

Institution: University of Illinois

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Activities

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

3.1. Changes in glucosinolate levels in crushed horseradish roots and broccoli (Mosbah Kushad)

Glucosinolates are thioglucosides found primarily in cruciferous vegetables like broccoli, horseradish, kale, Brussels sprouts, and cabbage. The health benefits of cruciferous vegetables have been linked to these compounds. So far about 150 of these compounds have been identified, however only about 15 have been detected in commonly consumed vegetables like broccoli and cabbage. For example, broccoli is rich in glucoraphanin, horseradish and cabbage are rich in sinigrin, while kale varieties contain a combination of both of these glucosinolates. Glucosinolates as they exist in intact plants have no biological activity against human diseases, however, when vegetables containing these compounds are cut, crushed or chewed, they become hydrolyzed by an enzyme called myrosinase. Glucosinolates and myrosinase are sequestered in different organelles in the intact cells. Disruption of these organelles results in myrosinase mediated deglucosylation of glucosinolates into isothiocyanates. Glucoraphanin in broccoli converts into sulforaphane, while sinigrin converts into allylisothiocyanate. Both sulforaphane and allylisothiocyanates have been shown to reduce several types of gastrointestinal cancers in laboratory animals.

During the last few years we have been evaluating the molecular and biochemical characteristics of the myrosinase enzyme in cruciferous vegetables, mainly horseradish. Using available methods, horseradish contains significantly higher myrosinase activity compared to broccoli and kale. For this reason, we have purified myrosinase from horseradish roots. The purified enzyme from horseradish has two identical subunits of a total molecular mass of about 130. The enzyme exhibited a very broad pH and temperature optimum. The enzyme reached its highest activity at pH 5.8 and maintained nearly 50% of its activity at pH 3.4 and 9. The optimum temperature for the purified enzyme was between 37°C and 50°C but maintained nearly 60% of its activity at 59 to 62°C. The broad pH and temperature range suggest that the enzyme is capable of surviving the environment of the stomach. It also suggests that the conversion of glucosinolates into bioactive isothiocyanates continues after the cruciferous vegetable are consumed.

In addition, we have found that horseradish myrosinase is capable of breaking down different types of glucosinolates in different vegetables and that the breakdown is very rapid. We have also been successful in cloning this enzyme in *Spodoptera frugiperda* insect cells.

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

Dr. Hao Feng, Department of Food Science and Human Nutrition

5.1. Inactivation of *Escherichia coli* O157:H7 with Peroxyacetic Acid, Acidic Electrolyzed Water, and Chlorine on Cantaloupes and Fresh-cut Apples

The necessity of conserving freshness and nutrition in fresh and fresh-cut produce precludes the use of high temperature sterilization or preservatives to control food borne pathogens. Therefore, an improved wash step for the removal of human pathogens and spoilage microorganisms is highly desirable. The main objective of this study was to investigate the inactivation of *E. coli* O157:H7 with peroxyacetic acid (POAA), acidified electrolyzed water (AEW), and chlorine on cantaloupes and fresh-cut apples. These two types of produce were chosen for their rough and porous surface characteristics so that limitations of the sanitizers for the inactivation of *E. coli* O157:H7 could be evaluated. Apple cylinders were dip inoculated with a cell suspension of *E. coli* O157:H7 and then treated with sterilized water (control), chlorine, AEW or POAA. Cantaloupe cylinders with skin were spot inoculated with *E. coli* O157:H7 before treatment with sterilized water (control), AEW or POAA. All sanitizer treatments showed a significantly ($P < 0.05$) higher inactivation than the control. The POAA treatment was more effective in the inactivation of *E. coli* O157:H7 compared to other sanitizers used in this study. The residual counts of *E. coli* O157:H7 on the surfaces of fresh-cut apples or cantaloupes exhibited a dual-phasic reduction behavior, with a fast inactivation (D-values of 0.8-5.0 min) in the first min (phase I) of a treatment followed by a much slower inactivation (D-values of 14.6-59.8 min) in the remaining time (phase II). The dual-phasic inactivation seems to be related to the fruit surface topography that determines the bacterial distribution and may be used to ascertain the optimal washing time in a sanitation treatment.

5.2. Washing Conditions Affect the Inactivation of *Escherichia coli* O157:H7 on Fruit Surfaces

Washing with sanitizers is an important step to reduce microbial populations during produce postharvest handling. The washing efficacy of sanitizers has been extensively studied in the contexts of produce-to-sanitizer ratio, washing time, and washing temperature. Little attention has been paid to the effect of flow conditions on the efficacy of a washing treatment. This study was undertaken to investigate effects of washing conditions such as flow rate, agitation rate, and treatment time on bacterial reduction on rough fruit surfaces. Cantaloupe and apples cylinders were prepared with and without skins respectively. The top surfaces of the cylinders were spot inoculated with *E. coli* O157:H7 before treatment with a sanitizer (Tsunami). Models were developed to determine correlation between *E. coli* O157:H7 inactivation and the wash conditions. The results showed that the residual bacterial population decreased with increasing agitation rate, flow velocity, and treatment time. A quadratic regression function was found to best fit the flow velocity/agitation rate to normalized log reductions (N/N_0 , with N the final pathogen population and N_0 the initial pathogen population). A logarithmic

relationship was found to best correlate the treatment times to $\log N/N_0$ at each flow rate. It is therefore important to maintain the optimum flow velocity, agitation rate, and washing time to achieve the maximum reduction of bacterial populations and ensure food quality and safety.

5.3 Examination of Cell Morphological Changes of *Escherichia coli* Treated with Acidic Electrolyzed Water, Peroxyacetic Acid and Chlorine Using a MFP-3DTM Atomic Force Microscope

The MFP-3DTM atomic force microscopy (AFM) is a newly developed AFM with high precision, accuracy and flexibility in acquiring and analyzing images and conducting specific measurements. The aim of this study was to investigate the bactericidal mechanism of acidic electrolyzed water (AEW), peroxyacetic acid (POAA) and chlorine on *Escherichia coli* K12 cells by examining the changes in the cell morphology of the bacterium with a MFP-3DTM AFM. One ml *E. coli* K12 cell suspension was treated with 9 ml AEW, chlorine or POAA for 30, 60, or 120 s. At the end of each contacting time, 0.1 ml treated mixture was diluted with sterilized water, from which 5 ml was taken and concentrated on an isopore filter. The filter was fixed to the center of a glass slide for AFM imaging in a tapping mode with a silicon tip. The force was measured in a contact mode with a silicon nitride tip. Viable bacteria were enumerated by the culturing method using MacConkey agar media. Cell morphology changes were observed on *E. coli* after a 30 s treatment. Extending contacting time to 120 s did not cause any additional morphological changes. POAA treatment caused separation of cell membrane from cell cytoplasm while AEW and chlorine treatments damaged cell surfaces as evidenced by significant changes in surface topography and morphology. Adhesion force between AFM tip and cell surface was sharply decreased when morphological changes were observed. The differences in cell morphological changes of *E. coli* cells treated with three sanitizers indicate that different bactericidal mechanism among the sanitizers may have existed. The similarity in cell morphological changes of *E. coli* treated with AEW and chlorine suggests a similar inactivation mechanism. MFP-3DTM AFM can be a useful tool to elucidate the bactericidal mechanism of sanitizers by examining the cell morphological changes.

Publications

- Li, Xian, Kushad, M. 2005. Purification and characterization of myrosinase from horseradish (*Aromracia rusticana*) roots. *Plant Physiol. Biochem.* 43:503-511.
- Abbasi, N. and Kushad, M. 2006. The Activities of SOD, POD, and CAT in 'Red Spur Delicious' Apple Fruit are affected by DPA but not Calcium in Postharvest Drench Solutions. *Fruit Variety Journal*. In press.
- Kim HJ, Feng H, Toshkov SA, Fan X. 2005. Effect of sequential treatment of warm water dip and low dose gamma irradiation on the quality of fresh-cut green onions, *Journal of Food Science* 70(3): M179-185.
- Feng H, Yang W. 2005. Power ultrasound. In *Handbook of Food Science, Technology, and Engineering*, 121-1-121-9, (ed.) Hui YH, CRC Press, New York.
- Lu S, Luo, Y. Feng H. 2006. Inhibition of apple polyphenol oxidase activity by sodium chlorite. *Journal of Agricultural and Food Chemistry*, 54:3693-3696.

- Kim HJ, Feng H, Kushad MM, Fan X. 2006. Effects of ultrasound, irradiation, and acidic electrolyzed water on germination of alfalfa and broccoli seeds and /Escherichia coli /O157:H7, *Journal of Food Science*, in press.
- Zhou B, McEvoy JL, Luo Y, Robert A. Saftner RA, Feng H, Beltran T. 2006. Application of 1- methylcyclopropene reverses the deleterious effect of exogenous ethylene on fresh-cut watermelon and controls microbial growth, *Journal of Food Science*, in press.
- Wang H, Feng H, Luo L. 2006. Dual-phasic inactivation of /Escherichia coli /O157:H7 with peroxyacetic acid, acidic electrolyzed water, and chlorine on cantaloupes and fresh-cut apples, *Journal of Food Safety*, accepted.