Progress Report

Title:

Inactivation of Pathogens by Dense Carbon Dioxide to Enhance Safety of Small Fruits

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Proposal Category: _X_ Research ___Outreach

Name(s), mailing and email address(s) of principal investigators

Qixin Zhong, Ph.D. (PI)
Assistant Professor in Food Biophysics
The University of Tennessee
Dept. of Food Science and Technology
2509 River Drive
Knoxville, TN 37996-4539
Phone: (865) 974-6196
Fax: (865) 974-7332
E-mail: qzhong@utk.edu

David A. Golden, Ph.D. (co-PI)
Associate Professor in Food Microbiology
The University of Tennessee
Dept. of Food Science and Technology
2605 River Drive
Knoxville, TN 37996-4591
Phone: (865) 974-7247
Fax: (865) 974-2750
E-mail: david.golden@t.edu

K.P. Sandeep, Ph.D. (co-PI)
Associate Professor in Food Engineering
North Carolina State University
Department of Food Science
129 Schaub Hall, Box 7624
Raleigh, NC 27695-7624
Phone: (919) 515-2957
Fax: (919) 515-7124
E-mail: kp_sandeep@ncsu.edu

Objectives:

(1). Our primary objective is to investigate the efficacy of dense phase carbon dioxide (DPCD) in inactivating pathogens of concern to small fruits;

(2). Our secondary objective is to evaluate the effects of DPCD processing on the quality of these fruits.

Justification:

Recent outbreaks of *Escherichia coli* O157:H7 in spinach and lettuce have rekindled the search for innovative techniques to grow, harvest, handle, process, and store fruits and vegetables. Some traditional foods, e.g. strawberry and blueberry, are consumed with minimal processing, and any preservation technique, regardless of its efficacy, is inapplicable in reality if the resulting products have quality attributes that are unacceptable to consumers. Chlorine washes are conventionally used by fruit and vegetable producers but this reduce microbial counts by 1 or 2 orders of magnitude.[1] The potential internalization of pathogens in plant tissue poses an even bigger concern in food safety because of the inability of washing and surface decontamination techniques such as radiation. Aruscavage *et al*.[2] suggested to research "sanitation practices that can disinfect protected sites (such as wounds where pathogens most likely attach and proliferate) and internal plant tissue more effective than washing."

Dense phase carbon dioxide (DPCD) is a non-thermal technology that combines the bactericidal efficacy of CO_2 and high pressure at a temperature much lower than that during conventional thermal treatments. The bactericidal function of DPCD was first noticed when the

CO_2 pressure was suddenly released from 3.4 MPa to ambient pressure.[3,4] Most developments of this technique however have occurred in the past decade. Various temperature and pressure combinations in DPCD have been researched for their efficacy in reducing microorganisms in different products.[5,6] The efficacy of DPCD varies significantly with processing conditions i.e. temperature, pressure, equipment, treatment time, food matrices, and bacterial strains. For fruit juices, buffers, or growth media, screening of processing parameters may enable the reduction of microorganisms greater than 5 orders of magnitude (regulatory standards for pasteurization) at a temperature lower than 50 °C. But the feasibility of inactivating pathogens in small fruits has not been studied.

In this project, we proposed to investigate the efficacy of DPCD in inactivating *Escherichia coli* O157:H7 and *Salmonella* spp., common foodborne pathogens to fruits and vegetables, inoculated on strawberries. The effects of DPCD processing on strawberry firmness will also be investigated.

Methodologies:

Strawberry will be used as the model small fruit in this project. The technique and protocol developed however can be extended to other small fruits in a more extensive future project. Dr. Zhong will be responsible for processing inoculated strawberry under different combinations of temperature, pressure, and treatment time. Dr. Golden will be in charge of inoculation and detection of pathogens. Dr. Sandeep will use his engineering expertise to evaluate the efficacy of DPCD processing and compare it to other conventional processes such as chlorine washing so that the researched parameters can be used in future production.

Equipment and processing protocol

The supercritical CO_2 unit (Thar Technologies, Pittsburgh, PA) is housed in the Department of Food Science and Technology at the University of Tennessee. The system is schematically illustrated in Figure 1. Bone-dry grade CO_2 (Airgas, Chicago, IL) from a cylinder will be cooled to 3 °C and delivered by a CO_2 pump continuously at a controlled mass flow rate. CO_2 will be heated to a set temperature by a heat exchanger before entering the pressure vessel (500 ml volume). The vessel pressure is regulated by an automatic pressure regulator downstream and the vessel temperature is controlled by an outer heat jacket. In a typical experiment, three strawberry replicates will be contained in a sample holder (see below), which will then be placed into the pressure vessel. The pressure vessel cap will then be closed and the valve to the CO_2 cylinder will be opened. All processing parameters are controlled by a computer. After treatment, the vessel will be depressurized and the sample collected for microbial and quality tests.

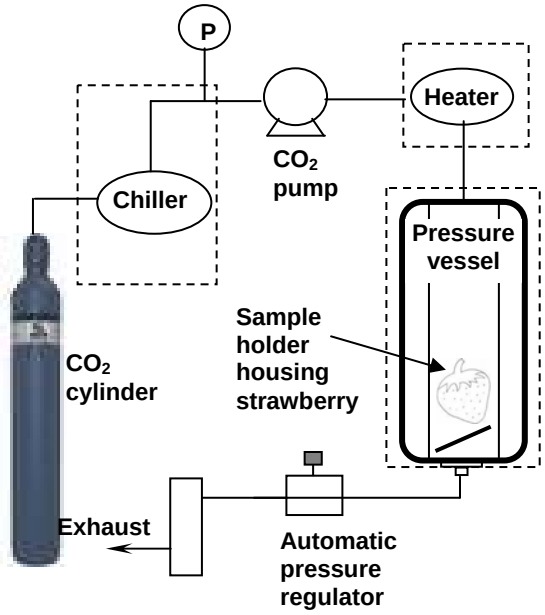

Figure 1. Schematics of DPCD process setup.

Screening of processing parameters.

Parameters to be screened include temperature (20-50 °C), residence time (10-60 min), and pressure (1 – 40 MPa). Two parallel tests will be performed. One set of tests will be to evaluate total plate counts of indigenous bacterial populations before and after DPCD processing. Samples in the other set will be inoculated with *Salmonella* or *E. coli* O157:H7. Data will be statistically analyzed using SAS 9.1 (Cary, NC).

Microbial inoculation and detection techniques

Test organisms and culture maintenance. Five strains each of *E. coli* O157:H7 and *Salmonella* serovars will be used throughout this study. All test strains are held in culture collections at the University of Tennessee. Test cultures will be maintained on tryptone soya agar (TSA) slants at 4°C and grown in tryptone soya broth (TSB) at 35°C. A minimum of two successive 24 h transfers will be performed before experimentation with 24 h cultures. Prior to experimentation, the five test strains (for each organism) will be combined to obtain mixed populations of *E. coli* O157:H7 or *Salmonella* consisting of approximately equal proportions of each test strain. Inoculated, untreated strawberry will serve as the control for each experimental procedure. A 100 μL inoculum of *E. coli* O157:H7 or *Salmonella*, at a level of $7\log_{10}$ CFU/g of fruit will be deposited on the skin or stem scar tissue of strawberries; the inoculated strawberries will be air dried for 2 h in a biosafety hood and stored at 4 °C for 20 h before being processed by DPCD.[7] Processed samples will be shaken with a sonicator and washed with 100 ml of phosphate-buffered saline (PBS; pH 7.2) to recover microorganisms for examination.[8] The protocol will also be used to determine indigenous bacterial populations in strawberries.

Microbiological enumeration. *E. coli* O157:H7 populations will be determined by surface plating (0.1 mL), serially diluted recovered samples onto modified eosin methylene blue agar (MEMB), which contains: 10 g peptone, 2 g potassium phosphate, 0.4 g eosin Y, 0.065 g methylene blue, 10 g sorbitol, 5 g sodium chloride, 0.02 g sodium novobiocin, and 15 g agar/L of deionized water. Rocelle *et al*.[9] demonstrated that MEMB provides good recovery of sublethally injured *E. coli* O157:H7. *Salmonella* populations will be determined similarly but by plating onto XLT4 agar (Difco). Aerobic bacterial populations and yeast populations will be determined using TSA and YM agar (yeast and mold agar) supplemented with 0.1% chloramphenicol. When *E. coli* O157:H7 and *Salmonella* populations are not detected by direct plating, samples will be enriched in modified TSB and tetrathionate broth, respectively, followed by streaking onto MEMB and XLT4. Representative presumptive colonies of *E. coli* O157:H7 selected from MEMB will be confirmed using the API 20E miniaturized diagnostic test kit (bioMérieux Vitek, Inc., Hazelwood, MO) and the *E. coli* O157 latex agglutination test (Oxoid). Representative presumptive colonies of *Salmonella* selected from XLT4 will be confirmed using the API 20E miniaturized diagnostic test kit.

Effects of DPCD on strawberry quality

Although the budget is limited in the current project, we plan to use a texture analyzer to compare the firmness of strawberry before and after DPCD processing and a Hunter colorimeter to compare the color changes. In future research, taste panels will be conducted to get a complete profile of processing effects on organoleptic properties.

Kinetic analyses of DPCD processing

Based on the detected microorganism populations after processing for different times at a constant pressure and temperature, the decimal reduction time (D-values) will be estimated. The D-values at a constant pressure but different temperatures will be used to estimate the thermal

resistance constant (z-values). These values will be used to compare the results with the use of other processes such as thermal processing, chlorine washing and will be necessary information for practical production.

Results:

In a relevant project of PIs Zhong and Golden, we built a sample holder (Figure 2) to contain three produce sample replicates that can be placed in the high pressure vessel and processed simultaneously. That project focused on spinach leaves, referred as the spinach project hereafter, and *E. coli* strain K-12 inoculated on spinach leaves was inactivated to a non-detectable level (Figure 3). The spinach project, although not directly related to this SRSFC project, provided some preliminary information that can be used in this SRSFC project.

Figure 2. Custom-made sample holder.

The student assigned to this project started her study in August 2008 and has learned fundamental microbiological methodologies and the operation of the supercritical CO_2 system. She will focus on the SRSFC project in Jan. – Mar. 2008, based on the three-month payments of her salary requested in the proposal. We expect to submit a final report in April 2008.

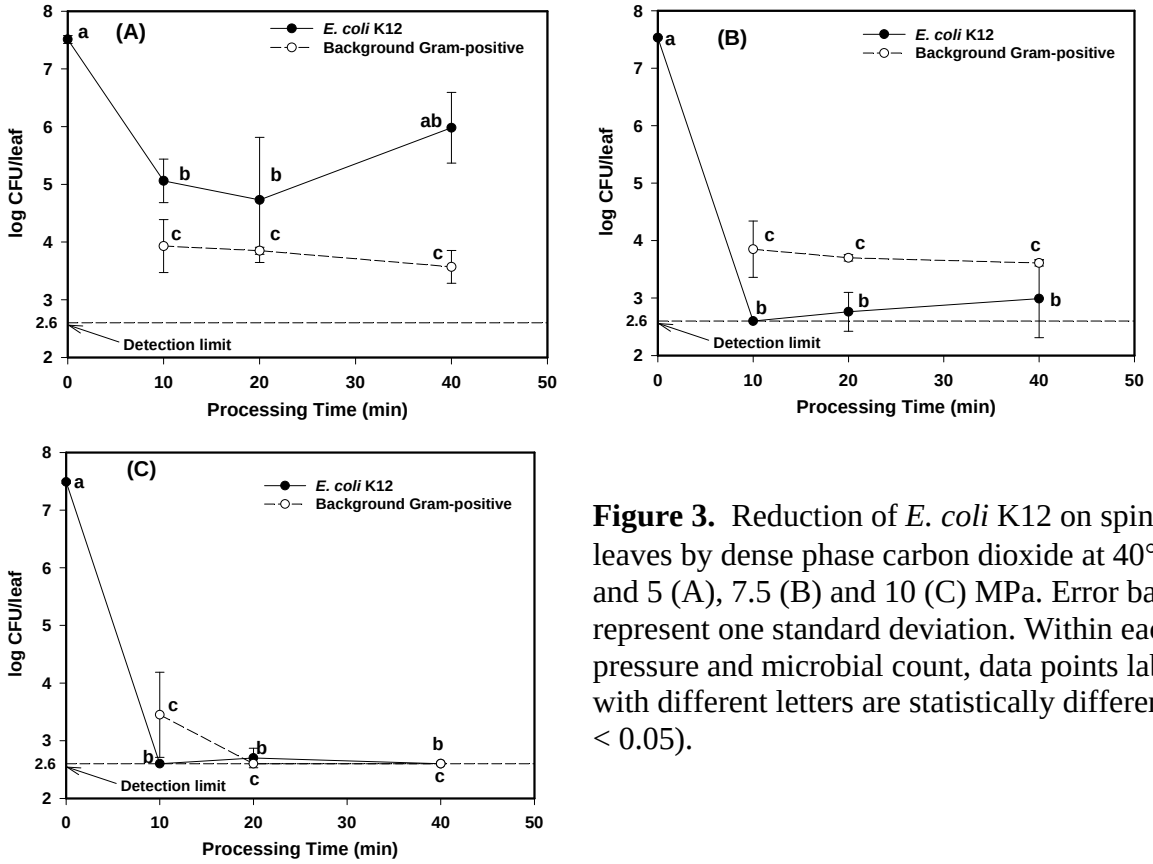

Figure 3. Reduction of *E. coli* K12 on spinach leaves by dense phase carbon dioxide at 40°C and 5 (A), 7.5 (B) and 10 (C) MPa. Error bars represent one standard deviation. Within each pressure and microbial count, data points labeled with different letters are statistically different ($P < 0.05$).

Conclusions:

Although we do not have data to report towards the specific objectives of the SRSFC project, we have generated some preliminary information from a separate relevant project that provided promising results of developing dense phase carbon dioxide into a non-thermal technology to enhance the safety of produce. We have built a sample holder that can be used for this project and trained a graduate student ready for this project.

Impact Statement:

Developing novel non-thermal pasteurization techniques will ensure that fresh produce products are safe and nutritious. This will reduce the outbreaks of foodborne illness, protecting consumers and reducing economic losses due to recalls, diseases and lawsuits.

Citation(s) for any publications arising from the project:

No report at this stage.

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