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Microbiological effects of high pressure processing on food

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Abstract

In recent years high pressure (HP) processing has been investigated as an alternative method for food preservation. HP technology allows inactivation of microorganisms while maintaining sensory and nutritional properties of foods. Consumers have increased their demand for high-quality foods that are convenient and nutritious, that have fresh flavour, texture, colour and minimal or no chemical preservatives, and above all, that are safe. The use of non-thermal methods for food preservation is due to consumer demands for microbiological safe products, without changes in the sensory and nutritional qualities of the product. High hydrostatic pressure (HHP) has emerged as an alternative totraditional thermal processing methods for foods. High-pressure processing (HPP) entails the pasteurization of food using pressure in the 100-600 MPa range, which results in a reduction of microbial loads and thus extends the shelf life of the processed food. The scientific theories behind HPP should be fully understood before appropriate parameter conditions such as pressure, temperature, time, and pH can be accurately selected. Among these, the pressure-resistant characteristics of various microorganisms, as well as their potential physiological response to HPP, are key factors that must be considered when developing HPP foods.

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Introduction

High hydrostatic pressure processing (HPP) is an emerging nonthermal technology that can ensure the same level of food safety as heat pasteurization and produces fresher-tasting, minimally processed foods. This technology reportedly increases shelf life, while minimizing loss of quality. Additionally, it maintains the nutritional value and quality of food and therefore does not result in any undesirable changes associated with thermal processing (Chiao *et al.*, 2014; Bermúdez-Aguirre and Barbosa-Cánovas, 2011). Under HPP, natural flavors can be retained to provide food of superior quality and nutritional value. All food-processing techniques must conform to sanitation and safety-related specifications before they can be employed in food and commercially applied. The pasteurization settings and control of conventional thermal processing techniques have been practically applied. Furthermore, appropriate heatresistant strains can be selected as target microorganisms to verify the sanitation and ensure effective food safety. However, HPP is still an emerging processing technique, necessitating further investigation of its related scientific theories, parameter standards, and commercial applications (Huang *et al.*, 2014). With non-thermal processing technologies, more fresh-like products can be obtained. HHP is considered to be an alternative to thermal pasteurization for fruit juices and other products when this process is used alone or in combination with traditional techniques. The major benefit of pressure is its immediate and uniform effect throughout different media, avoiding difficulties such as nonstationary conditions typical for convection and conduction processes. HHP is an attractive non-thermal process because the pressure treatments required to inactivate bacterial cells, yeasts, and molds have a minimal effect on the sensory qualities associated with fresh-like attributes such as texture, colour, and flavour (Aannou *et al.,* 2010; Chung and Yosuef, 2010). The knowledge of foods properties in the high-pressure range is important to develop and optimize such processes by means of mathematical modeling and simulation. HHP involves the use of pressures of approximately 300e700 MPa for periods of approximately 30 s to a few minutes to destroy pathogenic bacteria such as Listeria, Salmonella, Escherichia coli, and Vibrio and other bacteria, yeasts, and molds that cause foods to spoil (Jofrè *et al.*, 2010).

The effects of high pressure on inhibiting the growth of various microorganisms have been extensively published in the literature. Table 1 shows the survival rates of various bacterial vegetative and spores after applying high-pressure sterilization.

Table 1. Viability loss of bacterial vegetative and spores by HHP with different time, temperature and pressure combinations (Ref: Huang *et al.*, 2014).

Strain	Substrate	Temperature (°C)	Pressure (MPa)	*D* value (min)	Reference
Vegetative					
E. coli O157:H7	Mango juice	20–23	250	8.73	Hiremath & Ramaswamy, 2012
E. coli O157:H7	Fish slurry	20–25	400	3.19	Ramaswamy, Zaman, & Smith, 2008
S. aureus	Phosphate buffer	23	500	1.6	Tassou, Galiatsatou, Samaras, & Mallidis, 2007
S. aureus	Ham	23	550	2.2	Tassou et al., 2007
L. monocytogenes	BHIB	25	500	2.62	Dogan & Erkmen, 2004
L. monocytogenes	Fish slurry	20–23	350	4.16	Ramaswamy et al., 2008
S. typhimurium	Raw milk	25	300	9.21	Erkmen, 2009
Spores					
C. sporogenes	Beef	80	700	15.8	Ramaswamy et al., 2008
C. sporogenes	Milk	90	700	13.6	Shao & Ramaswamy, 2011
C. sporogenes	Sodium phosphate buffer	91	600	5.95	Koutchma, Guo, Patazca, & Parisi, 2005
B. amyloliquefaciences	Deionized water	105	700	0.4	Ahn, Balasubramaniam, & Yousef, 2007
B. stearothermophilus	Egg patty	105	400	0.72	Rajan et al., 2006
B. coagulans	Tomato juice	75	600	2.1	Daryaei and Balasubramaniam, 2013

Based on the literature, 50 MPa of pressure can inhibit protein synthesis in microorganisms and reduce the number of ribosomes. A pressure of 100 MPa can induce partial protein denaturation, and 200 MPa causes damage to the cell membrane and internal cell structure. Increasing the pressure to 300 MPa or more induces irreversible denaturation of enzymes and proteins, which causes rupturing of the cell membrane and the excretion of internal substances, resulting in bacterial death (Abe, 2007). Therefore, the effects that high pressure have on microorganisms can be categorized primarily as a change to the cell morphology, an inhibition of metabolic reactions essential for cell maintenance, and genetic mechanisms. Microorganisms possess strong environmental adaptability. Under adverse conditions, microorganisms employ various mechanisms for protection, such as adapting to the environment, changing to a dormant status (endospores), activating the regulation of stress-resistant genes, or producing adaptive mutations. Thus, the stress tolerance of microorganisms is not fixed; instead, it is affected by several internal and external factors, including the microorganism type, growth period, and environmental conditions. These factors are introduced below (Huang *et al.*, 2014).

Table 2. Inactivation of viruses by HP treatment (Ref: Laura *et al.*, 2005).

Virus	Treatment conditions	Suspending medium	Log reduction	Units	Reference
Adenovirus	400 MPa; 20 °C; 15 min	Cell culture medium	5	$TCID_{50}$	Wilkinson et al., 2001
BacteriophageΦX	300 MPa; 40 °C; 120 min	Buffer[a]	4	PFU ml^{-1}	Brauch et al., 1990
Bacteriophage λ	300 MPa; 40 °C; 120 min	Buffer[a]	5	PFU ml^{-1}	Brauch et al., 1990
Bacteriophage T4	300 MPa; 40 °C; 120 min	Buffer[a]	3	PFU ml^{-1}	Brauch et al., 1990
Bacteriophage T4	300 MPa; 30 °C; 240 min	Buffer[a]	4	PFU ml^{-1}	Carl and Ludwig, 1991
Bacteriophage T4	400 MPa; 30 °C; 240 min	Buffer[a]	4	PFU ml^{-1}	Groß and Ludwig, 1992
Bacteriophage T4	400 MPa; 4 °C; 10 min	Buffer[a]	8	PFU ml^{-1}	Groß and Ludwig, 1992
Classical swine fever virus	170 MPa; 12 h	Cell culture medium	>4	$TCID_{50}$	Freitas et al., 2003
Cytomegalovirus (Human)	400 MPa; 25 °C; 10 min	Cell culture medium	>4	PFU ml^{-1}	Nakagami et al., 1992
Feline calicivirus	275 MPa; ambient; 5 min	Cell culture medium	>6	$TCID_{50}$	Kingsley et al., 2002
Foot and mouth disease virus	240 MPa; ambient; 120 min	Buffer[b]	>4	PFU ml^{-1}	Oliveira et al., 1999
Foot and mouth disease virus	240 MPa; −15 °C; 120 min	Buffer[b]	>6	PFU ml^{-1}	Oliveira et al., 1999
Hepatitis A virus	450 MPa; ambient; 5 min	Cell culture medium	>6	PFU ml^{-1}	Kingsley et al., 2002
Hepatitis A virus	450 MPa; ambient; 5 min	Seawater	>3	PFU ml^{-1}	Kingsley et al., 2002
Herpes simplex virus type-1	400 MPa; 25 °C; 10 min	Cell culture medium	>7	PFU ml^{-1}	Nakagami et al., 1992
HIV-1 (IIIB-laboratory strain)	450 MPa; 25 °C; 10 min	Cell culture medium	>5	$TCID_{50}$	Otake et al., 1997
HIV-1 (KK-1 clinical strain)	450 MPa; 25 °C; 10 min	Cell culture medium	3.75	$TCID_{50}$	Otake et al., 1997
HIV-1 (KK-2 clinical strain)	450 MPa; 25 °C; 10 min	Cell culture medium	2.75	$TCID_{50}$	Otake et al., 1997
Immunodeficiency virus (Simian)	200 MPa; 21.5 °C; 180 min	Cell culture medium	5	$TCID_{50}$	Jurkiewicz et al., 1995
Infectious bursal disease virus	230 MPa; 0 °C; 10 min	Cell culture medium	4.5	$TCID_{50}$	Tian et al., 2000
Influenza virus	250 MPa; 37 °C; 180 min	Buffer[c]	Reduced to undetectable level	HU ml^{-1}	Gaspar et al., 2002
Mayaro virus	250 MPa; 22 °C; 480 min	Buffer[d]	6	PFU ml^{-1}	Gaspar et al., 2002
Poliovirus	600 MPa; 25 °C; 10 min		No reduction in infectivity		Nakagami et al., 1992
Poliovirus	240 MPa; ambient; 120 min	Buffer[b]	No significant reduction	PFU ml^{-1}	Oliveira et al., 1999
Poliovirus	240 MPa; −15 °C; 120 min	Buffer[b]	No significant reduction	PFU ml^{-1}	Oliveira et al., 1999
Poliovirus	600 MPa; 20 °C; 60 min	Cell culture medium	No significant reduction	$TCID_{50}$	Wilkinson et al., 2001
Poliovirus	600 MPa; ambient; 5 min	Cell culture medium	No significant reduction	PFU ml^{-1}	Kingsley et al., 2002
Rotavirus (Bovine)	250 MPa; 60 min	Cell culture medium	>5	PFU ml^{-1}	Pontes et al., 1997
Rotavirus (Human)	300 MPa; 25 °C; 2 min	Cell culture medium	8	$TCID_{50}$	Khadre and Yousef, 2002
Rotavirus (Simian)	250 MPa; 30 min	Cell culture medium	>4	PFU ml^{-1}	Pontes et al., 1997
Rotavirus (SA11-4S)	250 MPa; 60 min		>6	PFU ml^{-1}	Pontes et al., 2001
Sindbis virus	700 MPa; −2 to 20 °C; 20 min	Cell culture medium	No significant reduction	PFU ml^{-1}	Butz et al., 1992
Sindbis virus	250 MPa; 25 °C; 480 min	Buffer[e]	5	PFU ml^{-1}	Gaspar et al., 2002
Vesicular stomatitis virus	260 MPa; 20 °C; 12 h	Buffer[e]	>5	PFU ml^{-1}	Silva et al., 1992

Principles of HP processing

HP is widely used in materials science and engineering, providing existing experience in the design and use of commercial-scale equipment (Hoover *et al.*, 1989). HP technology offers food processors several advantages over conventional processing methods. For example, pressure is transmitted instantaneously and uniformly throughout a system (Marquis, 1976); consequently, in contrast to thermal processing, products are treated evenly throughout, regardless of the shape of packaging or volume of product (Knorr, 1999). From the consumer's viewpoint, the major advantage of HP technology is the production of safer foods that retain the appearance, flavour, texture and nutritional qualities of the unprocessed product (Farkas and Hoover, 2000).

When microorganisms are affected by high pressure, the cell membrane is typically the first organelle to be damaged. The primary function of the cell membrane is to maintain cell morphology, balance or mitigate differences between the internal and external pressures, and regulate substance entry and exit. When high pressure damages the cell membrane and structure, the microorganism's absorption of nutrition is affected, elimination of the waste accumulated inside the cell is hindered, and normal metabolic pathway are disrupted (Torres and Velazquez, 2005; Huang *et al.*, 2014). The breaking of covalent bonds is associated with an increase in volume, and so covalent bonds are not disrupted by HP. Hydrogen bonds also tend to be stabilised by relatively low pressures, but are disrupted by extreme pressures. The secondary structure of proteins, which is governed mainly by hydrogen bonds, is therefore only disrupted at very high pressures, leading to irreversible denaturation (Laura *et al.*, 2005; Knorr, 1999).

Some of the microorganisms' membrane proteins had deteriorated following HPP. Other studies have reported that high pressures inhibit the ATP synthesis of microorganisms. Previous studies also reported that HPP may either activate or inactivate enzymes, depending on the enzyme's inherent ability to withstand pressure stress. The enzymes responsible for synthesizing ATP fall away from the cell membrane after deactivation, reducing ATP synthesis. In addition, high pressures can also denature functional proteins and lead to a limited proton flow, reducing the intracellular pH (Huang *et al.*, 2014).

The disruption of ionic (and possibly hydrophobic) bonds is associated with decreases in volume, attributable to the presence of water in biological systems Considerable changes in the tertiary structure of proteins, which is maintained principally by hydrophobic and ionic interactions, are usually observed at pressures above 200 MPa (Laura *et al.*, 2005). Therefore, although parallels have been drawn between the effects of heat and HP, their mechanisms of action can lead to significant differences in products processed by these methods (Gudmundsson and Hafsteinsson, 2002; Laura *et al.,* 2005).

HP-induced inactivation of bacteria

It is now known that HP can damage membranes, denature enzymes and cause changes in cell morphology (Isaacs and Brooker, 1994). The bacterial cell membrane appears to be one of the targets of high-pressure treatment. Increase of pressure in the cell environment disrupts membrane permeability, which is followed by the loss of membrane integrity and swelling, and this eventually leads to cell death. The disruption of membrane integrity results in changes in the cell morphology and modification of physical characteristics of the cell. The composition of HPP-treated Salmonella typhimurium membrane protein showed an aggregated cytoplasm, indicating extensive protein denaturation (Mohamed *et al.,* 2012).

Cell membranes are thought to be a primary target for HP inactivation of bacteria andevidence for this is provided by the relationship between pressure resistance and membrane fluidity (Smelt *et al.*,

2002). Furthermore, it has been suggested that susceptibility to HP of Gram-negative compared to Gram-positive bacteria is due to the complexity of Gram-negative cell membranes (Shigehisa *et al.*,1991).

Marina *et al.*, (2013) research about L. monocytogenes suspensions in different high pressure treatments, ranging between 350 and 450 MPa at a constant temperature of 25º C. Fig. 1 depicts the effects of the different pressures applied for several minutes.

L. monocytogenessuffered 2 log cfu/mL reductions when it was treated at 350 MPa for 3 min. A longer time applied resulted in significantly greater reductions, achieving approximately 7 log cfu/ mL after 16 min of treatment.

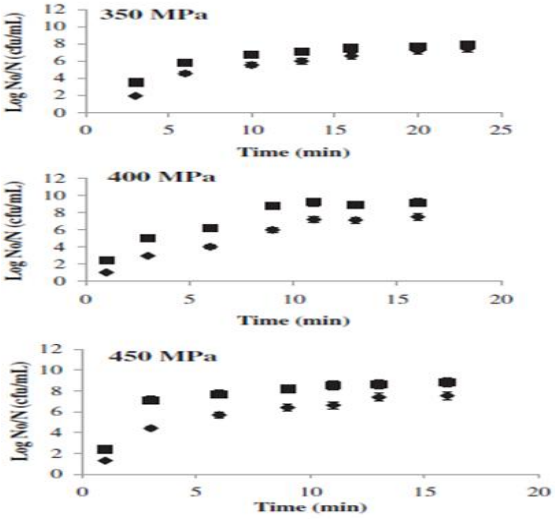

Fig. 1. Logarithmic viability reduction (log No/N) of L. monocytogenes after HHP treatments (350, 400 and 450 MPa) recovered on nonselective (♦) and selective (■) media. N_0 and N represent respectively the plate count of initial microbial load and after treatment (Ref: Marina *et al.*, 2013).

The effect of increasing pressure from 350 to 450 MPa was significant only after short exposure times and no difference in the inactivation level achieved were found at the pressures tested after 6, 13 or 16 min of treatment (Fig. 2). It is well known that microorganisms can develop adaptive responses and resistances when exposed to sublethal stresses. Furthermore Marina *et al.*, (2013) investigated the effect of NaCl concentration (0, 0.5 and 1%) and pH (5, 6 and 7), individually or in combination, in the recovery medium of cells of L. monocytogenes CECT 5672 (previously treated by HHP) was studied (Fig. 3). The lag phase increased when the environmental conditions became more severe. This trend could be confirmed for both conditions, NaCl concentration and pH. After 3 min at 350 MPa the duration of the lag phase of L monocytogenes CECT 5672 increased significantly with the decrease of the pH in the recovery medium. The effect of adding NaCl concentrationwas significant as the mean lag phase increased to 24.5 h when the NaCl concentration was 1.0% at pH 5. On the other hand, exposure to higher HP resulted in an increased level of injury, and subsequently a longer lag phase. The largest increase of lag phase was found with combinations of acid pH (pH 5) and 1% NaCl in the recovery medium, reaching lag values of approximately 50 and 100 h for cells treated at 450 Mpa for 3 and 16 min, respectively. The level of inactivation was similar in both treatments. Histograms of the lag values for different treatments are shown in Fig. 2.

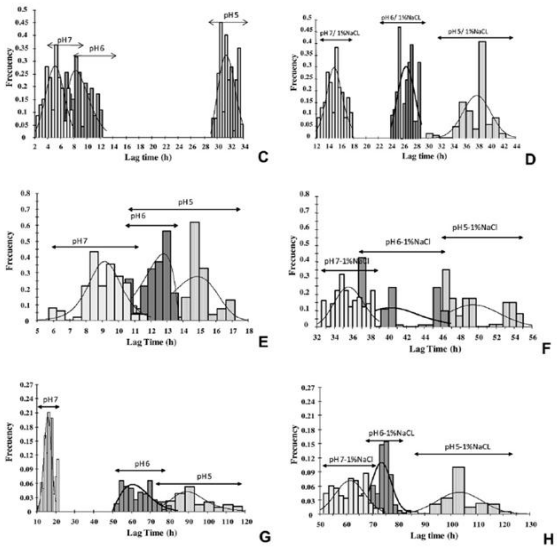

Fig. 2. Histograms of distribution of lag phase of L. monocytogenes exposed to HHP (A and B: 350 MPa/3min; C and D: 350 MPa/23 min; E and F: 450MPa/3 min; G and H: 450 MPa/ 16 min) and recovered in the conditions indicated (Ref: Marina *et al.*, 2013).

Huang, and Haiqiang. (2013) investigated the survival of Escherichia coli O157:H7 and Salmonella spp. In frozen strawberry puree and to assess the application of high pressure processing (HPP) to decontaminate strawberry puree from both pathogens. Fresh strawberry puree was inoculated with high (~6 log CFU/g) and low (~3 log CFU/g) levels of E. coli O157:H7 or Salmonella spp. and stored at −18 °C for 12 weeks. Both pathogens were able to persist for at least 4 weeks and samples with high inoculums were still positive for both pathogens after 12 weeks. Pressure treatment of 450 MPa for 2 min at 21 °C was able to eliminate both pathogens in strawberry puree. Frozen storage at −18 °C after pressure treatment substantially enhanced the inactivation of both pathogens and 4–8 days of frozen storage was able to reduce the pressure level needed for elimination of both pathogens to 250–300 MPa. Natural yeasts and molds in strawberry puree were effectively reduced by pressure of 300 MPa for 2 min at 21 °C. No adverse impacts on physical properties such as color, soluble solids content, pH and viscosity of strawberry puree was found for pressure-treated samples. Therefore, the treatment of 300 MPa for 2 min at 21 °C followed by 4 days frozen storage at−18 °C was recommended for the minimal processing of strawberry puree with great retention of fresh-like sensory properties. HPP could be a promising alternative to traditional thermal processing for berry purees.

The results of Huang, and Haiqiang. (2013) showed that both pathogens (Escherichia coli O157:H7 and Salmonella spp) were unable to grow in strawberry puree, but they were able to survive for a long period of time (Fig. 3). For both pathogens, there was a sharp decline in bacterial population in the first 3 days and a slower but steady decline of bacterial counts was observed during the following 12 weeks. The tailing effect observed in the survival curve could be due to differences in resistance to acid/frozen storage among the strains included in the cocktails. For samples with low initial inoculation level (~3 log CFU/g), E. coli O157:H7 and Salmonella were not detectable by enrichment (detection limit of 1 CFU/5 g) after 8 and 12 weeks frozen storages, respectively. However, in strawberry puree with high initial inoculation level (~6 log CFU/g), those pathogens were still viable even after 12 weeks frozen storage.

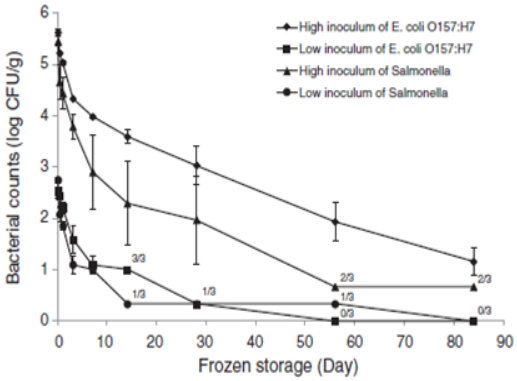

Fig. 3. Survival curves of E. coli O157:H7 and Salmonella spp. in strawberry puree during 12-week frozen storage. Strawberry puree inoculated with high and low levels of the two pathogens were stored at −18 °C. Error bars shown in figures represent one standard deviation. Enrichment was conducted when the bacterial counts were below the detection limit by the plating method (1 log CFU/g). Numbers in fraction represent the number of samples testing positive after enrichment out of a total of 3 trials (Ref:Huang and Haiqiang, 2013).

Furthermore they evaluated HPP on the inactivation of E. coli O157:H7 and Salmonella. Inoculated strawberry puree (~3 or 6 log CFU/g) was pressure treated at 200–300 MPa for 2 min at 21 °C and immediately stored at −18 °C for 0 (before frozen storage), 2, 4 or 8 days. As shown in Figs. 4 and 5, a significant decrease in the population of both pathogens was observed during the subsequent frozen storage after HPP treatment. The inactivation achieved during the frozen storage was sometimes rather substantial compared to the inactivation caused by pressure treatment alone. For example, pressure treatment at 200 MPa for 2 min only reduced E. coli O157:H7 by 1.2 log and a further 3.6 log reduction was achieved during the 8-day frozen storage (Fig. 4A). In the control sample, 8 days of frozen storage reduced E. coli O157:H7 by 2.3 log

units; therefore, an extra 1.3 log reduction of E. coli O157:H7 was achieved by the synergistic effect of HPP and subsequent frozen storage. Compared to the pressure level (450 MPa) required for elimination of ~5.5 log CFU/g E. coli O157:H7 in strawberry puree by HPP alone, only 250 MPa was needed if the pressure treatment was followed by 8 days frozen storage at −18 °C. Similarly, for samples inoculated with ~6 log CFU/g Salmonella, pressure at 350 MPa for 2 min at 21 °C was needed to get a complete kill by HPP alone. If combined with subsequent 8 days frozen storage, only 200 MPa was needed to achieve a complete elimination. Similar phenomenon was also observed for samples with low inoculation levels of E. coli O157:H7 and Salmonella. Those data indicate that a subsequent frozen storage after HPP could significantly enhance the inactivation of both pathogens in strawberry puree. Some previous studies have suggested that HPP can disrupt bacterial cell membrane and cause leakage of cytoplasm (Guerrero-Beltran *et al.,* 2005).

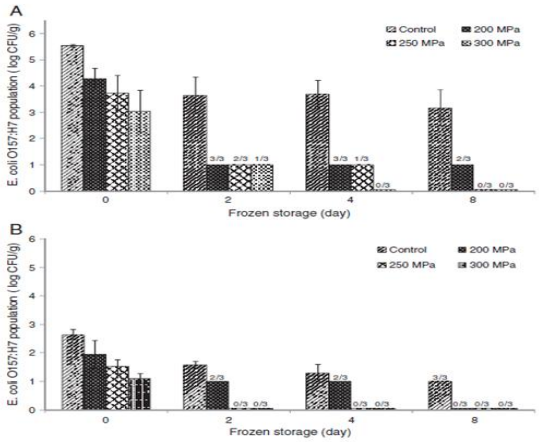

Fig. 4. Populations of E. coli O157:H7 in the control and pressure-treated strawberry puree with high (A) and low (B) inoculation levels during 8 days frozen storage. Inoculated samples were treated at 200–300 MPa for 2 min at 21 °C and then stored at −18 °C. Error bars shown in figures represent one standard deviation. Enrichment was conducted when the bacterial counts were below the detection limit by the plating method (1 log CFU/g). Numbers in fraction represent the number of samples testing positive after enrichment out of a total of 3 trials (Ref:Huang and Haiqiang, 2013).

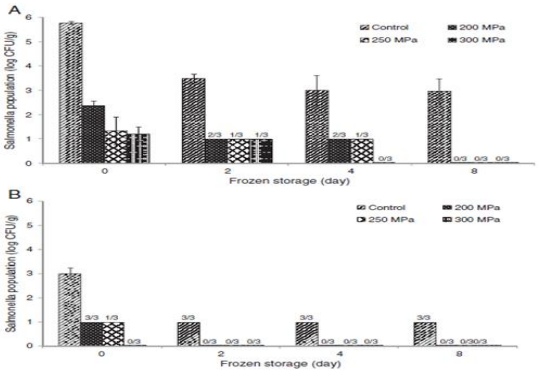

Fig. 5. Populations of Salmonella spp. in the control and pressure-treated strawberry puree with high (A) and low (B) inoculation levels during 8 days of frozen storage. Inoculated samples were treated at 200–300 MPa for 2 min at 21 °C and then stored at−18 °C. Error bars shown in figures represent one standard deviation. Enrichment was conducted when the bacterial counts were below the detection limit by the plating method (1 log CFU/g). Numbers in fraction represent the number of samples testing positive after enrichment out of a total of 3 trials (Ref:Huang and Haiqiang, 2013).

HP disrupts membrane function and causes leakage through the inner and outer membranes, as demonstrated for HP-treated cells by their increased sensitivity to sodium chloride and bile salts, uptake of propidium iodide and ethidium bromide leakage of ATP. HP can also denature or displace membrane-bound enzymes (Laura *et al*., 2005).

Myers *et al.*, (2013) evaluated growth of Listeria monocytogenes for up to 182 days after inoculation on ready-to-eat (RTE) sliced ham and turkey breast formulated with sodiumnitrite (0 or 200 ppm), sodiumchloride (1.8% or 2.4%), and treated (no treatment or 600 MPa) with high hydrostatic pressure (HHP). HHP at 600 MPa for 3 min resulted in a 3.85–4.35 log CFU/g reduction in L. monocytogenes. With formulations at similar proximate analyses, one of the evaluation days (day 21) without HHP showed significantly greater growth of L. monocytogenes in ham than in turkey breast, but there were no significant differences on other evaluation days or with HHP. There were no

differences in growth of L. monocytogenes due to sodium chloride level. Sodium nitrite provided a small, but significant inhibition of L. monocytogenes without HHP, but addition of sodium nitrite did not significantly affect growth of L. monocytogenes with use of HHP. The pooled least squares means for low salt and high salt for the 3 log inoculations are shown in Fig. 6. There was no significant difference ($p>0.05$) in L. monocytogenes for the two concentrations of salt at any of the evaluation days.

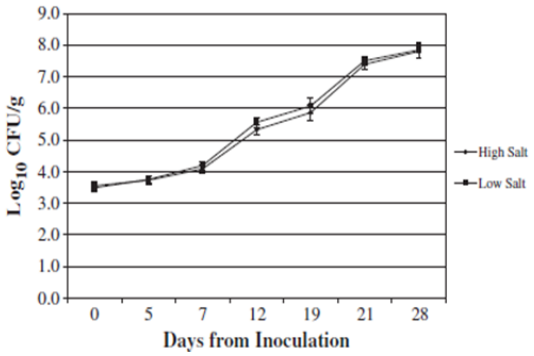

Fig. 6. Least squares means by salt level after inoculation with a 5-strain mixed culture of L. monocytogenes at a level of 103 CFU/g, and with non-HHP treatment (Ref: Myers *et al.*, 2013).

In addition, HP induces changes in morphology and internal organisation of cells, including cell lengthening, contraction of the cell wall and pore formation, separation of the cell membrane from the cell wall, and compression of gas vacuoles (Laura *et al.*, 2005). Altered distributions of DNA and ribosomes, and ribosome destruction have also been observed in HP-treated cells, and a correlation between cell death and ribosome damage has been suggested (Niven *et al.*, 1999). Although nucleic acids are more resistant than proteins to HP, condensation of nuclear material has been observed following treatment at very high pressures (Wouters *et al.*, 1998).

Kyung *et al.*, (2014) investigated the characterization of flavor, physicochemical properties and biological activities of garlic extracts prepared by high hydrostatic pressure (HHP) treatment (500 MPa) at various HHP reaction times and pH conditions. The evaluation of flavor revealed that HHP treated garlic samples in acidic condition were most effective to reduce the pungent flavor of garlic among all conditions. After HHP treatment, the hardness and color values of L*(lightness), a*(redness), and b*(yellowness) of garlic samples decreased, while the cohesiveness value of garlic samples was increased ($P < 0.05$). The antioxidative, antimicrobial and antitumor activities of HHP treated garlic samples were decreased compared with control. A rapid decrease in antimicrobial and antioxidative activities was observed over 3 min HHP reaction time. No antitumor activities were observed after 3 min HHP reaction time. Up to 56 s HHP reaction time, the alliinase activity was not changed significantly but it was dramatically decreased at a longer HHP reaction time compared with control, showing higher stability in acidic condition than alkaline condition.

There is also evidence that HP can cause degradation of bacterial DNA, due to the action of endonucleases not normally in contact with DNA (Chilton *et al.*, 1997). Many studies have shown that pressures in the range of 300–600 MPa can inactivate many fungi and vegetative bacteria (Smelt, 1998); however microorganisms can differ widely in their intrinsic susceptibility to HP. Gram-negative bacteria tend to be more sensitive to HP than Gram-positive species (Farkas and Hoover, 2000), but there are many exceptions to this generalisation, for example, certain strains of E. coli O157 are exceptionally pressure resistant (Patterson *et al.*, 1995).

Kyung *et al.*, 2014 examined changes in antimicrobial activities of HHP treated garlic samples with different HHP reaction times and pH of solutions. The HHP treatment reduced antimicrobial activities compared with control. The decrease in antimicrobial activities was found as HHP reaction time increased. In particular, the antimicrobial activities of HHP treated garlic samples were decreased over 3 min. It seems that denature of protein and irreversible cell membrane decomposition could take place with longer HHP reaction time. There was no correlation

between changes in antimicrobial activities and a difference in the pH of solutions. Kim *et al.* (2004) reported around 30% decrease of antimicrobial activities in heat treated garlic extract. Moreover, Chung *et al.* (2003) also indicated lower antimicrobial activities in heat treated garlic extract than those of control. The reducing antimicrobial effects of HHP treated garlic samples may be attributed to the release of antimicrobial substances from inner garlic or inactivation of alliinase in garlic samples.

HP-induced inactivation of viruses

HP induced dissociation of viruses may be fully reversible or irreversible, depending on the virus and treatment conditions and typically more extreme treatments lead to irreversible changes in virus conformation (Gaspar *et al.,* 1997). Viruses are a structurally diverse group of organisms that also differ widely in their sensitivities to HP (Table 2). For example, feline calicivirus (a norovirus surrogate) is inactivated by treatment at 275 MPa for 5 min. In contrast, poliovirus is very resistant to HP, with no significant reductions in infectivity reported after relatively severe treatments, such as 600 MPa at 20 - C for 60 min (Kingsley *et al.*, 2002; Wilkinson *et al.*, 2001). The resistance of poliovirus may be related to the size and shape of the virus particle or its high thermodynamic stability (Wilkinson *et al.,* 2001). The HP-induced conformational changes in membrane spike proteins of influenza and Sindbis viruses are also similar to receptoractivated changes involved in the normal binding process, except that in the case of HP-induced changes viruses become trapped in a Ffusion intermediate state (Gaspar *et al.,* 2002; Silva *et al.,* 2002). The reduced infectivity of picornaviruses following HP-treatment is caused by the loss of a receptor-binding protein (VP4) and/or small "pocket factors" from the capsid, forming P-particles (Silva *et al.*, 2002). HP causes little or no disruption to rotavirus capsids, and reduced infectivity is attributed to a loss of functional integrity of hemagglutinin (VP4), which forms spikes in the outer shell and is involved in receptor recognition (Pontes *et al.*, 2001). HP-inactivated virus particles retain many of the physical and chemical characteristics of the native virus, and remain highly immunogenic (Pontes *et al.*, 2001). Treatment at pressures above 300 MPa damages the envelope of human immunodeficiency virus (HIV) and cytomegalovirus and prevents the binding of virus particles to cells (Laura *et al.*, 2005).

Sindbis and influenza viruses also do not dissociate under pressures that inactivate them, and loss of infectivity is again credited to conformational changes in spike proteins (Gaspar *et al.*, 2002). Likewise, it has been proposed that HP induced inactivation of vesicular stomatitis virus is due to a conformational change in G protein, which although allows attachment with a host cell to occur, prevents internalisation and migration to the nucleus (Da Poian *et al.,*1996), and hepatitis A virus, may be inactivated by the denaturation of capsid proteins associated with receptor attachment, penetration or virus uncoating mechanisms (Kingsley *et al.*, 2002).

Conclusions

High pressure can inhibit the growth of pathogens and maintain a level of food quality and freshness similar to that of minimal processing. HP is an emerging technique in many countries. All new processing techniques must conform to food safety standards before commercial application. When applying HP, many parameters conditions such as pressure, temperature, time, and pH value must be considered. These parameters need to be set to a range that can effectively control microorganism safety risks. Using High Hydrostatic Pressure in cells of L. monocytogenes showed that surviving cells are damaged. E. coli O157:H7 and Salmonella were able to persist in frozen strawberry puree for a long period of time. High pressure processing of strawberry puree at 200–500 MPa for 2 min at 21 °C brought about different levels of inactivation of pathogens depending on the pressure level. The use of short term post-pressure frozen storage was able to effectively lower the pressure level to 250–300 MPa

for elimination of both pathogens in strawberry puree. HHP is effective for reducing the number of L.monocytogenes on ready-to-eat processed meat products.

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