuconostoc mesenteroides Strain b-1355 synthesizing alpha (1-2) glucosidic linkages. Applied and Environmental Microbiology.

Smith, Michael R. US Patent Disclosure 0155.97
Smith, Michael R.; Zahnley, J. US Patent Disclosure 0157.97
Smith, Michael R.; Zahnley, J. US Patent Disclosure 0201.97

PROJECT SUMMARY
Project Info Sheet

Prj. Number: 5325-41430-007-00D FY: 97 Mode Code: 5325-38-00
Title: Improvement of Citrus Quality and Enhancement of Citrus Byproduct Utilization
RL: G. Robertson INVESTIGATORS: S. Hasegawa, G. Manners, G. Robertson, R. Wong
SY Time: 2.15 NTL: $ 485,755
Start Date: 10/27/94 Term Date: 10/26/99

ACCOMPLISHMENTS:
This research program is focused on identifying new uses for citrus juice processing by-products such as the limonoids. The demand for limonoid glucosides for biological assessment and as food additives has increased significantly in recent years because they demonstrate anticancer activity. About 500 g of limonoid glucosides were purified from citrus molasses and sent to collaborators to test their biological activity in laboratory animals. The hamster test results indicate that, when added to the diets of the animals, the limonoid glucosides significantly inhibited the development of DMBA-induced oral tumors. The limonoids did not show toxic effects in any of these feeding tests. The chemical analysis of the blood of the test animals, including SGOT, LDH, total protein, glucose, creatine, uric acid and calcium, were normal. Ames tests showed no mutagenic activity. The limonoids have also been used as taxonomic markers in the search for new rootstocks and evaluation of new citrus varieties. Citrus limonoids are widely spread in the Rutaceae plants. Six non-citrus members of the Rutageae plants possessed citrus like limonoids. *Skimmia japonica*, a very distant relative of citrus, possesses unusual combination of *Citrus-, fortunella*-and *Papeda*-like limonoids, suggesting that this Rutaceae plant could be used for development of a cold hardy rootstock. This research impacts citrus growers, processors, and breeders as well as consumers.

RELEVANCE TO NATIONAL RESEARCH PROGRAM:
This CRIS project can be directly related to ARS research program mission in human nutrition, quality product production, by-product utilization (waste reduction) and marketing. Technology transfer research includes the production of limonoid bitterness free citrus fruits, the identification of anticarcinogenic activity of limonoids, development of foods or drinks with anticancer compounds recovered from processing by-products.

STP 4.1.2.1 New Foods: Develop technologies for producing new food products with increased nutrition, ease of preparation, and other quality traits consistent with consumer demands and export market efficiencies.

STP 4.3.2.1 Development Processes: Develop knowledge of the interrelation of developmental processes in pests and commodities that might be exploited for more effective quarantine treatments.

IMPACT:
The research of this project will benefit consumers by improving the flavor quality of citrus juices (transgenic citrus fruits free from limonoid bitter flavor), by exploring and establishing the potential of citrus limonoids for anticarcinogenic activity. The research will have further potential impact on consumers through the development of citrus based beverages, foods or pharmaceutical product containing anticarcinogenic compounds, limonoid glucosides, isolated from citrus juice processing by-products.

The project will benefit growers by providing data for citrus breeders to create rootstocks resistant to virus attack with improved cold-hardiness and breeders by classifying the currently ill-defined citrus taxonomy through the utilization of citrus limonoids as chemotaxonomic markers. The research will potentially benefit agricultural and consumers alike through development of the potential of citrus limonoids as promising insect antifeedants.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Institute of Food Technologists Meeting, American Chemical Society Meetings, Citrus Research Conferences, Phytochemical Society of North America, Institute of American Dental Research Meetings, California Citrus Research Board Meetings, American Association for Cancer Research Meetings, Citrus Chemistry and Technology Conferences, Pacifichem 95 Congress. Florida and California Citrus Growers and Processors.

RESEARCH PARTNERS:
California Citrus Research Board (Trust), Baylor College of Dentistry (Collaborator), LKT Laboratories, Inc. (Collaborator), University of Western Ontario (Collaborator), National Fruit Tree Research Institute, Japan (Collaborator), University of California at Riverside.

GOALS FOR NEXT YEAR:
Establish comprehensive analytical procedures for limonoid aglycones and glucosides, isolate limonoid glucoside mixtures and individual glucosides for cancer chemopreventative bioassays. Study absorption of limonoids and limonoid glucosides through digestive tract. Isolate limonoid glucosyltransferase DNA for creation of transgenic citrus trees free of limonoid bitterness.

SUMMARY OF *PUBLICATIONS/PATENTS*:
Record, M. T.; Miller, E. G.; Binnie, W. H.; Hasegawa, S. Systemic effects of limonoid glucosides on oral carcinogenesis, IADR, Orlando FL

Wright, J. L.; Miller, E. G.; Binnie, W. H.; Guo, I. Y.; Hasegawa, S. Further studies on the anticancer activity of citrus limonoids. J. Dent. Res. 75:436s IADR San Francisco, 3/14/96.

Hasegawa, S., Suhayda, C., Omura, M., Berhow, M. Creation of transgenic citrus free from limonin bitterness. Chapter 7 in Biotechnology for improved foods and flavors. Ed. Takeoka, G. R., Teranishi, R., Williams, P. J., Kobayashi, A. ACS Symposium Series 637, pp 79-87. 1996.

Hasegawa, S., Berhow, M. A., Fong, C. H. Analysis of bitter principles in citrus. in Modern Methods of Plant Analysis, vol 18, Fruit Analysis Edited by H. -F, Klinskens and J. F. Jackson. Springer-Verlag, Berlin pp 59-80 1996.

Hasegawa, S., Miyake, M., Ozaki, Y., Berhow, M. Limonin: A nonvolatile bitter principle in citrus juice. In “The contribution of Low and Nonvolatile Materials to the Flavor of Foods” ed by Pickenhagen W., Ho, C. T. And Spanier, A. M. Allured Publishing Corp. Carol Stream, IL, USA p 137-145 (1996).

Miller, E. G., Porter-Wright, J. L., Binnie, W. H., Guo, I. Y., and Hasegawa, S. The importance of the B-ring of the limonoid nucleus to the cancer chemopreventative activity of citrus limonoids. In “Hypernutritious Foods” Ed. by Finley, J.W. W., Armstrong, D. J., Nagy, S. And Robinson, S. F. Agscience, IN. Auburndale, FL, p 191-204. (1996)

Berhow, Mark, A., Fong, Chi H., Hasegawa, Shin. Limonoid and flavonoid composition in varieties of Papeda and Papedocitrus. Biochemical Systematics and Ecology, 24(3):237-242 (1996).

Hasegawa, Shin, Fong, Chi H., Miyake, M., Keithly, James H. Limonoid glucosides in orange molases. J. Food Science. 61(3): 560-561 (1996).

Miyake, M., Hasegawa, Shin. New functional food additives; limonoid glucosides. Chemistry and Biology 34: 289-291. 1996.

Hasegawa, S., Miyake, M. Biochemistry and biological functions of citrus limonoids. Food Reviews International. 12(4): 413-435. 1996.

In addition the following were published by Dr. Manners who recently joined this project:

Manners, G.D., Wong, R.Y., Benson, M., Ralphs, M.H., Pfister, J.A. The characterization and absolute stereochemistry of barbaline, a diterpenoid alkaloid from Delphinium barbeyi. Phytochemistry 42:875. 1996.

Manners, G.D. Plant toxins: The essence of diversity and a challenge to research. In: Natural toxins 2: Sturcture, mechanism of action and detection. Advances in Experimental Medicine and biology, vol 391 Eds. B.R. Singh and A.T. Tu, Plenum Press, pp 9-35. (1996)

Manners, G.D., Pfister, J.A., Ralphs, M.H. Larkspur: A poisonous problem on the range. Chem Tech. 26: 49-54. 1996.

Gardner, D.R.; Manners, G.D.; Ralphs, M.H.; Pfister, J.A. Quantitative analysis of diterpenoid alkaloids in larkspur (Delphinium sppp.) by Fourier transform infrared spectroscopy technique. Phytochem. Anal. 8:55-62. (1997).

Pfister, J.A., Provenza, F.D., Manners, G.D., Gardner, D.R., Ralphs, M.H. Tall larkspur ingestion: Can cattle regulate intake below toxic levels? J. Chem. Ecol. 23: 759-777. 1997.

Pfister, J.A.; Manners,G.D.; Gardner, D.R.; Price, K.W.; Ralphs, M.H.Influence of alkaloid concentration on acceptability of tall larkspur (Delphinium spp.) to cattle and sheep. J. Chem. Ecol. 22: 1147-1168. 1996.

Pfister, J.A., Panter, K.E., Manners, G.D. Effective dose in cattle of toxic alkaloids from tall larkspur (Delphinium barbeyi) Vet. Human Toxicology 36: 10-11. 1996.

Approved:

Hasegawa, Shin; Lam, Luke K.; Miller, Edward, G. Citrus limonoids: Biochemistry and biological functions. The Chemical Congress of North America, Cancan, Mexico (11/05/97).

Hasegawa, Shin; Suhayda, C. G.; HS, W. J.; Robertson, G. H. Purification of limonoid glucosyltransferase from naval orange albedo tissues. Phytochemistry. 46:33-37. 1997.

Hasegawa, Shin; Miyake, Masaki; Robertson, George H.; Berhow, Mark A. Limonoids in Skimmia Japonica. Japan Soc. Hort. Sci.

Hsu, Wan Jean; Berhow, M.; Robertson, G. H.; Hasegawa, S. H. Limonoids and flavonoid in juices of oroblanco and melogold. Journal of Food Science.

Hasegawa, Shin. Method of manufacturing limonoid glucosides. US Patent: application Docket number 0210.95 sn: 08-595607.

Manners, G., Hasegawa, Shin. A new normal phase HPLC analysis of neutral citrus limonoids. Phytochemical Analysis. 1997.

PROJECT SUMMARY
Project Info Sheet

Prj Number: 5325-42000-016-00D FY: 97 Mode Code: 5325-38-00
Title: Treatment and Reuse of Water in Commercial Food Processing Operations
RL: George H. Robertson INVESTIGATORS: L. Tsai, B. Hernlem
SY Time: 2.25 NTL: 496,088
Start Date: 10/01/92 Term Date: 10/26/99

ACCOMPLISHMENTS:
New strain of fungus, *Phytophthora infestans,* that causes the late blight disease triggered the Irish potato famine of the 1840's, has been found to spread throughout all major potato producing area of the US. The new strain resists to the fungicide that controls the original type of late blight and can destroy a crop within weeks. Even if the late blight infection did not destroy the growing plants, the infected tubers are reported to have problems during storage, which is threatening the processing industry that relies heavily on stored potatoes for a year-round supply of raw materials. We have developed a process to control potato spoilage by introducing chlorine dioxide gas to the air circulation system of the storage facility. Chlorine dioxide was demonstrated to be capable of killing microorganisms in laboratory tests. It controlled the spoilage of potatoes inoculated with heavy dose microorganisms without leaving any measurable amounts of residuals. The process is being reviewed by the Idaho Agricultural Department, which is anxious to solve that state's urgent need.

RELEVANCE TO NATIONAL RESEARCH PROGRAM (NRP):
STP 4.2.2.2 Processing Waters & Solid Wastes: Develop environmentally acceptable, economically means to prevent the occurrence of or remove hazardous materials of chemical or microbial origin in food processing waters and solid wastes.

STP 4.2.2.3 Extrinsic Toxic Factors in Foods: Develop faster, safer, and more accurate, precise, and cost-effective analytical methods to detect chemical pesticides, drugs, and other extrinsic toxic factors in foods.

IMPACT:
This research will impact the consumer through the improved safety of bulk-processed foods, the food processing industries through more cost effective processing technology and improved product reliability, and workers through safer working conditions. Food processing industries impacted include poultry and meat processors and a number of vegetable processing industries including potato, fresh lettuce, etc.

As the research contributes to minimizing disinfectant use, it will impact international trade decisions to the use of chlorine disinfectants.

This research will also impact understanding of the complex chemistries of disinfection with chlorine and chlorine dioxide, to the development of rational control strategies for food disinfection, and to improvements in separation technology with applicability to other non-food industries.

SIGNIFICANT INTERACTIONS WITH CUSTOMERS:
Research results were summarized and presented to Bio-Cide International, Inc., which supported the program by contributing financially through a CRADA. The Agricultural Department of Idaho is very interested in the development of this research because of the threat of the disease. Personnels of Idaho Agricultural Department has discussed with us the possibility of implementing this process under a "Special Local Need" program, providing all scientific requirements are met.

RESEARCH PARTNERS: CRADA w/Bio-Cide International, Inc.

GOALS FOR THE NEXT YEAR:

1. Work with industry and regulatory agencies to evaluate and implement the chlorine dioxide treatment process for potatoes.
2. Expand a novel process that disinfects and removes solids from food processing water. The preliminary study with a proto-design demonstrated that suspended solids, possibly some soluble solids, were efficiently removed and the water was disinfected.

SUMMARY OF PUBLICATIONS/PATENTS:
Approved:
Tsai, Lee-Shin; Wilson, R. E.; Randall, V. Mutagenicity of poultry chiller water treated with chlorine dioxide and chlorine. Journal of Agricultural and Food Chemistry.