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Pathogen reduction on mung bean reduction of *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* on mung bean using combined thermal and chemical treatments with acetic acid and hydrogen peroxide

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ABSTRACT

Mung bean sprouts were implicated in several foodborne outbreaks worldwide in recent years. The objectives of this study were to (i) assess the efficacy of individual (mild heat) and combined treatments (mild heat followed by acetic acid or/and hydrogen peroxide) for the inactivation of enteric bacterial pathogens on mung bean intended for sprout production and (ii) determine the impact of the treatments and storage conditions on germination. Mung bean was co-inoculated with *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* to achieve initial populations of approximately 5–6 log CFU of each species/g bean. The inoculated bean was then subjected to eight different treatments immediately after inoculation and after four weeks of storage at 22 °C. Selective media were used to estimate residual populations of each pathogen after treatment and subsequent to germination. The results showed that all combined treatments achieved a minimum 3-log CFU/g reduction in *E. coli* O157:H7, *S. enterica* and *L. monocytogenes* on freshly inoculated bean. The combined treatment with hot water followed by exposure to H_2O_2 and acetic acid resulted in a > 3-log reduction on mung bean stored for four weeks. The bactericidal effect of the combined treatments was significantly ($P < 0.05$) impacted by the duration of treatment and bean storage time. These data suggest that the combined use of mild heat, acetic acid and H_2O_2 may serve as a choice for organic sprouts industry in the disinfection of mung bean.

1. Introduction

The cultivation of sprouted vegetables as a source of food originated in Asia and has spread to other parts of the world in the past decades (Weiss et al., 2007). A range of seed types are now used to produce sprouted vegetables that are valued for their high content of nutrients (e.g., phytochemicals and phenolic compounds) which may have positive effects on human health (Benincasa et al., 2015). Unfortunately, sprouts also present a considerable public health challenge. Enteric bacterial human pathogens have been shown to survive on seeds and conditions during the germination process that are conducive to microbial growth (Sadler-Reeves et al., 2016; Symes et al., 2015). Numerous outbreaks of foodborne illness have been linked to sprouted vegetables, which are often consumed raw (Ding and Fu, 2016). A summary of outbreaks reported between 2000 and 2011 shows that alfalfa sprouts was the most common vehicle of transmission, followed by bean sprouts, including mung bean sprouts (Yang et al., 2013). *S. enterica* and *Escherichia coli* O157 were the infectious agents responsible for most of the outbreaks, although *Listeria monocytogenes* was also implicated in some incidents (CDC, 2014; FDA, 2016).

Seed disinfection remains the most crucial step in the production of safe sprouted vegetable products. The Canadian Food Inspection Agency (CFIA) currently recommends the application of a sanitation step to achieve a minimum 3 log reduction of microbial pathogens of concern before sprouting (CFIA, 2007). Several

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strategies have been explored to minimize the risk associated with bacterial pathogens on seeds and beans used for sprouting, including physical (e.g. dry heat, hot water, high hydrostatic pressure, irradiation), biological (e.g. antagonistic microorganisms and their metabolites) and chemical processes (i.e. chlorine, ozone and organic acids) (Ding and Fu, 2016; Sikin et al., 2013). According to Ding et al. (2013), combined treatments (i.e. more than one hurdle to reduce the level of undesirable microorganisms on seed) are more effective than individual treatments. Nei et al. (2013) reported reductions exceeding 5 log CFU/g of both *S. enterica* and *E. coli* O157:H7 on mung bean treated with hot water at 85 °C for 40 s followed by soaking in a 2000 ppm chlorine solution for 2 h. Germination rate was not significantly affected by the combined treatment.

Organic food production is carried out according to standardized principles and guidelines. Mechanical, physical or biological processing methods are preferred to minimize the use of non-agricultural ingredients, food additives and processing aids (CAN/CGSB-32.310, 2003). The CAN/CGSB guidelines allow certain chemicals to be used to maintain food quality and stability. Taking into account these requirements, chemical disinfectants sanctioned for use in organic food production or processing and thermal treatments may find value in the formulation of combined treatments for seed disinfection. Acetic acid and hydrogen peroxide (H_2O_2) are among the agents that can be used for disinfection of organically produced foods (CAN/CGSB-32.311, 2003). Both have well-characterized biocidal properties and are generally recognized as safe (GRAS) (FDA, 2015a, 2015b). The efficacy of acetic acid and hydrogen peroxide H_2O_2 against bacterial human pathogens on alfalfa seed has been documented. Beuchat (1997) used 6% H_2O_2 for 30 s and observed a 3.7 log CFU/g reduction in *S. enterica*. Higher concentrations (8%) and longer treatment times (10 min) were applied in a study conducted by Holliday et al. (2001), although *S. enterica* was reduced by only 3.27 log CFU/g. Hong and Kang (2016) applied combined treatments that included dry heat at 60, 70 or 80 °C for 0, 12, 18 or 24 h followed by treatment with a 2% hydrogen peroxide solution (10 min). *S. enterica* reductions in this study ranged from 1.66 to 3.60 log CFU/g. In contrast, Lang et al. (2000) treated seed inoculated with *E. coli* O157:H7 with 5% acetic acid for 10 min at 42 °C and achieved a 6.3 log CFU/g reduction. To date, the efficacy of treatments based on the combined effects of H_2O_2, acetic acid and heat have not been evaluated on mung bean, which is considerably different from alfalfa seed in size and physicochemical characteristics, factors that are known to affect sanitation efficiency (Studer et al., 2013).

Inhibition of microorganisms by acetic acid is believed to involve a number of mechanisms including cell membrane disruption, interference with essential metabolic processes, stress on intracellular pH homeostasis and the accumulation of toxic anions (Brul and Coote, 1999). Like chlorine and peracetic acid, H_2O_2 primarily inhibits microorganisms through chemical oxidation of cellular components but there are considerable differences in the antimicrobial efficacy of these oxidizing agents (Finnegan et al., 2010).

The objectives of this study were (i) to assess the antimicrobial efficacy of combined thermal and chemical treatments against *Escherichia coli* O157:H7, *S. enterica* and *L. monocytogenes* on mung bean intended for sprout production; and (ii) to determine the impact of these treatments on germination rates and yield as affected by bean storage. The treatments evaluated included hot water at 60 °C, hot water followed by acetic acid, hot water followed by hydrogen peroxide, and a combined treatment with hot water, acetic acid, and hydrogen peroxide.

2. Materials and methods

2.1. Bacterial cultures and bean inoculation

Certified organic mung bean were obtained from a local retailer and stored at 20 °C until used. The bean was simultaneously inoculated with (i) three *S. enterica* serotypes (*S.* Agona, alfalfa seed isolate, British Columbia, Canada, 1996; *S.* Enteriditis, poultry litter isolate PARC 29, Summerland Research and Development Centre culture collection; *S.* Typhimurium, ATCC 14028). (ii) three *L. monocytogenes* strains isolated from irrigation water in British Columbia (LMFS-L-JF 006, LMFS-L-JF 013 and LMFS-L-JF 067, UBC Wang Lab of Molecular Food Safety collection), and (iii) two *E. coli* O157:H7 strains (C7929, apple cider isolate; bovine isolate PARC 43, Summerland Research and Development Centre culture collection). All cultures were maintained in brain heart infusion (BHI) broth (BD, Difco, East Rutherford, NJ) supplemented with 20% glycerol at −80 °C.

To prepare the inocula, the bacterial strains were cultured on xylose lysine deoxycholate agar [XLD agar, BD, Difco™, Sparks, MD] for *Salmonella*; PALCAM *Listeria* Agar [Acumedia, Lansing, MI] for *L. monocytogenes* and MacConkey Agar with sorbitol, cefixime and tellurite [CT-SMAC, Oxoid, Basingstoke, Hampshire, England] for *E. coli* O157:H7 at 37 °C for 24 h. A single colony of each strain was transferred to 10 ml of Tryptic Soy Broth (TSB, BD, Difco, East Rutherford, NJ) which was incubated at 37 °C for 18 h with agitation (170 rpm) to achieve a cell density of approximately 9 log CFU/ml. The cultures were then spun at 3000 rpm for 10 min and the resulting pellet was re-suspended in 10 ml sterile 0.85% saline. Five ml of each bacterial strain were then mixed to prepare separate cocktails of *E. coli* O157:H7, *Salmonella* or L. *monocytogenes.* Five ml of each strain mixture (total 15 ml) were added to 100 g of bean to achieve an initial population of approximately 5–6 log CFU of each species/g. The bean was then mixed by hand for 5 min, spread evenly onto a sterile tray and placed in a biological safety cabinet under constant airflow for 3 h. The bean was stirred with a spatula twice during this time.

2.2. Sanitizers and treatments

Hydrogen peroxide (H_2O_2) (30% w/w in H_2O, Sigma-Aldrich Co., Canada) and acetic acid (AA) (5% distilled white vinegar, H.J. Heinz Company, Canada) were used to prepare solutions of 3 and 4% H_2O_2 and 5, 1 and 0.2% acetic acid. The treatments, which were designed to employ low concentrations of chemicals sanctioned for use in organic food production in conjunction with water heated to 60 °C, are provided in Table 1. The combined treatments consisted of the following: (1) sterile distilled water heated to 60 °C, (2) sanitizer solutions and (3) rinsing with sterile distilled water at room temperature. Untreated inoculated bean was used as the control. The inoculated bean was treated (i) immediately after inoculation or (ii) after four weeks of storage at 22 °C in Whirl-Pak™ bags.

The treatments were applied to 18 g of inoculated bean placed in conical test tubes. Where heated water was required, 36 ml were added to the bean in a test tube which was immediately placed in a water bath adjusted to 60 °C (Hurdle 1). The water was then decanted and 36 ml of the sanitizer solutions were added. For treatment combinations requiring both sanitizers the H_2O_2 solution was always applied first (Hurdle 2). The sanitizers were decanted and 36 ml sterile distilled water at room temperature was added (Hurdle 3). The tubes were shaken by hand for 1 min and the water was decanted. Ten g of treated bean were used for microbiological analysis and 8 g were retained for the assessment of germination rate and yield.