

Efficacy of High Hydrostatic Pressure Treatment in Reducing *Escherichia coli* O157 and *Listeria monocytogenes* in Alfalfa Seeds

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ABSTRACT: The application of high hydrostatic pressure (HHP) technology as a seed decontamination technique was evaluated. Alfalfa seeds inoculated with *Escherichia coli* O157 and *Listeria monocytogenes* were air-dried and subjected to independent HHP treatments of 275 to 575 MPa for 2 min or at 475 MPa for 2 to 8 min (40 °C). There were 1.4-log and 2.0-log reductions in *E. coli* O157 populations at 575 MPa (2 min) and 475 MPa (8 min), respectively. However, these treatments caused only 0.8-log and 1.1-log reductions in *L. monocytogenes* counts. Treated seeds took longer to germinate, achieving germination rate of up to 34%, whereas 95% of the control germinated. Results suggest that *L. monocytogenes* is more resistant to the bactericidal effects of HHP than *E. coli* O157. Although HHP treatments achieved a greater reduction in *E. coli* O157, it was at the expense of seed germination. Overall, our results indicate that although HHP treatments reduced the populations of *E. coli* O157 and *L. monocytogenes* in alfalfa seeds, they did not completely eliminate these microorganisms.

Keywords: high hydrostatic pressure, alfalfa seed, seed germination, *E. coli*, *L. monocytogenes*

Introduction

With the recent shift in consumer lifestyle toward healthy living and healthier foods, the consumption of raw sprouts in salads and sandwiches has increased in popularity (Feng 1997; Kurtzweil 1999). According to the International Sprout Growers Association (ISGA), some 475 sprout growers in the United States produce 300000 tons of sprouts annually contributing to a \$250 million market and as many as 10% of Americans eat sprouts regularly (Kurtzweil 1999). However, the popularity of sprouts dropped dramatically in 1995, when the consumption of sprouts was implicated in salmonellosis and *Escherichia coli* O157 outbreaks worldwide. Most of these outbreaks were caused by the consumption of sprouts grown using unsanitary practices and/or from contaminated seeds (Feng 1997). In 1998, the U.S. Food and Drug Administration (USFDA) declared alfalfa sprouts as a high-risk food (the 3rd food item after raw oysters and unpasteurized juices to carry such classification), making it unsuitable to be consumed by immunocompromised individuals, the elderly, pregnant women, and children (Burros 1998).

The USFDA recommends treating seeds with sanitizers before sprouting. This involves the use of highly concentrated sanitizers. Milder alternative processing methods need to be developed to produce microbiologically safe high-quality sprouts. Various seed decontamination methods studied in the past decade have not shown promising results in terms of bacterial inactivation capability. Techniques ranging from surface sterilization using gas to im-

mersion in chemicals caused sub-lethal cell injury instead of completely eliminating pathogens from seeds (Delaquis and others 1999; Weissinger and Beuchat 2000). Irradiation, which was considerably successful in inactivating pathogens in spices, satisfactorily eliminated pathogens from seeds but inadvertently lowered the yield ratio of seeds (Rajkowski and Thayer 2001). Sub-lethal injury is an important aspect when evaluating the efficacy of any food preservation method because the presence of injured bacterial cells could pose as much danger as fully viable ones. Under favorable conditions during storage, injured cells could undergo cellular repair and proliferate once recovery had been completed (Everis 2000). Studies have shown that sub-lethally injured pathogens present in cracks and crevices of seeds could proliferate to microbiologically hazardous levels during sprouting process where water and nutrients are plentiful (Feng 1997). Therefore, the development of a decontamination technique that could completely eliminate pathogenic contaminants in seeds is needed.

Past studies have demonstrated the efficacy of high hydrostatic pressure treatment (HHP) in inactivating a wide spectrum of Gram-negative and Gram-positive bacteria in suspensions, as well as in various solid food items (Patterson and others 1995; Arroyo and others 1997). Multidimensional HHP treatment incorporating a combination of time, pressure, heat, and antimicrobial compounds or performed consecutively with other decontamination methods create "hurdle" effects that could significantly eliminate various pathogens (Earnshaw and others 1995). Generally, bacterial inactivation by HHP is caused by damages on the cell membrane leading to alteration in membrane permeability, inactivation of intracellular enzymes, and even rupture of the cell wall (Hoover and others 1989; Vachon and others 2002). On the basis of these studies, HHP technology could present itself as an alternative technique for decontaminating seeds.

In this study, we sought to evaluate the feasibility of using HHP as a seed decontamination technique. The specific objectives of

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this research were to determine and to compare the effects of HHP on the survival of Gram-negative (*E. coli* O157:NM) and Gram-positive (*Listeria monocytogenes*) microorganisms inoculated on alfalfa seeds and to assess the effects of HHP on the germination capability of seeds.

Materials and Methods

Bacterial culture and inoculation of alfalfa seeds

Nonpathogenic variants of *E. coli* O157:NM (MF7123A) (Deng and Fratamico 1996) and *L. monocytogenes* (ATCC19113) (Pine and others 1991; Lathrop and others 2003) from our culture collection were streaked on trypticase soy agar slants (TSA) (Difco Laboratories, Detroit, Mich., U.S.A.) and stored at 4 °C before use. Strains were prepared independently by transferring inoculum from TSA slants to 100 mL of brain heart infusion broth (BHI) (Difco Laboratories) and incubated at 37 °C. Cells of *E. coli* O157:NM and *L. monocytogenes* obtained from 3 successive 24-h transfer cultures were collected by centrifugation (4000 × g, 20 min), resuspended in 50 mL 0.1% peptone, and pooled to 500 mL in separate sterile stomacher bags (Fisher Scientific, Pittsburgh, Penn., U.S.A.). Alfalfa seeds (500 g) purchased from the Sprout House (Forest Hills, New York, U.S.A.) were added into the stomacher bag and mixed with respective inoculum (500 mL) for approximately 5 min with a consistent gentle shaking to thoroughly mix the content. After decanting the suspension, seeds were air-dried at room temperature under a laminar flow hood (Biohazard Class II) on sterile cheesecloth for at least 5 h. Once dried, inoculated seeds were stored in the refrigerator (4 °C) for no more than 24 h and packed into plastic pouches for pressure treatments. From preliminary experiments, it was observed that inoculated seeds consistently retained approximately 10⁵ colony-forming units (CFU)/g of *E. coli* O157:NM and 10⁷ CFU/g of *L. monocytogenes*.

Sample packing and HHP treatment

HHP treatments were conducted using a Quintus Model QFP-6 High Pressure Food Processor (ABB Autoclave Systems, Inc., Columbus, Ohio, U.S.A.) with 50% glycol solution used as a pressure-transmitting medium. The initial temperature was adjusted so that the final process temperature of 40 °C was achieved for various pressure-holding time combinations (that is, final process temperature = initial temperature + compression heating factor × target pressure) (Balasubramanian and Balasubramanian 2003). Come-up time ranged from 85 to 145 s for pressures ranging from 275 to 827 Mpa, with depressurization completed in less than 2 s. In all cases, the duration of treatment does not include the pressure come-up or depressurization time. Seed treatments were performed as independent sequences consisting of (1) increasing pressure (275, 375, 475, and 575 MPa) at constant treatment duration of 2 min and (2) increasing treatment duration (2, 4, 6, and 8 min) at constant pressure of 475 MPa. Temperature during all treatments was continuously monitored using a thermocouple attached to top-closure of the HHP chamber. For the pressure treatment, 50 g of dried seeds were pressurized in a heat-sealed sterile polyethylene pouch (8 × 5 cm; Fisher Scientific). To avoid leakage during pressure treatment, each sample pouch was placed inside a larger pouch of a thicker material (FoodSaver® Rolls, Tilia®, San Francisco, Calif., U.S.A.) and then vacuum-packed (FoodSaver Vac350, Tilia).

Determination of bacterial inactivation and enumeration of injured cells

Pouches containing treated seeds were rinsed with 70% ethanol and then opened aseptically. Contents were stirred using a sterile

glass rod to obtain a homogeneous sample. Seeds (10 g) were added to 40 mL of sterile 0.1% peptone, stomached for 1 min at medium speed (Seward 400 Stomacher, Seward Medical Co., London, U.K.), serially diluted in sterile 0.1% peptone, and surface-plated (100 µL) in duplicate on Sorbitol MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, U.K.) supplemented with Cefixime (Dynal, Inc., Lake Success, N. Y., U.S.A.) (C-SMAC) or modified Oxford agar (MOA) (Difco Laboratories) containing antimicrobial supplement (Difco Laboratories). C-SMAC plates were incubated at 37 °C for 24 h whereas MOA plates at 37 °C for 48 h. Presumptive *E. coli* O157:NM colonies were confirmed using the *E. coli* O157 latex agglutination assay (Oxoid Inc., Ogdensburg, N.Y., U.S.A.) and presumptive *L. monocytogenes* colonies using the API *Listeria* assay (BioMerieux Vitec, Hazelwood, Miss., U.S.A.).

Injured cells of *E. coli* O157:NM were enumerated using the membrane transfer method (McCarthy and others 1998; Singh and others 2003). Appropriate diluents of seed slurries (100 µL) were surface-plated in duplicate on sterile polycarbonate filter membranes (0.4-µm pore size, 90-mm dia) (Osmonics Co., Westboro, Mass., U.S.A.) that had been placed on plate count agar plates (PCA) (Difco Laboratories). Following incubation at 37 °C for 4 h to resuscitate cells, membranes were aseptically transferred onto C-SMAC plates and further incubated at 37 °C for 20 h. Injured cells of *L. monocytogenes* were enumerated by the overlay method (Kang and Fung 1999). PCA plates inoculated with appropriate dilutions of *L. monocytogenes* were incubated at 37 °C for 4 h and then layered with liquefied MOA (tempered 38 °C). Once the MOA layer solidified, plates were incubated at 37 °C for 44 h. Presumptive colonies of *E. coli* O157:NM and *L. monocytogenes* were confirmed using the assays stated earlier.

Reduction in bacteria was calculated by subtracting log CFU/g recovered with resuscitation step from log CFU/g enumerated in positive control. The number of injured cells was determined by subtracting cell counts recovered without repair step from cells recovered after resuscitation.

Determination of seed germination

One hundred seeds were spread evenly between 2 pieces of water-saturated filter paper (nr 4; Whatman Intl. Ltd., Kent, U.K.) in a petri dish (90-mm dia; Fisher Scientific). Five plates were prepared for respective seed samples. Seeds were germinated at room temperature, and filter papers were moistened daily with sterile deionized water to maintain a high-moisture environment. Seeds were visually examined daily and considered germinated when 2 mm of radical protruded from seed coat. Germination percentage was calculated by averaging total number of seeds germinated in 5 plates at the end of day 7.

Statistical analysis

All experiments were replicated 3 times, and results were recorded as means ± standard deviations. One-way analysis of variance (ANOVA) and Duncan's multiple range tests were used to determine differences in the populations of *E. coli* O157:NM and *L. monocytogenes* recovered on treated alfalfa seeds, as well as to assess differences in germination percentage of seeds as a consequence of various pressure treatments.

Results and Discussion

Reduction of *E. coli* O157:NM and *L. monocytogenes* as a function of pressure and time

The inactivation of microorganisms by HHP is essentially a 3-dimensional process consisting of a combination of pressure, treat-

ment time, and temperature (Ting and others 2002). This design was adapted as the basis of our study. To compare the effects of HHP treatments on Gram-positive and Gram-negative microorganisms, *E. coli* O157:NM and *L. monocytogenes* were used in our study. Although *L. monocytogenes* has not been linked to illnesses associated with sprout consumption, this ubiquitous pathogen is a potential contaminant of seeds (NACMF 1999). In addition, being a psychrotroph, *L. monocytogenes* poses an additional safety hazard to the consumption of sprouted seeds because alfalfa sprouts are typically marketed under refrigeration.

The reduction of *E. coli* O157:NM and *L. monocytogenes* populations in alfalfa seeds after HHP treatments is summarized in Table 1 and 2. We observed that the highest reduction for both *E. coli* O157:NM and *L. monocytogenes* was obtained at the highest pressure of 575 MPa (2 min) and at the longest exposure to pressure (475 MPa, 8 min). Negative control did not contain any *E. coli* O157:NM and *L. monocytogenes*. Although HHP treatments had been reported to reduce the populations of *E. coli* O157:H7 and *L. monocytogenes* by 5 to 6 logs (at treatments up to 345 MPa) and 5 logs (300 MPa), respectively (Alpas and others 1999; Vachon and others 2002), our results did not corroborate with their findings. Overall, treatments at 575 MPa (2 min) reduced the populations of *E. coli* O157:NM by 1.4 logs and *L. monocytogenes* by 0.8 logs, whereas treatment for 8 min (475 MPa) produced 2.0-log and 1.1-log reduction of *E. coli* O157:NM and *L. monocytogenes*, respectively. We infer that this is most likely because of differences in test substrates used in our study and others. A higher bacterial reduction is usually observed when HHP treatment is subjected to culture-in-suspension than when bacteria is incorporated into food matrix because certain food components such as protein, carbohydrates, and lipids may confer baro-protective effects to bacterial cells (Patterson and others 1995). We also deduced that because seeds were inoculated with *E. coli* O157:NM and *L. monocytogenes* by soaking, these microorganisms may have been imbedded into the seeds and subsequently made them inaccessible to the bactericidal actions of HHP. Furthermore, cracks and crevices in the seed coat may have provided additional sites for bacterial attachment, as well as created a shield protecting these bacteria from the effects of HHP. This mode of contamination and bacterial attachment may or may not be true for naturally contaminated alfalfa seeds.

Comparative assessments on the number of *E. coli* O157:NM and *L. monocytogenes* recovered after HHP treatments (Table 1 and 2) suggest that lower populations of *L. monocytogenes* were eliminated than *E. coli* O157:NM for the same level of pressure or duration of exposure. These results indicate that *L. monocytogenes* may be more resistant to the effects of HHP than *E. coli* O157:NM, which reiterated previous findings involving pure culture-in-suspension and other food systems (Simpson and Gilmour 1997b; Alpas and others 1999). Thicker cell wall and complex cellular membrane composition have been speculated to confer Gram-positive bacteria a higher resistance to the bactericidal effects of HHP than Gram-negative bacteria (Arroyo and others 1997).

"Tailing off" phenomenon, which has been reported in other works involving HHP treatments (Hoover and others 1989; Patterson and others 1995; Simpson and Gilmour 1997a), was also observed in our study. The death rates of *E. coli* O157:NM and *L. monocytogenes* did not follow 1st-order kinetics both as a function of pressure and time (Table 1 and 2). Although this observation was not as evident in *L. monocytogenes* than *E. coli* O157:NM, the trend may be more apparent had we extended the processing time to longer than 8 min. However, we did not investigate this possibility because longer processing time may not be economically justifiable. Such "tailing off" phenomenon has been suggested to be a

Table 1—Survival of *Escherichia coli* O157:NM^a and *Listeria monocytogenes*^a in alfalfa seeds after high hydrostatic pressure (HHP) treatment at 275, 375, 475, and 575 MPa for 2 min at 40 ± 2 °C

Micro-organism	Pressure (MPa)	Control ^b	With repair step ^{c,e}	Without repair step ^d	Total log reduction ^{e,f}
<i>E. coli</i> O157:NM	275	5.1 ± 0.6	4.4 ± 0.8	3.7 ± 0.9	0.6 ± 0.4b
	375	5.0 ± 0.4	4.0 ± 0.8	3.4 ± 0.8	1.0 ± 0.5a,b
	475	5.1 ± 0.3	4.1 ± 0.4	3.4 ± 0.4	1.1 ± 0.2a,b
	575	5.1 ± 0.5	3.7 ± 1.1	2.9 ± 1.2	1.4 ± 0.6a
<i>L. monocytogenes</i>	275	7.2 ± 0.5	6.7 ± 0.8	6.5 ± 0.7	0.6 ± 0.3a
	375	7.3 ± 0.5	6.6 ± 0.7	6.5 ± 0.7	0.7 ± 0.2a
	475	7.2 ± 0.5	6.6 ± 0.7	6.3 ± 0.8	0.7 ± 0.3a
	575	7.4 ± 0.4	6.5 ± 0.7	6.3 ± 0.6	0.8 ± 0.4a

^aAll numbers are in log₁₀ colony-forming units (CFU)/g.

^bPopulations of *E. coli* O157:NM and *L. monocytogenes* recovered from positive control.

^c*E. coli* O157:NM were repaired using the membrane transfer method and *L. monocytogenes* using the overlay method.

^d*E. coli* O157:NM were surface-plated on sorbitol MacConkey agar with Cefixime and *L. monocytogenes* on modified Oxford agar containing antimicrobial supplement.

^eDifference in log₁₀CFU/g of bacteria in control and number recovered with repair step.

^fMeans ± S.D. with the same letter are not significant to each other ($n = 3$; $P > 0.05$); comparisons were made between rows (log₁₀CFU/g at 275 to 375 to 475 to 575 MPa) and independent comparison was made for each microorganism.

Table 2—Survival of *Escherichia coli* O157:NM^a and *Listeria monocytogenes*^a in alfalfa seeds after high hydrostatic pressure (HHP) treatment at 475 MPa for 2, 4, 6, and 8 min at 40 ± 2 °C

Micro-organisms	Time (min)	Control ^b	With repair step ^{c,e}	Without repair step ^d	Total log reduction ^{e,f}
<i>E. coli</i> O157:NM	2	5.2 ± 0.3	4.0 ± 0.5	3.4 ± 0.4	1.1 ± 0.3b
	4	5.5 ± 0.4	3.7 ± 0.9	3.2 ± 0.9	1.8 ± 0.6a,b
	6	4.7 ± 0.1	3.0 ± 0.1	2.4 ± 0.3	1.7 ± 0.2a,b
	8	4.6 ± 0.1	2.5 ± 0.7	2.0 ± 0.2	2.0 ± 0.7a
<i>L. monocytogenes</i>	2	7.2 ± 0.5	6.7 ± 0.6	6.5 ± 0.7	0.5 ± 0.2c
	4	7.1 ± 0.2	6.2 ± 0.1	6.2 ± 0.2	0.9 ± 0.1b
	6	7.3 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	0.9 ± 0.1b
	8	7.2 ± 0.2	6.0 ± 0.2	5.7 ± 0.4	1.1 ± 0.2a

^aAll numbers are in log₁₀ colony-forming units (CFU)/g.

^bPopulations of *E. coli* O157:NM and *L. monocytogenes* recovered from positive control.

^c*E. coli* O157:NM were repaired using the membrane transfer method and *L. monocytogenes* using the overlay method.

^d*E. coli* O157:NM were surface-plated on sorbitol MacConkey agar with Cefixime and *L. monocytogenes* on modified Oxford agar containing antimicrobial supplement.

^eDifference in log₁₀CFU/g of bacteria in control and number recovered with repair step.

^fMeans ± S.D. with the same letter are not significant to each other ($n = 3$; $P > 0.05$); comparisons were made between rows (log₁₀CFU/g at 2 to 4 to 6 to 8 min) and independent comparison was made for each microorganism.

normal feature associated with the mechanism of bacterial inactivation by HHP that is attributed to a small portion of the bacterial populations, which is relatively resistant to a certain pressure applied (Metrick and others 1989). These resistant cells pose potential microbial hazards because their recovery indicate that they were either totally not affected by HHP or suffered less damage than other less resistant cells and were able to undergo cellular repair under a suitable condition.

The effectiveness of HHP treatments was evaluated by comparing the number of *E. coli* O157:NM and *L. monocytogenes* recovered without repair step (surface-plating) to number enumerated with

repair procedure (membrane-transfer method or overlay method). For each treatment, lower numbers were recovered by surface plating than when repair step was incorporated (Table 1 and 2). This difference was more prominent in *E. coli* O157:NM (up to 0.8 log) than *L. monocytogenes* (up to 0.3 log). These observations suggest that surface plating on C-SMAC and MOA did not allow for resuscitation of injured cells. Consequently, the efficacy of a decontamination method may be overestimated when repair procedure is not incorporated into the enumeration protocol. This finding is consistent with the observations of other investigators (Simpson and Gilmour 1997a, 1997b; Kalchayanand and others 1998). These results also indicate that cells subjected to pressurization did not experience an equal degree of cellular damage even though HHP treatments are considered to be isostatic. In turn, this implied that the parameters of HHP treatments used in this study caused sub-lethal injury to bacterial cells instead of completely eliminating them.

Seed viability

Seed viability (that is the ability of seeds to germinate) was observed to be severely affected by HHP treatments. Reduction in germination was significantly correlated to increasing pressure level or exposure to HHP. Seeds treated at 275, 375, 475, and 575 MPa for 2 min achieved a germination rate of 34%, 34%, 27%, and 23%, respectively, whereas seeds treated for 2, 4, 6, and 8 min (at 475 MPa) attained a germination rate of 28%, 22%, 14%, and 9%, respectively. Untreated seeds, however, achieved a germination rate of 95%. In addition, treated seeds began to germinate after 48 h, whereas control seeds germinated within 36 h. When examined under a light microscope, the seed coats of treated seeds (475 MPa for 8 min; 575 MPa for 2 min) were damaged, showing cracks or completely broken seeds. On the basis of these observations, we concluded that the structure of dry alfalfa seeds may not be able to withstand pressures exerted by HHP. Similar to previous studies on seed decontamination techniques, this study found that alfalfa seeds were sensitive toward treatments (Delaquis and others 1999; Weissinger and Beuchat 2000; Rajkowski and Thayer 2001). Generally, longer exposure or higher concentration of chemicals used during treatment will lead to lower germination. Softening the seed coat with water or water-containing antimicrobials before HHP treatment may alleviate seed damage and thus improve seed germination. Wuytack and others (2003) reported a higher germination rate when they included sterile, deionized water in seed pouches during HHP treatments.

Conclusions

Results of this study suggest that Gram-positive bacteria (*L. monocytogenes*) are more resistant to the effects of HHP than Gram-negative bacteria (*E. coli* O157:NM). In addition, HHP treatment of alfalfa seeds at pressure levels of 575 MPa (2 min) or 475 MPa (8 min) did not produce the recommended 5-log reduction (NACMF 1999) in both *E. coli* O157:NM and *L. monocytogenes*. Instead of complete elimination, HHP sub-lethally injured the bacterial cells at the expense of seed germination. Pretreatment procedures to soften the seed coat and/or incorporation of liquid-based antimicrobials in seed pouches may alleviate damage to seeds and improve germination capabilities after pressure treatments. Given these overall findings, HHP treatment by itself had limited success eliminating bacterial contaminants in alfalfa seeds.

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References

- Alpas H, Kalchayanand N, Bozoglu F, Sikes A, Dunne CP, Ray B. 1999. Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Appl Environ Microbiol* 5(9):4248–51.
- Arroyo G, Sanz PD, Préstamo G. 1997. Effect of high pressure on the reduction of microbial populations in vegetables. *J Appl Microbiol* 82:735–42.
- Balasubramanian S, Balasubramanian VM. 2003. Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing. *Food Res Int* 36(7):661–8.
- Burros M. 1998. FDA issues warning on a 60's health food, alfalfa sprouts. *The New York Times*. September 30, 1998.
- Delaquis PJ, Sholberg PL, Stanich K. 1999. Disinfection of mung bean seed with gaseous acetic acid. *J Food Prot* 62(8):953–7.
- Deng MY, Fratamico PM. 1996. A multiplex PCR for rapid identification of Shiga-like toxin-producing *Escherichia coli* O157:H7 isolated from foods. *J Food Prot* 59(6):570–6.
- Earnshaw RG, Appleyard J, Hurst RM. 1995. Understanding physical inactivation processes: combined preservation opportunities using heat, ultrasound and pressure. *Int J Food Microbiol* 28:197–219.
- Everis L. 2000. Significance of injured microorganisms in food. Review nr. 19, Project nr 42224. Campden and Chorleywood Food Research Assn. Group. Gloucestershire, U.K.: Chipping Campden.
- Feng P. 1997. A summary of background information and foodborne illness associated with the consumption of sprouts. Washington, D.C.: U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition. Available from: <http://vm.cfsan.fda.gov/~mow/sprouts.html>. Accessed 2002 Feb 10.
- Hoover DG, Metrick C, Papineau AM, Farkas DF, Knorr D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. *Food Technol* 43:99–107.
- Kalchayanand N, Sikes T, Dunne CP, Ray B. 1998. Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. *Food Microbiol* 15:207–14.
- Kang DH, Fung DYC. 1999. Thin agar layer method for recovery of heat-injured *Listeria monocytogenes*. *J Food Prot* 62(11):1346–9.
- Kurtzweil P. 1999. Questions keep sprouting about sprouts. Washington, D.C.: U.S. Food and Drug Administration. Available from: http://www.fda.gov/fdac/features/1999/199_sprt.html. Accessed 2002 Feb 10.
- Lathrop AA, Jaradat ZW, Haley T, Bhunia AK. 2003. Characterization and application of a *Listeria monocytogenes* reactive monoclonal antibody C11E9 in a resonant mirror biosensor. *J Immunol Methods* 281:119–28.
- McCarthy J, Holbrook R, Stephens PJ. 1998. An improved direct plate method for the enumeration of stressed *Escherichia coli* O157:H7 from food. *J Food Prot* 61(9):1093–7.
- Metrick C, Hoover DG, Farkas DF. 1989. Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of *Salmonella*. *J Food Sci* 54:1547–9. [NACMF] Natl. Advisory Committee on Microbiological Criteria for Foods (NACMF). 1999. Microbiological safety evaluations and recommendations on sprouted seeds. *Int J Food Microbiol* 52:123–53.
- Patterson ME, Quinn M, Simpson R, Gilmour A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *J Food Prot* 58(5):524–9.
- Pine L, Kathariou S, Quinn F, George V, Wenger JD, Weaver RE. 1991. Cytopathogenic effects in enterocytelike Caco-2 cells differentiate virulent from avirulent *Listeria* strains. *J Clin Microbiol* 29(5):990–6.
- Rajkowski KT, Thayer DW. 2001. Alfalfa seed germination and yield ratio and alfalfa sprout microbial keeping quality following irradiation of seeds and sprouts. *J Food Prot* 64(12):1988–95.
- Simpson RK, Gilmour A. 1997a. The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. *J Appl Microbiol* 83:181–8.
- Simpson RK, Gilmour A. 1997b. The resistance of *Listeria monocytogenes* to high hydrostatic pressure in foods. *Food Microbiol* 14:567–73.
- Singh N, Singh RK, Bhunia AK. 2003. Sequential disinfection of *Escherichia coli* O157:H7 inoculated alfalfa seeds before and during sprouting using aqueous chlorine dioxide, ozonated water, and thyme essential oil. *Lebensm Wiss Technol* 36:235–43.
- Ting E, Balasubramanian VM, Raghubeer E. 2002. Determining thermal effects of high pressure processing. *Food Technol* 56(2):31–5.
- Vachon JF, Kheadr EE, Giasson J, Paquin P, Fliss I. 2002. Inactivation of foodborne pathogens in milk using dynamic high pressure. *J Food Prot* 65(2):345–52.
- Weissinger WR, Beuchat LR. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. *J Food Prot* 63(11):1475–82.
- Wuytack EY, Diels AMJ, Meersseman K, Micjels CW. 2003. Decontamination of seeds for seed sprout production by high hydrostatic pressure. *J Food Prot* 66(6):918–23.