Inactivation of *Escherichia coli* K-12 in Apple Juice Using Combination of High-Pressure Homogenization and Chitosan

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ABSTRACT: Apple juice and apple cider were inoculated with *Escherichia coli* K-12 and processed using a high-pressure homogenizer to study bacterial inactivation. Seven levels of pressure ranging from 50 to 350 MPa were used in the high-pressure homogenizer. Two types of chitosan (regular and water soluble) with 2 levels of concentration 0.01% and 0.1% were investigated for synergistic effect with high-pressure homogenization for the bacterial inactivation. *E. coli* K-12 inactivation was evaluated as a function of homogenizing pressure at different concentration of 2 types of chitosan in apple juice and cider. High-pressure homogenization (HPH) induced significant inactivation in the range of 100 to 200 MPa, while thermal inactivation was the primary factor for the bacterial inactivation above 250 MPa. Significant ($P < 0.05$) 2-way interactions involving pressure and type of substrate or pressure and chitosan concentration were observed during the study. The homogenization pressure and the incremental quantity of chitosan (both types) acted synergistically with the pressure to give higher inactivation. Significantly ($P < 0.05$) higher inactivation was observed in apple juice than apple cider at same homogenizing pressure. No effect of type of chitosan was observed on the bacterial inactivation.

Keywords: apple cider, apple juice, chitosan, *Escherichia coli*, high-pressure homogenization

Introduction

Apple juice and cider are popular drinks consumed by people of all ages for their sensory and nutritional qualities. They are low in sodium, cholesterol, and fat and are rich in vitamin C, polyphenols, and flavonoids contributing to good antioxidant properties (Lee and others 2003; Leontowicz and others 2003). Apple cider is the liquid obtained by crushing apples, and apple juice is the clarified apple cider where the suspended solids are removed by filtration, resulting in a transparent liquid. Mainstream grocery stores provide a wide selection of commercially sterile and frozen apple juice products when compared to fresh products due to the product shelf life, whereas consumers may prefer the unprocessed natural juice/cider due to its intact flavor.

Fruit and vegetable juices were traditionally considered as low-risk sources of foodborne pathogens. However, several outbreaks (FDA 2001) and an estimated 16000 to 48000 fruit and vegetable juice related illnesses/year prompted the FDA to establish new regulations for the juice industry in 2001, notably the implementation of the final rule for hazard analysis and critical control point (HACCP) processing of juices (FDA 2001). Under these regulations, the juice industry is required to use processes that achieve 5 log reduction of the most pertinent pathogen in their finished products, compared to levels that may be present in untreated juice (FDA 2001). The pertinent pathogen may vary with the type of juice and the type of treatment used, though typically it would be *Salmonella* serovars, *Escherichia coli* O157:H7, or *Cryptosporidium parvum*. Foodborne illness outbreaks of salmonellosis, enterohemorrhagic *E. coli* infections, and cryptosporidiosis have been attributed to consumption of unpasteurized apple juice and apple cider (Steele and others 1982; Besser and others 1993; CDC 1996, 1997).

Heat treatment (165 °F for 2.8 s, FDA 2001) is the most widely used method for preservation of apple juice and apple cider due to its effectiveness in reducing microorganisms, but deteriorates sensory and nutritive qualities of the juice products. Considerable research has been conducted in developing novel nonthermal preservation methods for inactivation of the microorganisms with minimal changes to the chemical and physiochemical qualities of the foods. The nonthermal technologies evaluated for preservation of fruit juices include UV light (Koutchma and others 2004), pulsed electric field (Evrendilek and others 1999), high hydrostatic pressure (Garcia-Graells and others 1998), and high-pressure homogenization (Lacroix and others 2005; Diels and Michiels 2006).

High-pressure homogenization is a physical process that involves subjecting fluid to a combination of pressure, shear, turbulence, and cavitation promoting the subdivision of particles or droplets to the micron size range, creating a stable dispersion or emulsion for further processing. Low-pressure homogenization (<50 MPa) has been widely used in the dairy industry to avoid cream separation in milk and ice cream, and in nonfood industries (for example, pharmaceuticals and cosmetics) for preparation and/or stabilization of emulsions.

Robust homogenizer designs that can generate up to 400 MPa pressure and are able to inactivate microorganisms have made this technology a new nonthermal application for assuring food safety. Gram-positive bacteria are more resistant to high-pressure

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homogenization compared to the Gram-negative bacteria possibly due to the differences in the cell wall structure (Kelemen and Sharpe 1979; Wuytack and others 2002).

Increase in consumer demand for minimally processed foods has resulted in a search for naturally occurring antimicrobial agents. Chitosan is a hydrophobic, natural biopolymer obtained by alkaline deacetylation of chitin, a natural polysaccharide found in the shells of crustacea such as crab, shrimp, and crawfish. Chitosan was shown to be effective against bacteria, yeasts, and molds (No and others 2002; Tsai and others 2002). Roller and Covill (1999) demonstrated antifungal properties of chitosan in laboratory media and apple juice.

The objective of this study was to evaluate synergistic effects of high-pressure homogenizing alone or in combination with chitosan (2 types) on the destruction of *E. coli* K-12 in apple juice and cider.

Materials and Methods

Bacterial strain

E. coli K-12 strain was obtained from The Univ. of Tennessee Food Science and Technology culture collection. The cells were revived and then further propagated by growing aerobically at 35 °C in tryptic soy broth (TSB; Becton Dickinson Co., Sparks, Md., U.S.A.) for 18 h and used for inoculation into either apple juice or apple cider.

Apple juice and apple cider

Shelf stable apple juice and cider were obtained from a local grocery store and stored at ambient temperature until use (2 wk). The pH of apple juice (Kroger Co., Ohio, U.S.A.) and apple cider (Knudson and Sons, Inc., Chico, Calif., U.S.A.) was measured using a pH meter (Model UB 10, Denver Instruments, Denver, Colo., U.S.A.) and was 3.81 and 3.71, respectively. Apple juice and apple cider were inoculated prior to the experiment each day by adding the 16 h bacterial culture (2% [v/v] inoculation) and mixing thoroughly.

Chitosan preparation

"Regular chitosan"—low molecular weight chitosan (75% to 85% deacetylated; Cat Nr 448869; Sigma-Aldrich, St. Louis, Mo., U.S.A.) solution was prepared by dissolving in 1% (v/v) acetic acid (Fisher Scientific, Pittsburgh, Pa., U.S.A.) solution in water. The "water soluble" chitosan (92% deacetylation; EZ Life Science Co. Ltd., Seoul, South Korea) solution was prepared by adding appropriate weight to distilled water and stirred to ensure proper mixing. Appropriate volumes of the 1% (w/v) solutions of the both, regular chitosan and the water-soluble chitosan were mixed with both apple juice or apple cider to obtain 0.01% and 0.1% (w/v) concentrations.

Equipment

Bench-top-high-pressure homogenizer (FPG 12500, Stansted Fluid power Ltd., Essex, U.K.) was used for homogenizing the juice and the cider. The homogenizer provides consistent homogenization pressure using 2 intensifiers. Real time data of pressure and temperature of the treatment region during was collected using Labview, v7.1 and Lookout, v6.0 (Natl. Instruments, Austin, Tex., U.S.A.). Samples of either apple juice or cider (25 °C initial temperature) were homogenized with the 1st stage set at pressures between 50 and 350 MPa (in 50 MPa intervals). The 2nd-stage pressure was maintained at 50 MPa for all the different 1st-stage pressures. Chilled water at 5 °C was circulated to cool the homogenizing valves in the valve head and the treated liquid was cooled immediately after homogenization using a tube in tube heat exchanger connected to a controlled temperature water bath set a 2 °C. The treated samples were collected and transferred to ice bath to reduce the temperature. Viscosity of processed and control samples was measured using a rheometer (Model AR 2000, TA instruments, New Castle, Del., U.S.A.) equipped with a cop and bob geometry and temperature set at 25 °C.

Microbial enumeration

Inoculated apple juice or cider and the treated samples were cooled to 2 °C. Serial dilutions (1:10) were prepared using 0.1% peptone water (Bacto Peptone, Beckton Dickinson, Sparks, Md., U.S.A.) and appropriate dilutions were plated on *E. coli* Petrifilm(tm) (3M, St. Paul, Minn., U.S.A.) in duplicate to evaluate inactivation of the cells during homogenization. The Petrifilms were incubated following the manufacturer's instructions (35 °C for 48 h) and enumerated. Microbial counts were averaged, converted, and expressed as log CFU/mL.

Statistical analysis

Three independent trials were performed for all treatments. Microbial data were analyzed by analysis of variance (ANOVA) using PROC MIXED procedure and model was written according to the experimental design explained further, with SAS (Release 9.1, SAS Inst. Inc., Cary, N.C., U.S.A.). Least significant difference (LSD) method was used to compare the means. A split–split plot design was used for the statistical analysis. The experimental design details are: main plot factors were type of juice (J) and type of chitosan (T), sub-plot factor was concentration level (C), sub-sub-plot factor was level of pressure (P), and repetition was used as a block (B).

Results and Discussion

A mean initial *E. coli* K-12 population of 7.5 ± log CFU/mL was achieved immediately after inoculation of the apple juice and apple cider. Homogenization of apple juice at pressures of 50, 100, 150, and 200 MPa resulted in *E. coli* K-12 reductions of 0.67, 1.30, 2.72, and 4.11 log CFU/mL, respectively. Higher pressures (>250 MPa) resulted in greater than 7 log CFU/mL reductions in *E. coli* K-12. The inactivation data for different combinations of substrate and type of chitosan used have been shown in Figure 1 to 4, wherein the error bars indicate the standard deviation for the 3 repetitions.

Homogenizing process involves forcing the liquid through an orifice and subsequent conversion of the pressure energy to thermal energy. Although heat was removed using chilled water circulation around the homogenization valve (5 °C), the temperature increase was proportional to the homogenization pressure (Figure 5). A quadratic relationship ($R^2 = 0.97$; $P < 0.05$) between pressure and temperature was observed, as reported by Taylor and others (2007). Using the D_{58} (4.04 min) and z values (5.4 °C) reported by Taylor and others (2007) for *E. coli* K-12 and the residence time of less than 20 s, the equivalent thermal destruction of *E. coli* K-12 at observed process temperature was calculated using thermal process calculation (Singh and Heldman 2003) and is presented in Figure 5. The nonthermal component of the microbial inactivation was predominant until 200 MPa. At 250 MPa, the bacterial inactivation due to high-pressure homogenization involved both thermal and nonthermal components. However, at homogenization pressures >250 MPa, the temperature of the apple juice or apple cider exceeded 70 °C (with a corresponding $D_{70} = 1.45$ s), indicating that thermal effect on *E. coli* K-12 inactivation was predominant at pressures >250 MPa.

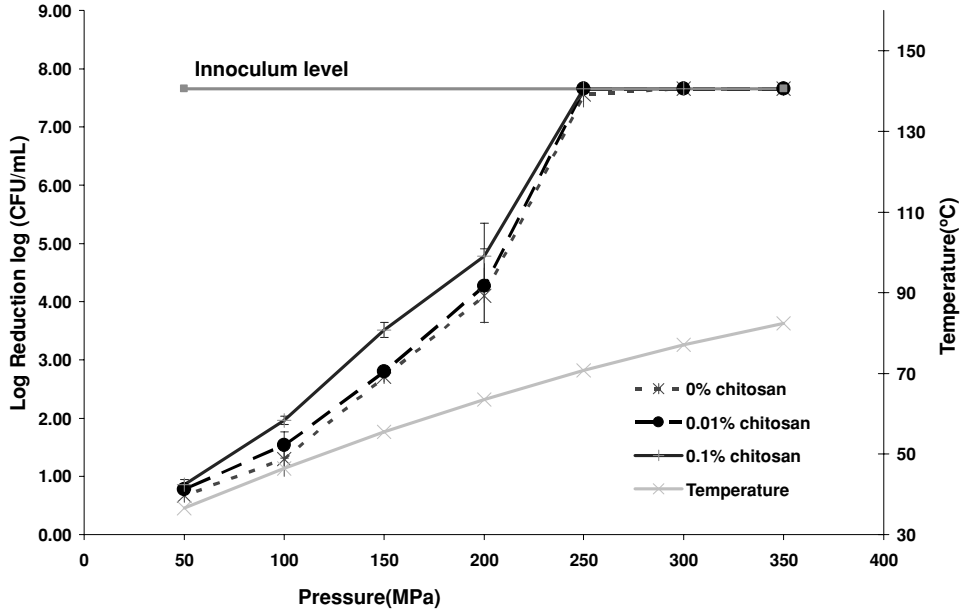

Figure 1—Effect of concentration of regular chitosan and homogenizing pressure on the inactivation of *E. coli* K-12 in apple juice.

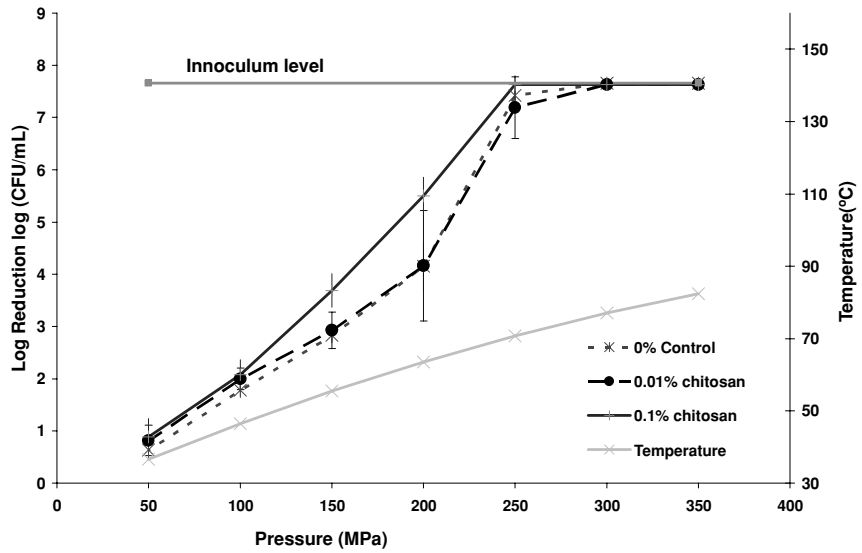

Figure 2—Effect of concentration of water-soluble chitosan and homogenizing pressure on the inactivation of *E. coli* K-12 in apple juice.

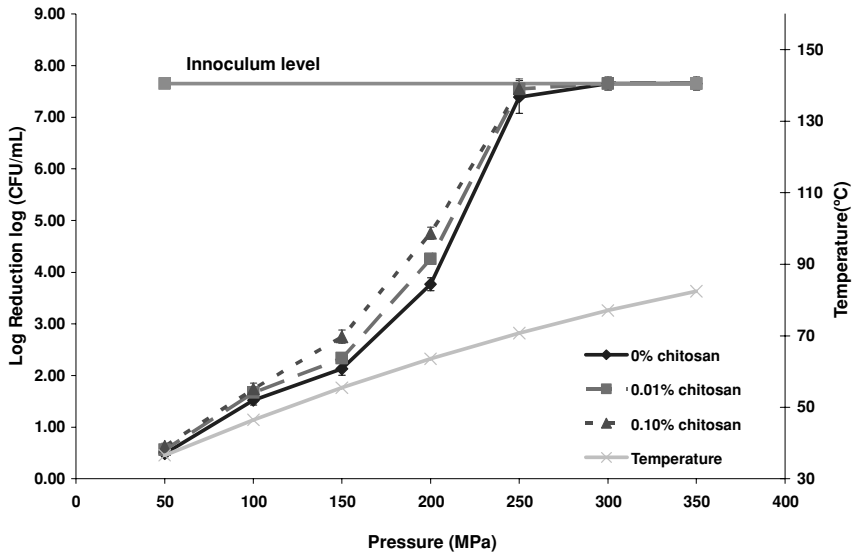

Figure 3—Effect of concentration of regular chitosan and homogenizing pressure on the inactivation of *E. coli* K-12 in apple cider.

The potential of high-pressure homogenization for inactivation of different microorganisms in different organisms in liquid food matrices such as *Listeria monocytogenes* in broth media (Lanciotti and others 1996), coliforms, and yeast and molds in goat cheese (Guerzoni and others 1999), and coliforms, total bacteria in milk (Pereda and others 2007) was shown in the literature. In this study, we evaluated the effect of high-pressure homogenization on apple juice and apple cider and found that the HPH effect was similar in microbial inactivation results. Kelemen and Sharpe (1979) and Wuytack and others (2002) reported that the Gram-positive bacteria were more resistant to high-pressure homogenization compared to Gram-negative bacteria indicating a potential correlation between cell wall structure and degree of inactivation by homogenization. In this study, we evaluated *E. coli* K-12, which is a Gram-negative bacterium, because, *E. coli* O157:H7 has been implicated in many apple juice and cider outbreaks and *E. coli* K-12 is a non-pathogenic strain and could be used in the engineering laboratory.

High-pressure homogenization process is still not a completely understood unit operation (Middelberg and others 1991). The mechanism of disruption has various theories and multiple mutually interacting mechanisms have been proposed to achieve the disruption. The rapid pressure drop near the entrance of the valve and the rate of this pressure drop were considered vital to the cell disruption (Brookman 1975). Further, Doulah and others (1975) studied the energy dynamics and found velocity fluctuations arising due to the random motions of the liquid and proposed turbulence as the major factor for the cell disruption. Engler and Robinson (1981) investigated the fluid dynamics of the liquid in a homogenizing valve and provided evidence that turbulence near the point of impact can enhance disruption. They proposed that shear, turbulence, or stress caused by impingement of a high velocity jet on a stationary surface could be responsible for disrupting cells. High temperature, cavitation, shock waves/pressure impulses produced as a result of cavity collapse are also theorized for cell disruption (Keshavarz-Moore and others 1990; Save and others 1994; Shirgaonkar and others 1998). Cavitation is a dynamic process of gas cavity growth and collapse and is proposed to occur when there is a difference in the pressure in the fluid and the saturation pressure of the dissolved gases. According to Henry's law, solubility of gas in a fluid is proportional to the pressure on the liquid, with

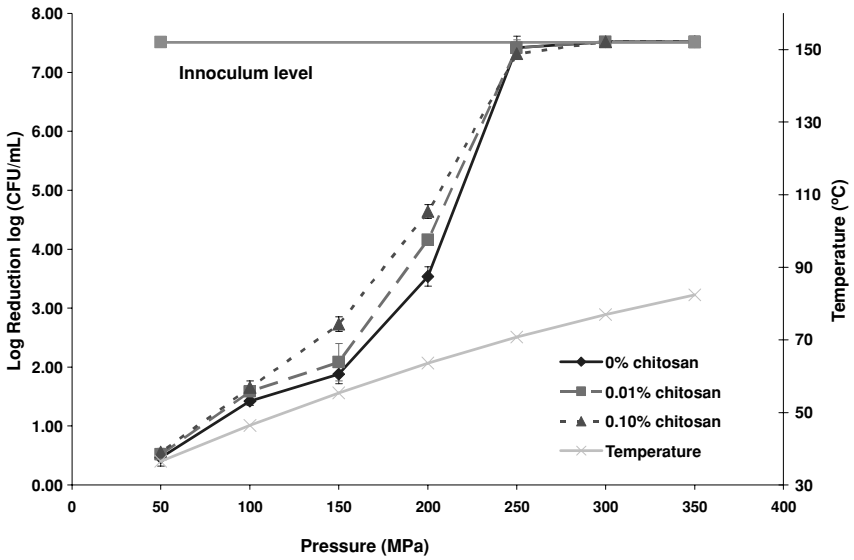

Figure 4—Effect of concentration of water-soluble chitosan and homogenizing pressure on the inactivation of *E. coli* K-12 in apple cider.

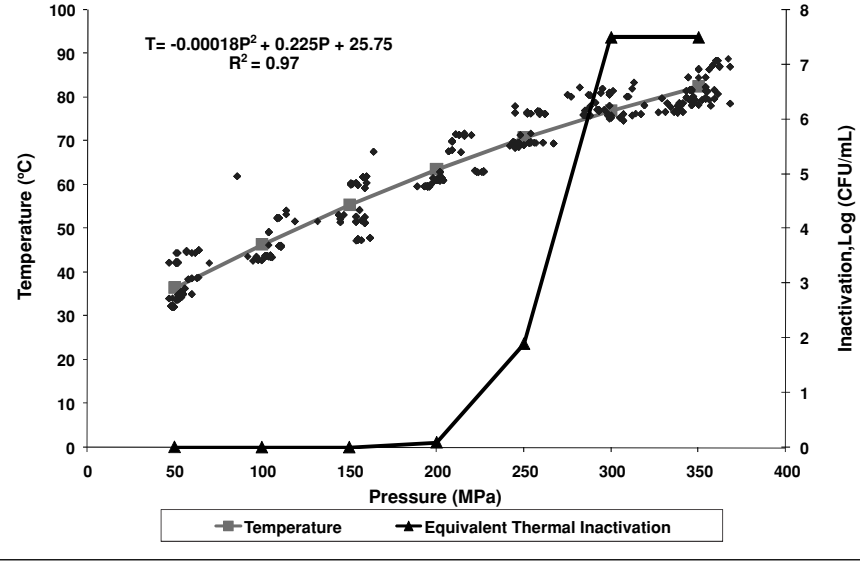

Figure 5—Influence of the pressure (*P*) on the temperature (*T*) of the valve head during the high-pressure homogenization and equivalent thermal inactivation.

gas bubbles forming and collapsing depending upon the pressure differential in the system (Diels and Michiels 2006). Chitosan has been reported to act on the microbial cell wall. The high-pressure homogenization also has been reported to act on cell wall, so possibly after the addition of chitosan the microorganisms become more sensitized to the effect of high-pressure homogenization.

The statistical analysis for the bacterial inactivation study was carried out as split–split plot design. The data for inactivation above 250 MPa were not included in the analysis, because there was complete inactivation above this pressure due to thermal effect and there was no variance among the data. The ANOVA table for the analysis is shown in Table 1. No 3-way interaction between the variables was observed. As expected, higher the homogenization pressure, higher the inactivation. Two-way interactions involving pressure with type of substrate (juice/cider) and chitosan (2 types) concentration level; type of substrate and chitosan concentration level were observed.

Addition of either type of chitosan to apple juice at 0.1% concentration resulted in increase in *E. coli* K-12 reductions at the respective homogenization pressures of up to 200 MPa (Figure 1 and 2). When the homogenization pressure was 150 MPa, the addition of regular chitosan at 0.1% increased the microbial inactivation by 0.64 log CFU/mL in apple juice (Figure 1) as compared to 0.01% level indicating synergism between the concentration level and homogenization pressure. Similar trend was observed with water-soluble chitosan in the pressure range of 100 to 200 MPa in both apple juice and apple cider. Addition of 0.01% chitosan (both types) has minimal increase (approximately 0.5 log CFU/mL) in inactivation, while addition of 0.1% has a relatively larger inactivation (approximately 1.5 log CFU/mL) between 100 and 200 MPa for both apple juice and cider (Figure 1 to 4). Significantly similar reductions in *E. coli* K-12 were observed in apple cider. The reductions in apple juice were significantly higher ($P < 0.05$) than in apple cider at same homogenizing pressure. The reductions in *E. coli* K-12 during homogenization were similar between apple juice containing regular chitosan and water-soluble chitosan. Chitosan has also been reported to inhibit the growth of microorganisms due to its chelating property (Skjak-Braek and others 1989). The chelating property of chitosan allows it to bind to the essential nutrients and metal ions, making them unavailable to microorganisms resulting in the inhibition of their growth rate. Liu and others (2001) reported the minimum inhibitory concentration (MIC) of chitosan for *E. coli* to be 20 ppm of chitosan (0.02%) in nutrient broth medium.

Differences in *E. coli* K-12 inactivation between the types of chitosan was minimal ($P > 0.05$) in both apple juice and apple cider. There was a significant ($P < 0.05$) interaction between the concentration level of chitosan (2 types) used and the homogenization pressure employed. The inactivation data for 0% and 0.01% regular chitosan concentrations appeared to be similar but the 0.1% regular chitosan concentration caused more inactivation at the same homogenizing pressure. Popper and Knorr (1990) reported no interaction ($P > 0.05$) of high homogenization pressure (<150 MPa) and chitosan concentration (up to 0.01%). It has also been reported that true synergism is dependent upon the concentration of the antimicrobial (Diels and others 2005). Diels and others (2005) also reported that HPH may induce a transient permeabilization of the outer membrane of *E. coli* making it more susceptible to lytic agents like lysozyme and nisin and that does not involve a physical disruption and is immediately repaired after the process. Similarly, in our study, such interaction between chitosan (2 types) and HPH caused synergistic inactivation; the higher concentrations of chitosan (2 types) in conjunction with high-pressure homogenization acted synergistically and enhanced microbial inactivation.

The exact mechanism for antimicrobial activity of chitosan is not fully understood but many researchers have proposed different mechanisms. Altering the cell permeability due to the interactions between positively charged chitosan and negatively charged cell wall leading to leakage of intracellular components is one of the proposed mechanisms (Young and others 1982; Leuba and Stössel 1986; Papineau and others 1991; Sudarshan and others 1992; Fang and others 1994; Chen and others 1998). Additional mechanism is the binding of chitosan with eukaryotic DNA leading to the inhibition of the mRNA via interference in protein synthesis (Hadwiger and Loschke 1981; Hadwiger and others 1986; Sudarshan and others 1992). Cuero and others (1991) proposed the chelation of metals, spore elements, and essential nutrients interaction with chitosan as the mechanism for chitosan antimicrobial activity.

The antimicrobial activity of chitosan is influenced by multiple factors. Chitosan has been reported to have stronger antimicrobial activity against bacteria compared to fungi and the type of bacterium also influences its antimicrobial efficacy (No and others 2002; Tsai and others 2002). The food matrix and temperature are also vital factors for the antimicrobial activity of chitosan (Devlieghere and others 2004; Zivanovic and others 2004). Devlieghere and others (2004) reported variable antimicrobial efficacy of chitosan in different food systems with different levels of oil, starch, whey protein, and salt at different pH. Other factors influencing the antibacterial effects of chitosan and chitosan oligomers are reported to be its molecular weight (Uchida and others 1989; Jeon and others 2001; No and others 2002) and degree of deacetylation (Tsai and others 2002).

Recent studies on antibacterial activity of chitosan and chitosan oligomers have revealed that chitosan is more effective in inhibiting growth. Uchida and others (1989) and Jeon and others (2001) reported that chitosan has better efficacy compared to chitosan oligomers. Roller and Covill (1999) reported that chitosan activity against the filamentous moulds and yeast was concentration, pH, and temperature dependent. The solubility of chitosan in solution is critical to its antimicrobial activity, and the chitosan solubility is adversely affected by pH > 5.5 (Skjak-Braek and others 1989; Popper and Knorr 1990).

The effect of apple juice and apple cider was also investigated for the influence of the 2 types of chitosan: regular and soluble, on the

Table 1 — ANOVA for the bacterial inactivation using split–split plot design for the statistical analysis.

Effect[a]	Num DF	Sum of squares	Mean square	*F* value	*Pr* > *F*
J	1	2.52	2.52	10.2	0.01
T	1	0.1	0.1	0.41	0.93
C	2	3.58	1.79	35.4	<0.0001
P	6	1570	261	7381	<0.0001
P∗J	6	3.18	0.53	14.2	<0.0001
P∗C	12	5.49	0.46	12.2	<0.0001
J∗C	2	0.34	0.17	2.98	0.04
P∗T	6	0.54	0.09	2.53	0.09
J∗T	1	0.3	0.3	1.21	0.23
T∗C	2	0.12	0.06	1.07	0.37
J∗T∗C	2	0.09	0.04	0.77	0.47
J∗T∗P	6	0.57	0.1	2.41	0.06
J∗C∗P	12	0.39	0.03	0.83	0.47
T∗C∗P	12	0.46	0.04	0.97	0.34

[a]J = type of juice; T = type of chitosan; C = concentration level; P = level of pressure; and B = repetition or block.

Table 2—Influence of type and concentration of chitosan and high-pressure homogenization on the viscosity (10^{-3} Pa-s) of apple juice.

		Pressure (MPa)							
Chitosan	Concentration (%)	0	50	100	150	200	250	300	350
Control	0.00	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Regular	0.01	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.6
	0.10	2.8	2.3	2.2	2.1	2.1	2	2	1.9
Water soluble	0.01	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.6
	0.10	1.8	1.8	1.8	1.7	1.7	1.7	1.7	1.7

microbial inactivation. The results indicated that there was no significant ($P > 0.05$) difference in the inactivation using either type of soluble chitosan in any of the 2 substrates. There was a significant ($P < 0.05$) interaction between the type of substrate used and concentration of chitosan (2 types) used. There was significantly ($P < 0.05$) higher inactivation in apple juice compared to apple cider for a particular concentration of chitosan (2 types) used. This may be attributed to the fact that cider had big particles, which may have decreased the efficacy of homogenization resulting in lower bacterial inactivation.

Viscosity of the apple juice containing 2 types of chitosan (regular and water soluble) at 0.01% and 0.1% concentrations before and after high-pressure homogenization at various pressure levels is shown in Table 2. The viscosity of the apple juice (control) is 1.7E-3 Pa-s. While the addition of water-soluble chitosan did not affect the viscosity of the apple juice, addition of regular chitosan at 0.1% concentration increased the viscosity of the apple juice to 2.8E-3 Pa-s. However, subsequent high-pressure homogenization reduced the viscosity to the level that was similar to the viscosity of the control apple juice. Similar results (data not shown) were obtained for apple cider. Thus, addition of chitosan (2 types) at these concentration levels did not considerably change the viscosity of apple juice and cider after high-pressure homogenization. Because viscosity is an important property affecting the energy required to pump the liquid (Singh and Heldman 2003), addition of chitosan (2 types) followed by high-pressure homogenization will not impact the processing (pumping and other operations) of apple juice.

Conclusions

High-pressure homogenization is a promising technology, which may be an alternative to thermal pasteurization for apple juice and apple cider. Whereas homogenization pressures of 100 to 200 MPa cause microbial inactivation due to high-pressure homogenization, homogenization pressures >250 MPa resulted in significant thermal inactivation. Homogenization pressures of >250 MPa resulted in greater than 7 log CFU/mL of *E. coli* K-12 inactivation in apple juice and apple cider mainly due to the thermal component of the high-pressure homogenization process. There were no significant ($P < 0.05$) 3-way interactions observed. However, significant ($P < 0.05$) 2-way interactions (pressure * type of substrate and pressure * chitosan concentration) were found during the study. The homogenization pressure was a critical factor in causing the inactivation and the incremental quantity of chitosan (2 types) acted synergistically with the pressure to give higher inactivation. Addition of chitosan (2 types) at 0.1% concentration resulted in enhancing *E. coli* K-12 inactivation in apple juice and apple cider up to 200 MPa. There was no significant ($P < 0.05$) effect of type of chitosan on the bacterial inactivation. Also, there was significantly ($P < 0.05$) higher inactivation in apple juice than apple cider using same homogenizing pressure. Future study will be carried out to evaluate the sensory and shelf-life studies to assess the impact of high-pressure homogenization on the apple juice.

References

Besser RE, Lett SM, Weber JT, Doyle MP, Barret TJ, Wells JG, Griffin PM. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA 269:2217–20.

Brookman JSG. 1975. Further studies on the mechanism of cell disruption by extreme pressure extrusion. Biotechnol Bioeng 17(4):465–79.

CDC. 1996. Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, October 1996. MMWR 45(44):975.

CDC. 1997. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. MMWR 46(44):4–8.

Chen C, Liau W, Tsai G. 1998. Antibacterial effects of N-sulfonated and N-sulfobenzoyl chitosan and application to oyster preservation. J Food Prot 61(9):1124–8.

Cuero RG, Osuji G, Washington A. 1991. N-carboxymethyl chitosan inhibition of aflatoxin production: role of zinc. Biotechnol Lett 13(6):441–4.

Devlieghere F, Vermeulen A, Debevere J. 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. Food Microbiol 21(6):703–14.

Diels AM, Michiels CW. 2006. High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. Crit Rev Microbiol 32(4):201–16.

Diels AMJ, De Taeye J, Michiels CW. 2005. Sensitisation of *Escherichia coli* to antibacterial peptides and enzymes by high-pressure homogenisation. Int J Food Microbiol 105(2):165–75.

Doulah MS, Hammond TH, Brookman JSG. 1975. A hydrodynamic mechanism for the disintegration of *Saccharomyces cerevisiae* in an industrial homogenizer. Biotechnol Bioeng 17(6):845–58.

Engler CR, Robinson CW. 1981. Disruption of *Candida utilis* cells in high pressure flow devices. Biotechnol Bioeng 23(4):765–80.

Evrendilek GA, Zhang QH, Richter ER. 1999. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. J Food Prot 62(7):793–96.

Fang SW, Li CF, Shih DYC. 1994. Antifungal activity of chitosan and its preservative effect on low-sugar candied kumquat. J Food Prot 57(2):136–40.

FDA. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice; final rule. Fed Reg 66(13):6137–202.

García-Graells C, Hauben KJA, Michiels CW. 1998. High pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices. Appl Environ Microbiol 64(4):1566–8.

Guerzoni ME, Vannini L, Chavez Lopez C, Lanciotti R, Suzzi G, Gianotti A. 1999. Effect of high pressure homogenization on microbial and chemico-physical characteristics of goat cheeses. J Dairy Sci 82(5):851–62.

Hadwiger LA, Loschke DC. 1981. Molecular communication in host-parasite interactions: hexosamine polymers (chitosan) as regulator compounds in race-specific and other interactions. Phytopathology 71(7):756–62.

Hadwiger LA, Kendra DF, Fristensky BW, Wagoner W. 1986. Chitosan both activates genes in plants and inhibits RNA synthesis in fungi. In: Muzzarelli R, Jeuniaux C, Gooday GW, editors. Chitin in nature and technology. New York: Plenum Press. p 209–14.

Jeon YJ, Park PJ, Kim SK. 2001. Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydr Polym 44(1):71–6.

Kelemen MV, Sharpe JEE. 1979. Controlled cell disruption: a comparison of the forces required to disrupt different microorganisms. J Cell Sci 35(1):431–41.

Keshavarz-Moore E, Hoare M, Dunnill P. 1990. Disruption of baker's yeast in a high-pressure homogeniser: new evidence on mechanism. Enzyme Microb Technol 12(10):764–70.

Koutchma T, Keller S, Stuart C, Parisi B. 2004. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. Inn Food Sci Emerg Technol 5(2):179–89.

Lacroix N, Fliss I, Makhlouf J. 2005. Inactivation of pectin methylesterase and stabilization of opalescence in orange juice by dynamic high pressure. Food Res Int 38(5):569–76.

Lanciotti R, Gardini F, Sinigaglia M, Guerzoni ME. 1996. Effect of growth conditions on the resistance of some pathogenic and spoilage species to pressure homogenization. Lett Appl Microbiol 22(2):165–8.

Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY. 2003. Major phenolics in apple and their contribution to the total antioxidant capacity. J Agric Food Chem 51(22):6516–20.

Leontowicz M, Gorinstein S, Leontowicz H, Krzeminski R, Lojek A, Katrich E, Cizm M, Martin-Bellooso O, Soliva-Fortuny R, Haruenkit R, Trakhtenberg S. 2003. Apple and

pear peel and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol-containing diets. J Agric Food Chem 51(19):5780–85.
Leuba JL, Stössel P. 1986. Chitosan and other polyamines: antifungal activity and interaction with biological membranes. In: Muzzarelli R, Jeuniaux C, Gooday GW, editors. Chitin in nature and technology. New York: Plenum Press. p 215–22.
Liu XF, Guan YL, Yang DZ, Li Z, Yao KD. 2001. Antibacterial action of chitosan and carboxymethylated chitosan. J Appl Polym Sci 79(7):1324–35.
Middelberg APJ, O'Neill BK, Bogle IDL. 1991. A novel technique for the measurement of disruption in high pressure homogenization: study on *E. coli* containing recombinant inclusion bodies. Biotechnol Bioeng 38(4):363–70.
No HK, Park NY, Lee SH, Meyers SP. 2002. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int J Food Microbiol 74(1–2):65–72.
Papineau AM, Hoover DG, Knorr D, Farkas DF. 1991. Antimicrobial effect of water soluble chitosans with high hydrostatic pressure. Food Biotechnol 5(1):45–57.
Pereda J, Ferragut V, Quevedo JM, Guamis B, Trujillo AJ. 2007. Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. J Dairy Sci 90(3):1081–93.
Popper L, Knorr D. 1990. Applications of high-pressure homogenization for food preservation. Food Technol 44:84–9.
Roller S, Covill N. 1999. The antifungal properties of chitosan in laboratory media and apple juice. Int J Food Microbiol 47(1–2):67–77.
Save SS, Pandit AB, Joshi JB. 1994. Microbial cell disruption—role of cavitation. Chem Eng J Biochem Eng J 55(3):B67–72.
Shirgaonkar IZS, Lothe RR, Pandit AB. 1998. Comments on the mechanism of microbial cell disruption in high-pressure and high-speed devices. Biotechnol Prog 14(4):657–60.
Singh RP, Heldman DR. 2003. Preservation processes. In: Singh RP, Heldman DR, editors. Introduction to food engineering. Glasgow: Academic Press. p 333–66.
Skjak-Braek G, Anthonsen T, Sandford P. 1989. Chitin and Chitosan. London, U.K.: Elsevier Applied Science. 560 p.
Steele BT, Murphy N, Arbus GS, Rance CP. 1982. An outbreak of hemolytic uremic syndrome associated with the ingestion of fresh apple juice. J Pediatr 101:963–5.
Sudarshan NR, Hoover DG, Knorr D. 1992. Antibacterial action of chitosan. Food Biotechnol 6(3):257–72.
Taylor TM, Roach A, Black DG, Davidson PM, Harte F. 2007. Inactivation of *Escherichia coli* K-12 exposed to pressures in excess of 300 MPa in a high-pressure homogenizer. J Food Prot 70(4):1007–10.
Tsai GJ, Su WH, Chen HC, Pan CL. 2002. Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. Fish Sci 68(1):170–7.
Uchida Y, Izume M, Ohtakara A. 1989. Preparation of chitosan oligomers with purified chitosanase and its application. In: Skjak-Bræk G, Anthonsen T, Sandford P, editors. Chitin and chitosan: sources, chemistry, biochemistry, physical properties and applications. London, U.K.: Elsevier. p 373–82.
Wuytack EY, Diels A, Michiels CW. 2002. Bacterial inactivation by high-pressure homogenization and high hydrostatic pressure. Int J Food Microbiol 77(3):205–12.
Young DH, Kohle H, Kauss H. 1982. Effect of chitosan on membrane permeability of suspension cultured *Glycine max* and *Phaseolus vulgaris* cells. Plant Physiol 70(5):1449–54.
Zivanovic S, Basurto CC, Chi S, Davidson PM, Weiss J. 2004. Molecular weight of chitosan influences antimicrobial activity in oil-in-water emulsions. J Food Prot 67(5):952–9.