5-2009

Use of high pressure homogenization in bacterial inactivation

Panchalee Pathanibul
University of Tennessee

To the Graduate Council:

I am submitting herewith a thesis written by Panchalee Pathanibul entitled "Use of high pressure homogenization in bacterial inactivation." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

P. Michael Davidson, Major Professor

We have read this thesis and recommend its acceptance:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Panchalee Pathanibul entitled "Use of High Pressure Homogenization in Bacterial Inactivation." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

P. Michael Davidson
Major Professor

We have read this thesis and recommend its acceptance:

Federico M. Harte

Arnold M. Saxton

Accepted for the Council:

Carolyn R. Hodges
Vice Provost and Dean of the
Graduate School

(Original signatures are on file with official student records.)

Use of High Pressure Homogenization in Bacterial Inactivation

A Thesis Presented for the
Master of Science Degree
University of Tennessee, Knoxville

Panchalee Pathanibul
May 2009

Acknowledgements

I would like to thank my major professor, Dr. P. Michael Davidson, for giving me the opportunity to pursue my M.S. degree in Department of Food Science and Technology, University of Tennessee. I would also like to thank my committees, Dr. Federico M. Harte and Dr. Arnold M. Saxton, for their guidance in completing this thesis. I am grateful for Dr. Federico M. Harte for his encouragement and assistance in accomplishing my first published paper and presenting a poster at an IAFP conference. I extend my appreciation to Dr. Williams C. Morris for his support, encouragement and guidance.

I would like to thank all lovely friends in Food Science department for their encouragement and friendship, especially, Helen Kerr, Adrienne Roach, and Vyku Ganesan for their help with my experiments. I would not have gone through these things without them. I would also like to thank my Thai friends, Koi, P'O, P'Nuch, P'Taal, Fern, for their support in everything and being with me when I need someone.

Last, but not least, I would like to thank my family in Thailand. Their love and care mean so much to me. I really appreciate everything they have done for me. They are always there beside me throughout the good and bad times.

Abstract

High pressure homogenization has been of growing interest as a nonthermal technology for the inactivation of microorganisms in fruit and vegetable juices. Cells of *Escherichia coli* and *Listeria innocua*, used as surrogates for foodborne pathogens, were inoculated into apple or carrot juice (~7 $\log_{10}$ CFU/ml) containing 0 or 10 IU/ml nisin and subjected to 350 to 0 MPa high pressure homogenization. At 50 MPa homogenization pressure intervals, juice samples were collected, immediately cooled to <10^{o}C, and then serially diluted and plated on nonselective recovery media. Following incubation, survivors were enumerated. As processing pressure increased, inactivation of *E. coli* increased, and a >5 log reduction of cells was achieved following exposure to pressures in excess >250 MPa. In contrast, little inactivation was observed for *L. innocua* with pressure <250 MPa and up to 350 MPa processing pressure was required to achieve an equivalent 5 log inactivation. The addition of 10 IU nisin, together with high pressure homogenization, did not exhibit significant additional *E. coli* inactivation, but interactions were observed with *L. innocua*. Results indicate that high pressure homogenization processing is a promising technology to achieve pathogen decontamination in fruit and vegetable juices.

Table of Contents

List of Figures

1. Literature review

1.1 *Escherichia coli* O157:H7 and *Listeria monocytogenes* as foodborne pathogens of concern

Foodborne diseases have continuously been a serious problem to public health all over the world. Both industrialized and developing countries are encountering large numbers of illnesses every year. Most foodborne illnesses are relatively mild, and are associated with gastrointestinal symptoms such as diarrhea and vomiting in which these illnesses can recover in a short period of time. However, foodborne diseases can sometimes be severe and life-threatening. In the United States, the occurrence of foodborne diseases causes up to 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year (Mead et al., 1999). *Campylobacter* and *Salmonella* are most commonly reported as bacterial pathogens causing foodborne illnesses in the United States (Mead et al., 1999).

Recently, greater awareness of food safety and changes in regulations and practices in food production had led to reduction in the incidence of particular foodborne diseases in some regions (Blackburn and McClure, 2002). In the UK, reported cases of salmonellosis were reduced by 54% in 2000 compared with the previous year. Similarly, a reduction in reported cases of salmonellosis was observed in the United States (Olsen et al., 2001). In 2004, surveillance data indicated an overall subsidence in the incidence of infections caused by foodborne pathogens such as *Campylobacter*, *Yersinia*, *Salmonella* and *Listeria* (Anonymous, 2005). However, many cases of the foodborne illnesses are sporadic and unreported, and therefore may not be accounted for in surveillance systems.

Escherichia coli O157:H7 and *Listeria monocytogenes* are two foodborne pathogens that have also been implicated in many foodborne diseases and outbreaks (Table 1). Both of them have been classified as emerging pathogens which can be defined as those pathogens that have increased in prevalence in recent decades, or are likely to do so in the near future (Altekruse and Swerdlow, 1996). Contamination of foods by these microorganisms followed by inadequate cooking and/or inappropriate food preparation could lead foodborne illness. That, in turn, may lead to financial losses through medical costs, recalls and/or lawsuits in addition to loss of reputation for food processors.

Table 1. Some incidents of foodborne outbreaks caused by *E. coli* O157:H7 and *L. monocytogenes* (Ciesielski et al., 1987; Blackburn and McClure, 2002; Strachan et al., 2006; Gandhia and Chikindas, 2007).

Foodborne pathogen	Food associated	Year	Place	Illnesses
Escherichia coli O157:H7	beefburgers	1992	UK	8 people
	hamburger	1992	Washington	501 cases of infections, 3 deaths
	burgers	1994	Fife	22 people
	lettuce	1996	Connecticut and Illinois	>61 cases of infections
Listeria monocytogenes	coleslaw	1981	Nova Scotia	34 perinatal cases and 7 adult infections
	pasteurized milk	1983	Massachusetts	49 cases of infections
	mexican-style cheese	1985	Southern California	93 perinatal cases
	turkey deli meat	2002	8 states in USA	46 cases of infections, 7 deaths

1.1.1 *Escherichia coli* characteristics

Escherichia coli was discovered and isolated from infant stools by Theodor Escherich in 1885. Genus *Escherichia* is described as Gram-negative facultative anaerobic non-sporing rods which are motile by peritrichous flagella. All species ferment glucose with the formation of acid or of acid and gas, are capable of reducing nitrates to nitrites, are oxidase negative, and catalase positive. *E. coli* are commonly found in the lower intestine of humans and animals. However, some strains may occur in other parts of the body, on plants, or in soil (Wilson and Miles, 1964).

E. coli is genetically related to some other genera of the *Enterobacteriaceae* and, based upon DNA homology, *E. coli* and the four species of the genus *Shigella* may be considered a single species (Jones, 1988). The first serogrouping scheme developed for *E. coli* divided the species into more than 170 different serogroups based on their somatic (O) antigens (Kauffmann, 1947). Subsequently, over 50 flagellar (H) antigens and around 100 capsular (K) antigens were also recognized. Subdivision of *E. coli* into serotypes can be performed using this information.

1.1.1.1 Gastroenteritis caused by *Escherichia coli*

E. coli was first recognized as a foodborne pathogen in 1971 when outbreaks of illness associated with consumption of imported cheese took place in 14 states in the United States and nearly 400 people became ill (Jay et al., 2005). Based on disease syndromes, different types of pathogenicity, and serological groupings, *E. coli* can be categorized into five virulence groups including enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC). Different virulence factors are expressed by the various groups including colonization factors, ability to invade

epithelial cells of small intestine, hemolysin production, and toxin production (Blackburn and McClure, 2002). A brief summary regarding the pathogenicity of different serogroups, types of diseases, and characteristics of the illnesses associated with each group of *E. coli* is displayed in Table 2.

1.1.1.2 Non-pathogenic strain: *Escherichia coli* K-12

Escherichia coli strain K-12 was originally isolated in 1922 (Riley et al., 2006). Since strain K-12 is non-pathogenic and easy to cultivate, it has been used in laboratories throughout the world. *E. coli* K-12 is the primary model organism for basic biology, molecular genetics, and physiology of bacteria (Riley et al., 2006). It is often used in studies as a surrogate for the foodborne and waterborne pathogenic strain *E. coli* O157:H7. Although strain K-12 and O157:H7 share the same species name, a genomic study on the complete DNA sequence of both strains indicated that they are not that similar. Strain K-12 contains 4.64 x 10^6 base pairs whereas the pathogenic strain O157:H7 contains 5.44 x 10^6 base pairs. *E. coli* K-12 has 528 genes that are not found in O157:H7 and O157:H7 has 1387 genes not present in K-12. This finding suggested that these two strains of the same species differ in some 25% of their genes.

1.1.2 Listeria monocytogenes characteristics

Listeria species are Gram-positive facultatively anaerobic non-spore forming rods. They are catalase-positive and produce lactic acid from glucose and other fermentable sugars (Jay et al., 2005). Currently, six clearly distinguishable species of *Listeria* are recognized including *L. monocytogenes, L. innocua, L. welshimeri,*

Table 2. Pathogenicity and characteristics of foodborne illnesses caused by each virulence group of pathogenic *E. coli*. Adapted from Bell and Kyriakides (1998) and Willshaw et al. (2000).

Pathogenic type of *E. coli*	Examples of serogroups	*E. coli*/host interaction	Symptoms
EPEC (enteropathogenic)	O18ab, O18ac, O26, O44, O55, O86, O114, O119, O125, O126, O127	EPEC attach to intestinal mucosal cells causing cell structure alterations and invade the mucosal cells	Severe diarrhea in infants, fever, vomiting, abdominal pain. In adults, severe watery diarrhea with a lot of mucus without blood, nausea, vomiting, fever, abdominal cramps
ETEC (enterotoxigenic)	O6, O15, O25, O27, O63, O78, O115, O148	ETEC adhere to the small intestinal mucosa and produce toxins that act on the mucosal cells	Watery diarrhea, low-grade fever, abdominal cramps, malaise, nausea. If severe, cholera-like diarrhea with rice water-like stools, leading to hydration
EIEC (enteroinvasive)	O28ac, O29, O112ac, O121, O124, O135, O144, O152	EIEC invade cells in the colon and spread laterally, cell to cell	Profuse diarrhea or dysentery, chills, fever, headache, muscular pain, abdominal cramps
VTEC (EHEC) (Vero-cytotoxigenic) (Entero-haemorrhagic)	O2, O4, O5, O6, O15, O18, O22, O23, O26, O55, O75, O91, O103, O104, O105, O111, O153, O157	EHEC attach to and efface mucosal cells and produce toxin	Hemorrhagic colitis: sudden onset of severe crampy abdominal pain, bloody diarrhea, vomiting Hemolytic uremic syndrome (HUS): bloody diarrhea, acute renal failure in children, thrombocytopenia, acute nephropathy, seizures, coma, death
EAEC (enteroaggregative)	O3, O44, O51, O77, O86, O99, O111, O126	EAEC bind in clumps to cells of the small intestine and produce toxins	Persistent diarrhea in children. Occasionally bloody diarrhea or secretory diarrhea, vomiting, dehydration

L. seeligeri, L. ivanovii, and *L. grayi.* The most commonly occurring species in foods are *L. innocua* and *L. monocytogenes* (Kozak et al., 1996).

L. monocytogenes is recognized as the primary human pathogen of the genus *Listeria*. It is widely distributed in the environment and occurs in almost all food raw materials from time to time (Blackburn and McClure, 2002). The exact factors regarding *Listeria* infection are still not totally understood. However, they involve host immunity, inoculum level, and virulence factors, including hemolysin production in *L. monocytogenes* strains (Blackburn and McClure, 2002).

1.1.2.1 Listeriosis

Listeria monocytogenes has been recognized as a foodborne pathogen since the early 1980s when outbreaks of foodborne listeriosis occurred with exceptionally high levels of mortality (Blackburn and McClure, 2002). When a healthy individual is infected from contaminated food they may have only a mild illness. This depends upon the dose of *L. monocytogenes* consumed. If food containing high levels ($>10^7$ viable cells per gram) of *L. monocytogenes* is consumed, persons may develop symptoms of vomiting and diarrhea which are generally self-resolving. These symptoms may progress to bacteremia in more severe cases (Bell and Kyriakides, 1998). In susceptible individuals, symptoms of listeriosis vary according to the type of infection and may involve the uterus, the bloodstream, or the central nervous system. Persons exceptionally vulnerable to listeriosis include fetuses, the elderly, and the immuno-compromised. In pregnant women, listeriosis can lead to spontaneous abortion, stillbirth, or birth of an infected newborn. Surprisingly, the mother does not usually get a severe infection with listeriosis as the disease seems to mainly focus on the fetus (Rocourt, 1996). Adults, particularly the elderly and the immuno-

compromised, may also contract listeriosis that often results in meningitis and/or septicemia.

1.1.2.2 Non-pathogenic strain: *Listeria innocua*

Listeria innocua is a non-pathogenic strain of the genus *Listeria*. It is widely distributed throughout the environment. However, its primary habitat is considered to be soil and decaying matter. *L. innocua* can survive at extreme pH and temperature, and in high salt concentration. Genetically, foodborne *L. innocua* is closely related to *L. monocytogenes*. *L. innocua* was found to be deficient in a 10-kb virulence locus, a cluster of genes that engenders pathogenicity to *L. monocytogenes* (Buchrieser et al., 2003).

Since *L. innocua* is closely related to *L. monocytogenes* and safe to handle, it is often used as a surrogate of *L. monocytogenes* in laboratory work. Moreover, it has been employed in comparative genome studies to investigate more about the virulence of *L. monocytogenes* and the evolution of genes within the genus *Listeria* (Chakraborty et al., 2000, Glaser et al, 2001; Buchrieser et al., 2003; Hain et al., 2006).

1.2 Fruit juices as foods susceptible to microbial contamination

Fruit juices contain sugars and other nutrients and have a high water activity making them a favorable environment for survival and or growth of many microorganisms. One unfavorable aspect for microorganisms in fruit juices is their low pH. However, some strains of *Escherichia coli*, including O157:H7, are resistant to acid, and can survive for long periods in acid foods, especially at low temperature (Glass et al., 1992; Miller and Kaspar, 1994; Jordan et al., 2001). *Listeria* are not

known to have been associated with foodborne disease via consumption of fruit juices, but they have been isolated from unpasteurized apple juice (Sado et al., 1998). The process of microbial contamination of juices begins during pressing or extraction of the whole fruit. Contamination comes from the natural microflora, exposure of juice to air and dust and cross-contamination from multiple sources such as equipment or personnel.

There have been a number of reports concerning the contamination of natural juices by pathogenic bacteria or parasites resulting in foodborne illness outbreaks. *E. coli* O157:H7, *Salmonella* Typhimurium (and other serotypes), *Cryptosporidium parvum*, and *Clostridium botulinum* have been associated with outbreaks in apple cider, orange juice, apple juice, and carrot juice, respectively (Parish, 1997; Cook et al., 1998; CDC, 1999; Cody et al., 1999; Krause et al., 2001). Most of the outbreaks occurred due to consumption of contaminated fresh unprocessed juices. This type of outbreak has increased as the trend for consumers to demand "fresh" and "natural" foods has increased. These microorganisms could be eliminated by pasteurization but heating changes the quality characteristics of juices. In an attempt to respond to the demands of consumers, newly developed juice processing techniques that do not involve heat have been developed in an attempt to produce juice with more "natural" characteristics and, at the same time, maintain microbiological still safety.

1.2.1 Current HACCP 5-log reduction requirement for juice processing (Adapted from FDA, 2001)

Juice processors HACCP plans are required to include the application of a process that results in a 5-log reduction of the most important or pertinent pathogen microorganism in their product (FDA, 2001). The pertinent microorganism is defined

as the microorganism that shows the most resistance to a particular processing technique and a specific type of juice. For instance, *Cryptosporidium parvum* has exhibited the highest resistance to heat in an apple cider pasteurization process. Therefore, *Cryptosporidium parvum* would be the pertinent microorganism of concern. However, if apple cider were processed with an alternative process, for example, high pressure, the pertinent microorganism may not necessarily the same as that processed with heat.

Manufacturers subject to the 5-log reduction performance standard must achieve 5-log reduction of the pertinent pathogen by treatment of the extracted juice. Application of the reduction standard must be done under good manufacturing practices (GMP) in a single production facility (place where the juice product is packaged into its final form prior to sale) and could be a single treatment or combination of treatments. Manufacturers must validate the process they use for the 5-log microbial decontamination. Validation involves end-product testing for appropriate process verification. Juice processors using cumulative surface treatments of fruits including cleaning, brush washing, and sanitizing steps must include a number of validation and monitoring steps which are to be documented in their HACCP plan. Aspects that must be addressed in the validation plans include concentration of the sanitizer chemicals, pH, temperature, and exposure time of the treatment(s). If the process to reach the 5-log performance standard is found to be inadequate after the verification process is complete, corrective actions must be performed accordingly. The validation process must also be repeated at least once a year to maintain records, and certify that the company is following the procedure and monitoring as required by the HACCP plan.

1.3 Control measures for pathogens in juices

There are numerous currently available methods that can be used for disinfecting juices of microbial foodborne pathogens. Among those, heat pasteurization seems to be the most common and well-studied process. Other novel food protection methods are being studied and are becoming more popular. For example, ultraviolet irradiation, ozone, high hydrostatic pressure, pulsed light, and carbon dioxide in combination with high pressure are all possible inactivation methods for foodborne pathogens (Morris et al., 2007). UV radiation and ozone are the two methods that were approved for use on foods by the US FDA in 2000 and 2001, respectively (Luck and Jager, 1995). Most other processes are still being developed and optimized to obtain a desirable level of microbial destruction while maintaining juice quality.

1.3.1 Non-thermal processing of foods for microbial inactivation

Heat processing of foods is one of the most common food preservation techniques. It is an efficient and economical process for inactivating pathogenic and spoilage microorganisms in all foods, including juices. However, one concern associated with the use of heat is that it often causes undesirable product attributes, such as development of off-flavors and off-odors, and loss of nutrients (Diels and Michiels, 2006). Therefore, over the 15-20 years, food processing technologies that inactivate microorganisms but do not utilize significant input of heat have been developed and optimized. These “nonthermal” technologies involve no heat or merely mild heat in food processing but still provide a satisfactory reduction of microbial load. Nonthermal techniques are often more energy efficient than the conventional thermal processes (Morris et al., 2007). Moreover, nonthermal processing of foods

results in retention of many product nutrients, quality attributes, and physiochemical properties.

1.3.1.1 Microbial inactivation by high pressure

High pressure processing is one of the nonthermal methods that has been successfully employed for microbial inactivation purposes. It is an attractive technology that can reduce the microbial load and prolong the shelflife of food while minimally impacting the nutritional and sensory properties of food.

There are generally two types of high pressure processing that are currently being studied and used, high hydrostatic pressure (HHP) and high pressure homogenization (HPH). The first type of treatment, HHP, is more common. When "high pressure" is mentioned in books or scientific journals it often refers to HHP. HHP was discovered more than a century ago. The term "hydrostatic" refers to the use of water (or other liquid) applied as a pressure transmitting medium to uniformly transfer high pressure to the food. Products to be treated by HHP can be either solid or liquid and can be packaged or not. Also, HHP can operate as a batch, semi-batch, or even continuous process, but continuous process is only applicable for liquid (Morris et al., 2007).

High pressure homogenization or HPH was developed after HHP from a conventional homogenizer that was primarily used as a machine to process dairy products. Its operation substantially differs from HHP. A liquid food is filled into the machine, and is then forced through a small orifice under pressure. The product type that is suitable for HPH is liquid and unpackaged. HPH is generally a continuous process but batch processing is also possible.

1.3.1.2 Development of homogenizing equipment

The traditional homogenizer was initially invented in 1900 by Auguste Gaulin, and was introduced into the food industry mainly for use in the production of dairy products and food emulsions to improve texture, taste, flavor, and shelflife characteristics (Diels and Michiels, 2006). Its primary use has been to break up fat globules in milk products to reduce the "creaming" effect. To serve the needs and respond to consumer demands for longer shelflife and products with better stability, a new generation of homogenizers with pressures 10 to 15 times higher than conventional homogenizers was developed in the early 1990s (Burgaud et al., 1990). The introduction of high pressure homogenizers expanded possibilities for new applications and product enhancement that could not be performed with lower pressure operations.

HPH is currently used in the cosmetic, pharmaceutical, chemical, and food industries, for preparation or stabilization of emulsions and suspensions. Another application is cell disruption of yeasts or bacteria in order to release intracellular products such as recombinant proteins (Pandolf, 1998; Paquin, 1999). Application of HPH in cell disruption inspired the initiation of use for microbial inactivation purposes. It was believed that HPH could also cause partial inactivation of the microorganisms while processing for emulsions or suspensions (Popper and Knorr, 1990; Lanciotti et al., 1994, 1996). Although microbial load reduction is not the main purpose of the process, it may result in an extended shelf life and improve the microbiological safety of the processed products. Consequently, application of HPH in food processing may reduce the need for other process steps that are designed to inactivate microorganisms, such as the use of antimicrobial additives, sanitizers or

heat, or decrease the intensity of such process steps which may also influence product quality and process cost in a beneficial way.

1.3.1.3 High pressure homogenization operation

Basically, a homogenizer consists of a positive displacement pump and a homogenizing valve. The pump is used to force the fluid into the homogenizing valve. The fluid under pressure is forced through a small orifice between the valve and the valve seat (Figure 1). The operating pressure is controlled by adjusting the distance between the valve and the seat or by the amount of fluid displaced by the pump. The product leaves the homogenizer at high velocity and atmospheric pressure, and is then chilled to minimize thermal damage caused by heat of friction which is generated due to high fluid velocity, increasing the product temperature by about 2-2.5˚C per 10 MPa (Engler and Asenjo, 1990; Popper and Knorr, 1990).

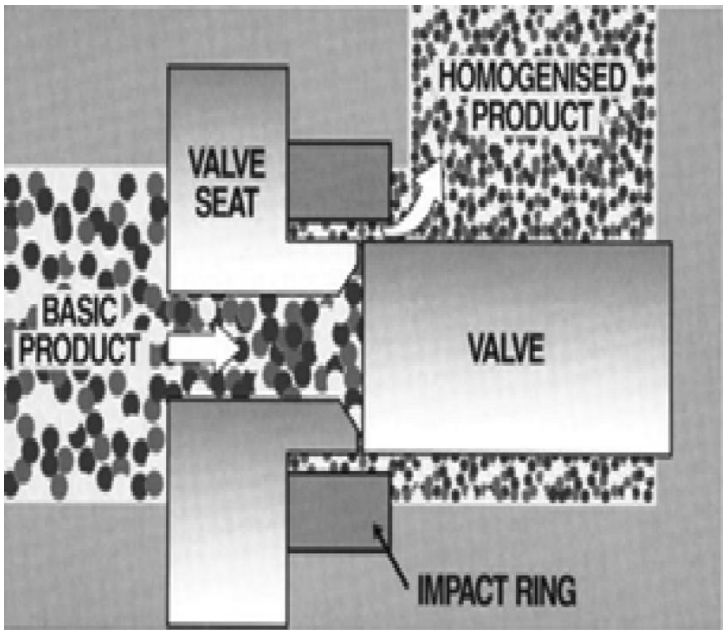

Figure 1. section of the homogenizer (Diels and Michiels, 2006).

1.3.1.4 Factors involved in microbial inactivation by high pressure homogenization

Factors related to the extent of microbial decontamination by HPH include process parameters, microbial parameters, and fluid-related parameters.

1) Process parameters

Process parameters that influence microbial inactivation include the maximum pressure achieved, temperature, number of passes through the HPH, and HPH valve geometry. An increase of process pressure results in an increase of microbial inactivation. Previous studies have found consistent results regarding this increasing inactivation with increasing pressure, but different types of relationships have been reported (Diels and Michiels, 2006). A study on the disintegration of *Saccharomyces cerevisiae* by Brookman (1974) found an exponential increasing rate of inactivation. Kelemen and Sharpe (1979) on the other hand found a sigmoidal increase of the percentage of disrupted cells with the applied pressure. Lanciotti et al. (1994) concluded that the relationship between the number of surviving cells and the process pressure applied in milk was linear.

Temperature also affects microbial inactivation by HPH. The level of inactivation increases with increasing process temperature. Vachon et al. (2002) explained that temperature changes the physical properties of cell membrane. It is likely that elevated temperature reduces the flexibility of the cell membrane so the cell becomes more susceptible to high pressure at high temperature.

The number of rounds or passes that the sample goes through HPH also affects the level of inactivation. Previous studies have shown that the enhanced microbial inactivation can be achieved with increasing number of rounds (Sauer et al., 1989; Baldwin and Robinson, 1994; Wuytack et al., 2002).

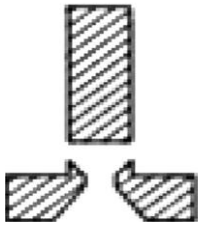

Figure 2. Knife edge valve design which was believed by most researchers to be the most effective type of valve (Diels and Michiels, 2006).

Homogenizing valve design is also understood to be important in order to get high level of microbial reduction. Changes in homogenizing valve geometry can cause a significant increase in microbial inactivation at the same pressure. Several studies have demonstrated that valves that have a simple flow path like knife edge valve (Figure 2) were found to be the most effective. However, the efficiency of the valve also depends on the sharpness of valve edge (Hetherington et al., 1971; Keshavarz-Moore et al., 1990).

2) Microbial parameters

Among various types of microorganisms, bacteria have been the most studied. From several experiments done in bacteria, it can be concluded that gram-positive bacteria are more resistant to HPH than gram-negative bacteria (Harrison et al., 1991; Lengler et al., 1999; Madigan et al., 2000). The reason behind the difference between two types of bacteria can be explained by the structure of bacterial cell wall. The cell wall of gram-positive bacteria has thicker peptidoglycan layer than that of gram-negative bacteria, and this appears to contribute to greater structural resistance to mechanical breakage by HPH in gram-positive bacteria. Bacterial spores are usually known to be resistant to most of food preservation techniques. Similar results were also obtained when subjected bacterial spores to HPH. Among few studies in spores with HPH, gram-positive spores of *Bacillus* spp. and *Clostridium* spp. were found to be very resistant to HPH (Popper and Knorr, 1990). There was a study trying to

destroy *Bacillus licheniformis* spores by HPH. Only 0.5 $\log_{10}$ CFU/ml was achieved at pressure 200 MPa (Feijoo et al., 1997). The most investigated yeasts are *Saccharomyces cerevisiae* and *Candida utilis*. Previous experiments have shown that *C. utilis* is less sensitive to HPH than *S. cerevisiae*, because *C. utilis* has larger cell size and different cell wall structure (Engler and Robinson, 1981). However, yeasts are generally less resistant to HPH than bacteria (Geciova et al., 2002). Currently, there are few studies on resistance of viruses to HPH. A study on hepatitis A virus displayed the resistance of the virus to HPH since 5 passes of 300 MPa were needed to reach more than 1 log unit inactivation (Jean et al., 2001). Another study on bacteriophages, the inactivation of bacteriophage could be explained by breaking of phage heads by HPH that made its genetic material lost (Moroni et al., 2002).

Most studies report that cell concentration or initial microbial load has no significant influence on cell disruption efficiency (Hetherington et al., 1971; Agerkvist and Enfors, 1990; Harrison et al., 1991). Nevertheless, Vachon et al (2002) found that highest degree of inactivation was gained with the lowest initial load when compared over a wide range of cell concentrations. Therefore, further investigation may be needed to clarify concerning this factor.

Growth phase of a microorganism can influence sensitivity to HPH. Previous studies have indicated the increased sensitivity of cell in exponential phase to HPH while stationary phase cell was found to be harder to kill by HPH. It was explained that cell in exponential phase grows rapidly, and extending the cell wall during cellular growth results in weakened areas that makes the cell becomes more susceptible to HPH (Harrison et al., 1991).

3) Fluid-related parameters

According to previous experiments, microbial destruction by HPH was inversely associated with the initial fluid viscosity. In other words, the inactivation of microbial cell decreases with increasing relative fluid viscosity.

The type of fluid in which microorganisms are suspended may influence inactivation. Vachon et al. (2002) compared inactivation of *L. monocytogenes* and *E. coli* O157:H7 in phosphate buffer and in milk. Results clearly revealed that more inactivation occurred in buffer than milk. Jean et al. (2001) compared HPH inactivation of hepatitis A virus in milk and apple juice. Results demonstrated greater reduction of hepatitis A virus in juice. This led to a subsequent study on *L. innocua* inactivation in milk with different fat contents (Kheadr et al., 2002). As expected, more inactivation of *L. innocua* was seen in skim milk than in full fat milk. All researchers attributed the higher resistance to the protective effect of milk fat on microorganisms (MacDonald and Sutherland, 1993; Garcia-Graells et al., 1999).

It is widely believed that microbial cell wall is the main target of HPH. Therefore, any antimicrobial additive or type of treatment that weakens the cell wall may increase the microbial sensitivity to HPH. A synergistic interaction between additives and HPH was discovered in HPH experiments where sodium dodecyl sulfate (SDS), EDTA, lysozyme, zymolyase (lytic enzyme), and nisin were used (Harrison et al., 1991). However, the synergistic effect of lysozyme and nisin were observed at only high pressures in some studies (Diels et al., 2005b). A study in milk by Zapico et al. (1999) found that HPH caused the loss of nisin antimicrobial activity. Hence, more investigation may be needed to better explain the association of additives and HPH.

1.3.1.5 Proposed mechanisms of microbial inactivation by high pressure homogenization

There have been numerous mechanisms proposed to describe cell disruption by HPH. However, the exact mechanism for the destructive effect of HPH on microbial cells remains under debate. Brookman (1975) suggested that the rate of the pressure drop near the entrance of the homogenizer influenced microbial inactivation. Later in the same year, Doulah et al. (1975) contradicted Brookman's hypothesis and proposed that turbulence was the most important parameter for cell disruption. He stated that as liquid flowed through the homogenizing valve, the applied compression energy was converted into two types of energy: kinetic energy and friction energy. He further explained that the majority of the compression energy altered the kinetic energy and only a small part of the compression energy changed to friction energy. He suggested that friction energy brought about the temperature rise but did not assist in cell disruption process (Figure 3). Contrary to Doulah hypothesis, recent researches and studies have shown that the increase of temperature in the HPH process does play a role in microbial inactivation by HPH (Diels and Michiels, 2006; Taylor et al., 2007).

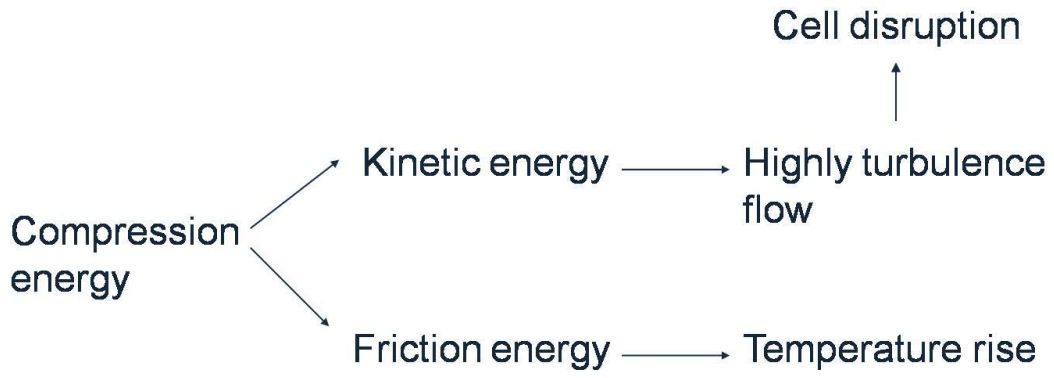

Figure 3. Doulah hypothesis diagram

Doulah believed that the kinetic energy created a highly turbulent flow which resulted in velocity fluctuations in liquid. As a result of these fluctuations, cells would be confronted with motions of various scales and intensities. When the kinetic energy of these motions exceeds the cell wall strength, the cell would be disrupted.

Engler and Robinson rejected these hypotheses in 1981, and proposed that impingement of a high velocity jet of suspended cells on a stationary surface results in effective disruption of cell walls by HPH. More recently in 1994, Save et al. (1994) suggested that cavitation, which is the process of gas cavity growth and collapse in liquid, and shock waves/pressure impulses that are produced as a result of cavity collapse are responsible for cell disruption. They indicated that cavitation conditions must exist for efficient cell disruption. The generation of free radicals owing to cavitation may also play a role in cell destruction by HPH.

Shamlou et al. (1995) proposed that cell stress is the reason for the death of the microorganisms caused by HPH. He justified that cells experience a lot of stress when it travels through the homogenizer. When the stress surpasses the mechanical cell wall resistance, the cell is destroyed.

Most recent in 2000, Lander et al. studied the mechanisms of microbial inactivation by HPH through using high molecular weight polysaccharides. They used this polysaccharide as a model compound instead of the microbial cells in their study, and found that the breakage of polysaccharide is primarily occurred by fluid shear.

1.3.2 Antimicrobial food additives

There are plenty of antimicrobial compounds available nowadays that are currently employed by food processors in order to prolong the shelf life of food and make food safe for consumers. Addition of food additives is one of chemical food

preservation techniques. These techniques do not necessarily kill the microorganisms, but they are at least capable to inhibit or prevent the growth of the microorganisms so as to reduce the rate of food spoilage (Luck and Jager, 1995). Food preservatives have been used since prehistoric times (e.g., salt). Advancement in chemistry and commencement of industrialization brought about innumerable newly innovated food preservatives. Examples of the widely used preservatives are nitrates, nitrites, chlorine, propionic acid, sorbic acid, and benzoic acid. Each preservative has different antimicrobial spectrum, and is obtained via different production process. A number of food preservatives are created by chemically synthesized process while some of them are produced by strains of bacteria (so called bacteriocins). Of bacteriocins, nisin has been commercially applied in some types of food products and it is sometimes used by combining it with other food processing techniques as well.

1.3.2.1 Nisin and its properties

Nisin ($C_{143}H_{230}N_{42}O_{37}S_7$) is naturally produced by several strains of *Lactococcus lactis* subsp. *Lactis*. It is a member of lantibiotic group, defined as a group of antimicrobial peptides produced by Gram-positive bacteria (Sahl and Bierbaum, 1998), with the presence of uncommon amino acids lanthionine and β-methyl-lanthionine, that name the lantibiotic family (Guiotto et al., 2003). Nisin is cationic, hydrophobic, and composed of 34 amino acid residues with a size of approximately 3.4 kDa. The main structure of nisin contains one lanthionine, four β-methyl-lanthionine rings, and unusual residues including dehydroalanine and dehydrobutyrine (Cheigh and Pyun, 2005). Two types of nisin, nisin A and nisin Z, have been identified. Their structures are overall similar (Figure 4). A slight

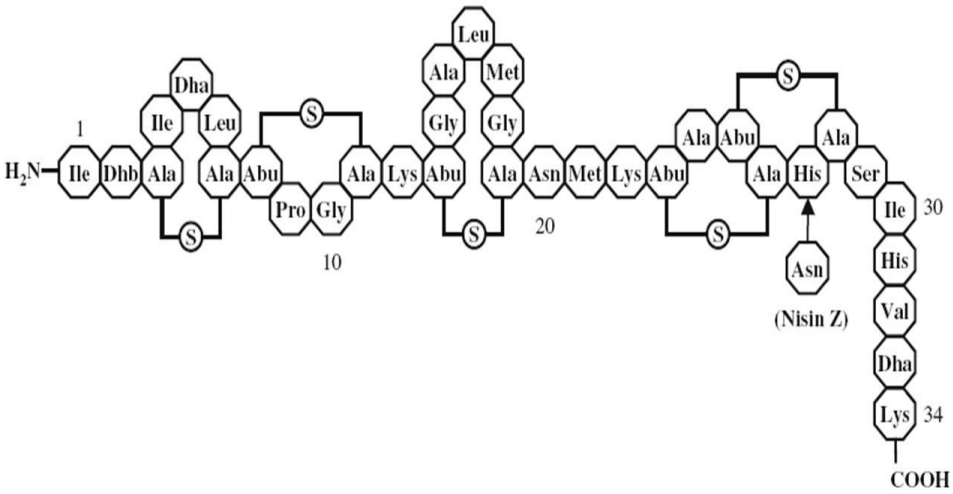

Figure 4. The structure of nisin. The shown molecule is actually nisin A, but the substitution of asparagine (nisin Z) for histidine at position 27 is indicated by the arrow. (Dha is dehydroalanine, Dhb is dehydrobutyrine, Ala-S-Ala is lanthionine and Abu-S-Ala is β-methyl-lanthionine) (Cheigh and Pyun, 2005)

difference is that the amino acid residues of position 27 in the structure of nisin A and nisin Z are histidine and asparagine, respectively. However, both types of nisin are believed to possess comparable antimicrobial activity (Mulders et al., 1991).

Nisin can be stable for years in its dry form. However, the stability of nisin relies on pH value in its solution form. Nisin is most stable in acid condition. Therefore, it is poorly soluble in neutral and mild alkaline solution. At pH 2 it can tolerate a high temperature of 121˚C for 30 min without losing its activity (Luck and Jager, 1995). Nisin is particularly sensitive to metabisulfite, titanium oxide, and certain proteolytic enzymes such as trypsin, pancreatin, and salivary and digestive enzymes (Luck and Jager, 1995; Jay et al., 2005).

1.3.2.2 Microbial inactivation mechanisms of nisin

Nisin is generally effective against Gram-positive but not Gram-negative bacteria (Breukink and de Kruijff, 1999). It has high antibacterial activity against many strains of Gram-positive bacteria, including staphylococci, streptococci, bacilli, clostridia, and mycobacteria (Jack et al., 1995). The resistance of Gram-negative bacteria to nisin is explained by many researchers (Sahl and Bierbaum, 1998; Brotz and Sahl, 2000; Helander and Mattila-Sandholm, 2000) as results of the relatively large size of nisin (~3.4 KDa) that limits its entry across the outer membrane of Gram-negative bacteria. Note that the outer membrane covers the layers of peptidoglycan and cytoplasmic membrane, the latter is conceived to be the target site of nisin attack (details will be further discussed). However, some studies have discovered that nisin was also able to exhibit its bactericidal effect against *Salmonella* spp. and *E. coli* when it was employed in combination with certain chelating agents such as ethylene diamino tetra acetic acid (EDTA) and citrates (Stevens et al., 1991; Stevens et al., 1992; ter Steeg, 1993; Boziaris and Adams, 1999).

The primary proposed mechanism of action for nisin relies on its capability of pore formation in cytoplasmic membrane of bacterial cell (Jack et al., 1995; Breukink and de Kruijff, 1999; Brotz and Sahl, 2000; Jay et al., 2005). Its mode of action is understood to comprise several steps. The first step is binding of nisin to the target cell membrane. Previous studies indicated that nisin favorably binds to membrane containing anionic lipids since it itself is cationic. Therefore, nisin initially binds with its C-terminus via electrostatic interactions with the anionic lipids (Breukink and de Kruijff, 1999). Additional results from a study by Breukink et al. (1997) found that the efficient binding of nisin had somewhat significant relationship with the anionic lipid content of the membranes. In other words, nisin is able to bind the cytoplasmic

membrane well when there is a large amount of anionic lipids present in it. This finding is in agreement with the fact that Gram-positive bacteria have higher concentrations of anionic lipids than Gram-negative bacteria do (Ratledge and Wilkinson, 1988). Hence, this could partially justify the greater activity of nisin towards Gram-positive bacteria (Breukink et al., 1997).

The next process after binding of nisin to the target membrane, is the insertion of nisin into the lipid phase of the membrane. Previous studies displayed the importance of the presence of anionic phospholipids for effective insertion of nisin (Demel et al., 1996; Breukink et al., 1997). Results from these studies also indicated that the N-terminal part of nisin is basically the part of nisin that inserts into the lipid phase of the membrane. Measurement of the molecular hydrophobicity potential of nisin further showed that the N-terminus of nisin is the most hydrophobic (Brasseur, 1991). Consequently, it is reasonable to say that hydrophobic interactions play a crucial role in inserting of the N-terminal nisin into the lipid phase of the membrane (Lins et al., 1999). Moreover, other experiments using nisin containing tryptophan residues at different positions in the molecule also supported these findings on the insertion of nisin. The obtained results showed that nisin at the N-terminal had the deepest location in the membrane, whereas nisin at the C-terminus was located close to the membrane surface (Van Den Hooven et al., 1996). The orientation of nisin was also found to be overall parallel with respect to the membrane surface (Breukink and de Kruijff, 1999).

The last step in the mechanism of action for nisin in bacterial inactivation is the cytoplasmic membrane pore formation. This foremost step occurs as a result of the insertion of nisin in the membrane. Pore formation by nisin appears to work closely related with the aggregation of nisin in the membrane in order to intrude into the

bacterial cell (Breukink and de Kruijff, 1999). Evidence from tryptophan and planar lipid bilayer studies clearly revealed the incident of nisin aggregation inside of the membrane (Giffard et al., 1996; Breukink et al., 1998). The pore formation by nisin was also studied using dye-leakage assays (Martin et al., 1996; Breukink et al., 1997; Giffard et al., 1997). Results suggested that the amount of bound nisin considerably influenced the amount of membrane leakage that occurred. Other studies from black-lipid membrane experiments additionally provided some more possible mechanisms for pore formation by nisin (Sahl et al., 1987). It was assumed that nisin pore was transient (had short pore-lifetime). Plus, the C-terminus of nisin was found to translocate across the membrane upon pore formation. The translocation of nisin then led to the relief of stress on the outer leaflet of the membrane. In addition, nisin selectively recruits negatively charged lipids, thus creating a locally higher concentration of these lipids in its surrounding area (Breukink and de Kruijff, 1999). The pore formation in the membrane by nisin will eventually cause the loss of accumulated amino acids and the inhibition of amino acid transport which may subsequently induce the cell death (Jay et al., 2005). Figure 5 shows the steps of nisin mode of action.

1.3.2.3 Application of nisin in juice and other food products

Nisin in food was initially used in Swiss cheese to prevent its spoilage with *Clostridium butyricum* (Hurst, 1981). It was used to maintain the quality properties of processed cheese. Nisin is the most widely used compound among all bacteriocins for food preservation, with approximately 50 countries allowing its use in foods to varying degrees (Delves-Broughton, 1990). It was approved for use by the Food and

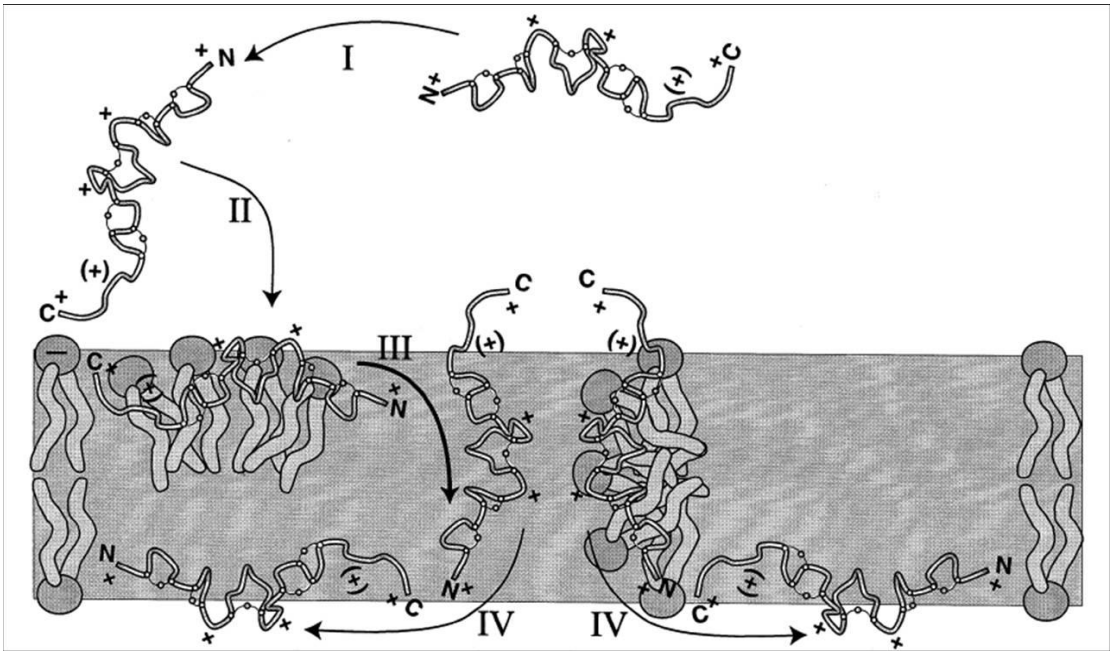

Figure 5. Model for nisin mode of action. Binding of nisin via its C-terminus (step I), insertion of nisin into the membrane (step II), pore formation by nisin (step III), and translocation of the whole peptide (step IV).

Drug Administration (FDA) in the United States in 1988 (Jay et al., 2005; Sobrino-Lopez and Martin-Belloso, 2008). In Europe, it was accepted into the European food additive list (Sobrino-Lopez and Martin-Belloso, 2008). At present, nisin is the only bacteriocin that has been approved by the World Health Organization (WHO) for use as a food preservative, and it is commercialized as a dried concentrated powder (Sobrino-Lopez and Martin-Belloso, 2008).

The use of nisin as a food preservative is gaining interest since it has desirable properties that are attractive to the food industry. For instance, it is nontoxic (generally recognized as safe: GRAS), does not lead to off flavors or off-odors in food products, it is heat stable, has excellent storage stability (Jay et al., 2005), and has a narrow spectrum of antimicrobial activity against the Gram-positive pathogens of concern involved in foodborne illnesses, such as *Listeria monocytogenes* or *Staphylococcus aureus*.

Aside from the use of nisin in cheese-making and some dairy products, it was also employed on a small scale as a sterilizing aid in canned food processing (Luck and Jager, 1995). Because of its ability in increasing the heat sensitivity of certain bacteria, it thereby contributes to the less intensity of sterilization conditions, resulting in enhanced product quality of low-acid canned foods. Examples of canned products with nisin supplemented are tomato, mushroom, soups, and other fruits and vegetables (Hawley, 1957). The goal of nisin use in these processes was to inactivate the endospores of both *C. botulinum* and other spoilage organisms (Luck and Jager, 1995).

Nisin may also be interesting to use in juices for microbial inactivation purposes. As suggested by Komitopolou et al. (1999), Yamazaki et al. (2000), and Pena and de Massaguer (2006), nisin could be added directly to the juices. However, the application of nisin to preserve fruit and vegetable juices is not as extensive compared to those types of foods mentioned earlier. The sole employment of nisin in juices is even more uncommon. As seen from previous studies, nisin is more likely to be used in combination with other antimicrobial agents, such as sodium benzoate, potassium sorbate, and cinnamon, to obtain the desired safety of the product (Yuste and Fung, 2004; Walker and Phillips, 2008). A study by Walker and Phillips (2008) found that nisin was not very effective when it was solely used. When used alone, concentrations up to 1000 IU/ml of nisin were not able to inhibit multiplication of *Propionibacterium cyclohexanicum*, while nisin concentrations as low as 2.5 IU/ml in combination with either sodium benzoate or potassium sorbate were shown to be efficient capable to retard growth of *P. cyclohexanicum* in orange juice for 29 days. Otherwise, nisin might be used along with adjusting other process factors. Pena and de Massaguer (2006) successfully prevented the development of *Alicyclobacillus*

acidoterrestris in orange juice by adding nisin and manipulating three other factors including pH, soluble solids concentration, and incubation temperature.

Another popular trend for nisin use in juices is its combination with other food processing technologies. As mentioned earlier, nisin can collaborate with thermal treatment to disinfect foods, in which nisin plays a role in reducing the intensity of heat treatment, ensuring food safety and better quality. This combination of heat processing in the presence of nisin has been used in canned foods and dairy products, but not in juices. Research efforts have been directed towards the use of nisin together with nonthermal food preservation technologies for juice processing. This may be possibly explained by the fact that thermal treatments are perceived to cause unfavorable changes in sensory and nutritional properties of juices. Accordingly, nonthermal juice processing is gaining more interests nowadays. Nevertheless, only a few reports have been published on the combined nonthermal treatments and nisin to disinfect juices. Pulsed Electric Fields (PEF) is one of the nonthermal technologies that has been used in juices with nisin added (Galvez et al., 2007). These reports showed the synergism between PEF and nisin in inactivating *E. coli* O157:H7 in fresh apple cider (Lu et al., 2001), and *Salmonella* spp. in orange juice (Liang et al., 2002).

2. Inactivation of *Escherichia coli* and *Listeria innocua* in apple and carrot juices using high pressure homogenization and nisin

2.1 Introduction

The ability of foodborne pathogens to contaminate fruit and vegetable juices has led the United States Food and Drug Administration (FDA) to impose Hazard Analysis and Critical Control Point (HACCP) requirements on juice processors. Current HACCP standards require processors of fruit juices to achieve a 5-log reduction of a target pathogen via processing (FDA, 2001). Enteric foodborne pathogens including *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium, parasitic protozoa such as *Cryptosporidium parvum*, and Gram-positive pathogens such as *Listeria monocytogenes* have all been reported capable of survival in raw fruit and vegetable juices (Burnett and Beuchat, 2001; Mak et al., 2001; Mutaku et al., 2005; Roering et al., 1999). Microorganisms associated with fruit juice outbreaks in the U.S. include *E. coli* O157:H7 and *Salmonella* spp. (CDC, 1999; Cody et al., 1999; Cook et al., 1998). Thermal processing (heat pasteurization) has been long recognized as an effective method to eliminate pathogenic vegetative cells in fluid foods such as juices. The general purposes of thermal processing are to make a food product safe for consumption by inactivating pathogenic microorganisms and to extend product shelf stability by destroying spoilage microorganisms. However, thermal processing of foods leads to some undesirable effects in juices (*e.g.,* loss of nutrients, development of off-flavors) that are traditionally consumed fresh (Diels et al., 2003; Vachon et al., 2002; Wuytack et al., 2002).

High pressure homogenization is an emerging nonthermal technology that has demonstrated capability to inactivate various types of bacterial and fungal

microorganisms without significant loss of product quality. Several reports have documented the efficacy of high pressure homogenization to inactivate both pathogenic and spoilage microbes (Bevilacqua *et al.*, 2007; Diels *et al.*, 2004a; Diels *et al.*, 2005a; Diels *et al.*, 2003; Engler and Robinson, 1981; Harrison *et al.*, 1991; Keshavarz Moore *et al.*, 1990; Lopez-Pedemonte *et al.*, 2006; Taylor *et al.*, 2007). High pressure homogenization is a process where fluid is forced through a narrow orifice under conditions of high hydrostatic pressure. Homogenization at relatively lower pressure (<50 MPa) has been extensively used in many industries to produce and/or stabilize emulsions and suspensions (Diels *et al.*, 2005a). The application of high pressure homogenization for microbial disruption and disinfection was originally inspired by researchers in the biotechnology field who demonstrated the ability of homogenization at high pressure to break cells and then release their intracellular contents (Diels *et al.*, 2005a). The development of valve homogenizers that can perform at much higher pressures (>300 MPa) spurred intensive research into homogenization-driven reduction of microbial loads in various foodstuffs. Despite several hypotheses, the exact mechanism of how high pressure homogenization inactivates a microorganism has not yet been fully elucidated. Mechanistic explanations have attributed microbial disruption to the combined effects of turbulent flow, cavitation, impact of cells with solid surfaces at high velocity, and shear stress (Doulah et al., 1975; Engler and Robinson, 1981; Keshavarz Moore et al., 1990; Save et al., 1994; Shamlou et al., 1995).

The addition of antimicrobial compounds to juice products enhances the inactivation of microbial contaminants by other processes. Taylor et al. (2007) reported accelerated inactivation of *E. coli* K12 cells in phosphate buffer containing 10 IU/ml nisin as compared to buffer that contained no antimicrobial following

exposure to 200 MPa homogenization pressure. Populations of stationary and mid-log phase cells exposed to nisin + high pressure homogenization were between one and three logs lower than cells treated with high pressure homogenization alone (Taylor et al., 2007). Diels et al. (2005a), investigating the combination of high pressure homogenization processing (≤300 MPa) and nisin exposure (100 IU/ml), concluded that synergism of antimicrobial and high pressure homogenization depends upon antimicrobial concentration. Other researchers have reported decreased survival for pathogenic or spoilage microbes in juice products supplemented with nisin or other antimicrobials (Kisko and Roller, 2005; Komitopoulou et al., 1999).

The primary objective of this study was to explore the efficacy of high pressure homogenization processing, with or without added nisin, to disinfect apple and carrot juices inoculated with *E. coli* K12 and *L. innocua*, surrogates for the foodborne pathogens *E. coli* O157:H7 and *L. monocytogenes*, respectively.

2.2 Materials and methods

2.2.1 Bacterial culture maintenance

Listeria innocua ATCC 51742 (American Type Culture Collection, Manassas, VA, USA) and *Escherichia coli* K12 were obtained from the Department of Food Science and Technology, University of Tennessee, culture collection. *L. innocua* culture was transferred daily in brain heart infusion broth (BHI) (Becton Dickinson, Sparks, MD, USA) and incubated aerobically without agitation at 35°C. Confirmation of correct phenotype was performed by inoculating a sterile tube of Fraser Broth (FB) (Becton Dickinson). Following overnight incubation (35°C, aerobic, static) and confirmation of phenotype, Petri dishes containing PALCAM agar (Becton

Dickinson) were streaked for isolation and incubated aerobically at 35°C. Following 24 hr incubation, correct colony morphology was confirmed. *E*. coli K12 cells were transferred daily in tryptic soy broth (TSB) (Becton Dickinson) and incubated aerobically without agitation at 35°C. Correct culture phenotype was confirmed via streaking for isolation on Petri dishes containing Levine's Eosin Methylene Blue medium (EMB) (Becton Dickinson). Both cultures were grown aerobically without agitation at 35ºC in non-selective media for 24 hr prior to use. Both types of cells were subsequently diluted in 0.1% peptone water (Fisher Scientific, Fairlawn, NJ). Then, 7 ml of 1:10 culture was added into 693 ml of the juice to obtain a further 1:100 dilution of cells in the juice.

2.2.2 Bacteriocin preparation

Powdered nisin (10^6 IU/g; 2.5% actual nisin) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Nisin (0.1 g) was mixed with 10 ml of 20 mM hydrochloric acid (10,000 IU/ml) (Thermo-Fisher Scientific, Waltham, MA). The stock solution was immersed in boiling water (100°C) for 4-4.5 min. The stock solution was then refrigerated until ready for use; stock solutions were never refrigerated more than six days after their preparation.

2.2.3 Juice preparation and HPH experimentation

Apple juice (100% apple juice; pH~3.8; Regular Apple Juice, White House, Winchester, VA, USA) and carrot juice (100% carrot juice; pH~5.2; Odwalla, Dinuba, CA, USA) were purchased from a local retail store and frozen upon returning to the laboratory. Two days prior to the experiments, the juices were placed in a refrigerator (~5°C) and allowed to thaw. Once fully thawed, 693 ml of juice was filled

into a 1 l screw cap glass bottle; bottles were then autoclaved (121ºC, 15 min). After autoclaving, the bottles were cooled to room temperature. When appropriate, immediately prior to inoculation with *E. coli* K12 or *L. innocua* ATCC 51742 cultures, stock nisin solution was added to a final concentration of 10 IU/ml juice. Bacterial cultures were inoculated into the juice to a concentration of 6 $\log_{10}$ CFU/ml juice. Juice samples were stirred vigorously for approximately 2 minutes and then aseptically loaded into the high pressure homogenization apparatus.

Samples were processed using an FPG 12500 bench-top high-pressure homogenizer (Stansted Fluid Power, Ltd., Essex, United Kingdom) equipped with two positive displacement high pressure pumps in series with a two stage homogenization valve, and a tubular heat exchanger (set at 4^{o}C) connected immediately after the homogenization valve to minimize shear-induced thermal effects. The supervisory control and data acquisition software package Lookout, version 5.1, and Labview, version 7.1 (National instrument, Austin, TX, USA) were used to record the actual chamber pressure and temperature during experimentation. Samples were collected at the outlet of the homogenizer starting from the highest experimental pressure (350 MPa) to zero gage pressure, at intervals of 50 MPa. Sample collection tubes were then placed immediately on ice.

2.2.4 Enumeration of survivors

Immediately following processing, bacterial survivors were serially diluted in 0.1% Peptone water (Thermo-Fisher Scientific) and plated on tryptic soy agar supplemented with 0.6% yeast extract (TSA-YE) (Becton Dickinson) (*L. innocua* ATCC 51742) or tryptic soy agar (TSA) (Becton Dickinson) (*E. coli* K12). Following 48 hr aerobic incubation at 35°C, survivors were enumerated.

2.2.5 Statistical analysis

Analysis of variance (ANOVA) was conducted for each combination of juice source (apple, carrot), microorganism (*E. coli*, *L. innocua*), and antimicrobial (0 and 10 IU/ml nisin). Results were analyzed as a randomized complete block design with two replications for *E. coli* and three replications for *L. innocua*. A block was defined as a volume of inoculated juice subjected to 0 to 350 MPa homogenization pressure. The Student's t-distribution was used to generate 95% confidence intervals for means in cases where data analysis showed significant differences ($P<0.05$). Data were analyzed using Statistical Analysis Software (SAS), version 9.1 (SAS Institute, Cary, NC, USA).

2.3 Results and discussion

The inactivation of the Gram-negative *E. coli* K12 by high pressure homogenization, with or without added nisin at 10 IU/ml, in apple and carrot juice is depicted in Figure 6A and 6B, respectively. A steady decrease in the number of survivors was observed with increasing processing pressures. However, for both juice products, at least 250 MPa homogenization pressure was required to achieve the minimum 5-log reduction in target bacterium as required by HACCP regulations. Whereas apple and carrot juice had different product properties and characteristics such as acidity, clarification, soluble solids, and density, the type of juice in which the microorganism was suspended had no apparent influence on the effectiveness of high pressure homogenization to inactivate *E. coli* K12. The number of bacterial survivors recovered from homogenized apple and carrot juices did not significantly differ ($p<0.05$) between juices supplemented with nisin and juices with no nisin added (Fig. 6A-B). Our results were consistent with other researchers who demonstrated the lack

of effect of 10 IU of nisin on Gram-negative bacteria such as *E coli* (Branen and Davidson, 2004). Nonetheless, these results are contrary to other reports that demonstrated enhanced inhibition of *E. coli* K12 suspended in buffer, exposed to 10 IU/ml nisin, and processed via high pressure homogenization (Taylor et al., 2007).

The inactivation of the Gram-positive *L. innocua* by high pressure homogenization with or without added nisin at 10 IU/ml, in apple and carrot juice, is shown in Figure 7A and 7B, respectively. The number of *L. innocua* survivors recovered from apple and carrot juices did not significantly change with processing pressure up to 200 MPa, indicating greater resistance to high pressure homogenization. When both juices were exposed to homogenization pressures greater than 200 MPa, the numbers of survivors sharply decreased and a 5-log reduction was finally achieved following homogenization pressures approaching 350 MPa (Fig. 7A-B). The number of survivors was not significantly different ($p<0.05$) for juices supplemented with nisin versus juices without nisin, with processing pressure up to 200 MPa. However, large differences in bacterial survivor numbers between nisin-added and nisin-free apple and carrot juices were observed with processing pressure in the 200 to 300 MPa range, indicating potential antimicrobial-pressure interaction. The lantibiotic nisin was particularly effective in promoting further reduction of *L. innocua* subjected to 250 MPa homogenization pressures where any thermal effect was still negligible.

Nisin is mainly effective against Gram-positive microorganisms through two killing mechanisms: permeabilization of the cell membrane and inhibition of cell wall synthesis (Lubelski et al., 2008). Due to the short exposure time of the bacteria to nisin prior to and during homogenization, it is hypothesized that the former

mechanism (permeabilization) would better explain the observed positive effect of nisin during homogenization.

Recorded temperature immediately after the homogenization valve followed a quadratic regression with respect to pressure ($T = 25 + 0.225P - 0.00018P^2$, where T is temperature in $^{\circ}C$; P is pressure in MPa; Figure 6 & 7). To minimize the potential shear-induced thermal effect on microbial survivors, the high pressure homogenizer was equipped with a heat exchanger connected immediately after the homogenization valve, that dropped the sample's temperature to $<10^{o}C$ in less than 2 seconds. The equivalent thermal induced inactivation in the homogenization valve was estimated by transforming the first order kinetic inactivation equation by Bigelow and Esty (1920) to the form:

$$Log\ Thermal\ Inactivation = \frac{Maximum\ Residence\ Time}{D_{T_{ref}} \cdot \left(10^{\left(\frac{T-T_{ref}}{z}\right)}\right)}$$

where D_{Tref} is the decimal reduction time (minutes) at a reference temperature, T is the temperature (^{o}C) at the homogenization valve, and T_{ref} is the reference temperature (^{o}C) for which D_{Tref} was calculated, the maximum residence time was 1/30 min (or 2 s), and z is the temperature difference for a log change in the decimal reduction time (^{o}C). For the case of *E. coli* $D_{58^{o}C}$=4.04 min (Black, 2008) and z=5.4^{o}C (Blackburn et al., 1997) and for the case of *L. innocua* $D_{60^{o}C}$=2.7 min and z=7.3^{o}C (Miller et al., 2006). Figures 6 and 7 show the estimated equivalent thermal death caused by the shear-induced increase in temperature at the homogenization valve. With pressure up to 200 MPa, little thermal inactivation was estimated for both microorganisms and juices. The shear-induced temperature increase was able to inactivate microbial cells at pressure above 200 MPa, but the thermal contribution to

microbial inactivation was minor for pressure between 200 to 250 MPa due to the short residence time (2 s maximum), but significant above ~275 MPa homogenization pressure for *E.* coli and ~325 MPa for *L. innocua*. Our results indicate that as homogenization pressure approached 350 MPa, all observed inactivation could be explained by shear induced high temperature (~80^{o}C) in the homogenization valve, even with the very short residence times.

The susceptibility to high pressure homogenization by *E. coli* K12 over *L. innocua* was consistent with previous findings using homogenization at pressure lower that 300 MPa (Vachon et al., 2002; Wuytack et al., 2002) and with previous reports on other microorganisms and food products (Bevilacqua et al., 2007; Diels et al., 2004a; Diels et al., 2005a; Diels et al., 2004b; Diels et al., 2005b; Diels et al., 2003; Engler and Robinson, 1981; Harrison et al., 1991; Keshavarz Moore et al., 1990; Lopez-Pedemonte et al., 2006). Gram-negative bacteria have traditionally been considered more susceptible to disintegration by mechanical stress than their Gram-positive counterparts (Shiu et al., 2001). This is most likely due to the relatively thin peptidoglycan layer found in Gram-negative bacteria. However, the physical properties of cells, *e.g.*, cell size, shape and wall strength, are highly species, strain and physiological state dependent and will influence the relative resistance of different microorganisms to high pressure homogenization (Harrison et al., 1991; Madigan et al., 2000).

Juices are recognized as having favorable conditions for microorganisms to survive and potentially grow. Our results show the potential of using high pressure homogenization to fulfill HACCP requirements for fruit juice pasteurization. Furthermore, this technology responds the consumer demands for alternative processing technologies that retain nutritional quality, sensory attributes, and physicochemical properties of foods (Mittal and Griffiths, 2005; Mosqueda-Melgar et al., 2008).

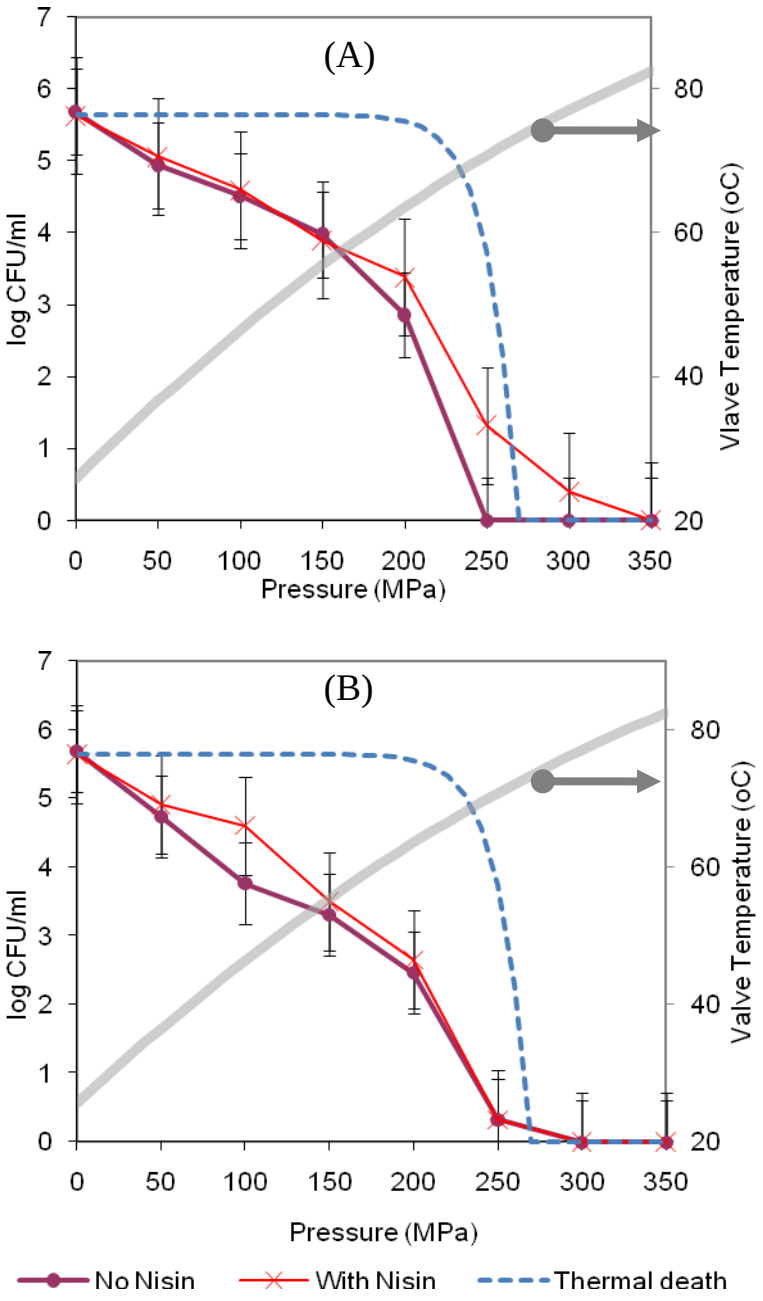

Figure 6. Inactivation of E. coli K12 in (A) Apple Juice and (B) Carrot Juice by high pressure homogenization with and without 10 IU/mL Nisin. Bars are 95% confidence intervals for any mean. Thermal death is the equivalent due to 2 s exposure to the corresponding homogenizating valve temperature

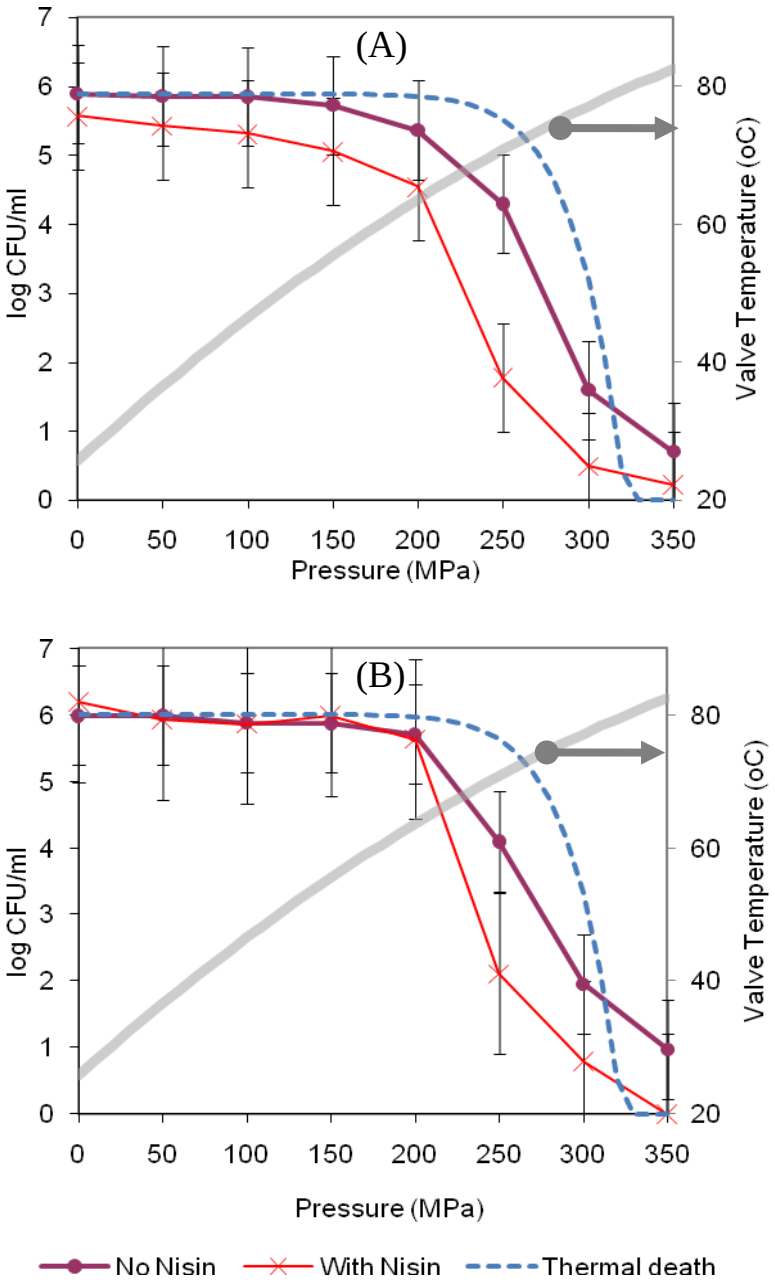

Figure 7. Inactivation of L. innocua in (A) Apple Juice and (B) Carrot Juice by high pressure homogenization with and without 10 IU/mL Nisin. Bars are 95% confidence intervals for any mean. Thermal death is the equivalent due to 2 s exposure to the corresponding homogenizing valve temperature.

2.4 Conclusion

A 5-log reduction of cells was accomplished by high pressure homogenization in both *E. coli* K12 and *L. innocua* as required by juice HACCP regulations. *L. innocua* (Gram-positive) showed a stronger resistance to high pressure homogenization than *E. coli* K12 (Gram-negative). No additional inactivation effects of nisin were observed when combined with high pressure homogenization against *E. coli* K12 cells. On the other hand, interaction effects were observed in the case *L. innocua* subjected to high pressure homogenization in the presence of 10 IU nisin. Based on estimates of the shear induced increase in temperature at the homogenization valve, it was concluded that a combination of homogenization and short term exposure to high temperature are responsible for the bacterial inactivation at pressure >200 MPa. Results clearly showed the potential of high pressure homogenization as an alternative for juice processing in order to make the product safe from harmful microorganisms. However, experiments using pathogens and shelf-life studies are still needed.

References

Agerkvist, I. and Enfors, S. 1990. Characterization of *E. coli* cell disintegrates from a bead mill and high-pressure homogenizers. Biotechnol. Bioeng. 36: 1083-1089

Altekruse, S.F., and Swerdlow, D.L. 1996. The changing epidemiology of foodborne diseases. Amer. J. Med. Sci. 311: 23-29.

Anonymous. 2005. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 sites, United States. Morb. Mortal. Wkly. Rep. 54: 352-356.

Baldwin, C. V. and Robinson, C. W. 1994. Enhanced disruption of Candida utilis using enzymatic pretreatment and high-pressure homogenization. Biotechnol. Bioeng. 43: 46-56.

Bell, C., and Kyriakides, A. 1998. *E. coli*: A practical approach to the organism and its control in foods. Blackwell Science, Oxford

Bevilacqua, A., Cibelli, F., Corbo, M.R., and Sinigaglia, M. 2007. Effects of high-pressure homogenization on the survival of *Alicyclobacillus acidoterrestris* in a laboratory medium. Letters in Applied Microbiology 45, 382-386.

Bigelow, W.D., and Esty, J.R. 1920. The thermal death point in relation to typical thermophilic organisms. Journal of Infectious Diseases 27, 602-617.

Black, D.G. 2008. A review and application of key modeling concepts utilized in predictive microbiology for food processing. PhD Dissertation, University of Tennessee, Knoxville.

Blackburn, C.W., Curtis, L.M., Humpheson, L., Billon, C., and McClure, P.J. 1997. Development of thermal inactivation models for *Salmonella enteriditis* and *Escherichia coli* O157:H7 with temperature, pH, and NaCl as controlling factors. International Journal of Food Microbiology 38, 31-44.

Boziaris, I.S., and Adams, M.R. 1999. Effect of chelators and nisin produced in situ on inhibition and inactivation of Gram negatives. Int. J. Food Microbiol. 53: 105-113.

Branen, J.K., and Davidson, P.M. 2004. Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. International Journal of Food Microbiology 90, 63-74.

Brasseur, R. 1991. Differentiation of lipid-associating helices by use of three-dimensional molecular hydrophobicity potential calculations. J. Biol. Chem. 266: 16120-16127.

Breukink, E., and de Kruijff, B. 1999. The lantibiotic nisin, a special case or not. Biochimica et Biophysica Acta 1462: 223-234.

Breukink, E., Van Kraaij, C., Demel, R.A., Siezen, R.J., Kuipers, O.P., and de Kruijff, B. 1997. The C-terminal region of nisin is responsible for the initial interaction of nisin with the target membrane. Biochemistry 36: 6968-6976.

Breukink, E., Van Kraaij, C., Van Dalen, A., Demel, R.A., Siezen, R.J., and de Kruijff, B., and Kuipers, O.P. 1998. The orientation of nisin in membranes. Biochemistry 37: 8153-8162.

Brotz, H., and Sahl, H.G. 2000. New insights into the mechanism of action of lantibiotics-diverse biological effects by binding to the same molecular target. J. Antimicrobial Chemotherapy 46: 1-6.

Buchrieser, C., Rusniok, C., The Listeria Consortium, Kunst, K., Cossart, P., and Glaser, P. 2003. Comparison of the genome sequences of *Listeria monocytogenes* and *Listeria innocua*: clues for evolution and pathogenicity. FEMS Immunology and Medical Microbiology. 35: 207-213.

Burgaud, I., Dickinson, E., and Nelson, P.V. 1990. An improved high-pressure homogenizer for making fine emulsions on a small scale. Int. J. Food Sci. Technol. 25: 39-46.

Burnett, S.L., and Beuchat, L.R. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. Journal of Industrial Microbiology and Biotechnology 27, 104-110.

Brookman, J. S. G. 1974. Mechanism of cell disintegration in a high-pressure homogenizer. Biotechnol. Bioeng. 16: 371-383.

CDC. 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice. Morbidity and Mortality Weekly Reports 48, 582-585.

Chakraborty, T., Hain, T., and Domann, E. 2000. Genome organization and the evolution of the virulence gene locus in *Listeria* species. Int. J. Med. Microbiol. 290: 167-174.

Cheigh, C., and Pyun, Y. 2005. Nisin biosynthesis and its properties. Biotech. Letters 27: 1641-1648.

Ciesielski, C.A., Swaminathan, B., and Broome, C.V. 1987. *Listeria monocytogenes* —A foodborne pathogen. Clinical Microbiology Newsletter 9: 149-152.

Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J.H., Swaminathan, B., Bryant, R.G., and Vugia, D.J. 1999. An outbreak of *Escherichia coli* O157:H7 infection from unpasteurized commercial apple juice. Annals of Internal Medicine 130, 202-209.

Cook, K.A., Dobbs, T.E., Hlady, W.G., Wells, J.G., Barrett, T.J., Puhr, N.D., Lancette, G.A., Bodager, D.W., Toth, B.L., Genese, C.A., Highsmith, A.K., Pilot, K.E., Finelli, L., and Swerdlow, D.L. 1998. Outbreak of *Salmonella* serotype Hartford

infections associated with unpasteurized orange juice. The Journal of the American Medical Association 280, 1504-1509.

Delves-Broughton, J. 1990. Nisin and its uses as a food preservative. Food Technol. 44: 100, 102, 104, 106, 108, 111-112, 117.

Demel, R.A., Peelen, T., Siezen, R.J., de Kruijff, B., and Kuipers, O.P. 1996. Nisin Z, mutant nisin Z and lacticin 481 interactions with anionic lipids correlate with antimicrobial activity: A monolayer study. Eur. J. Biochem. 235: 267-274.

Diels, A.M., Callewaert, L., Wuytack, E.Y., Masschalck, B., and Michiels, C.W. 2004a. Moderate temperatures affect *Escherichia coli* inactivation by high-pressure homogenization only through fluid viscosity. Biotechnology Progress 20, 1512-1517.

Diels, A.M., Callewaert, L., Wuytack, E.Y., Masschalck, B., and Michiels, C.W. 2005a. Inactivation of *E. coli* by high-pressure homogenization is influenced by fluid viscosity but not by water activity and product composition. International Journal of Food Microbiology 101, 281-291.

Diels, A.M., De Taeye, J., and Michiels, C.W. 2004b. High-pressure homogenisation sensitises *Escherichia coli* to lysozyme and nisin. Community for Agricultural and Applied Biological Sciences 69, 115-117.

Diels, A.M., De Taeye, J., and Michiels, C.W. 2005b. Sensitisation of *Escherichia coli* to antibacterial peptides and enzymes by high-pressure homogenisation. International Journal of Food Microbiology 105, 165-175.

Diels, A.M., and Michiels, C.W. 2006. High-pressure homogenization as a non-thermal technique for the inactivation of microorganisms. Crit Rev Microbiol 32, 201-216.

Diels, A.M., Wuytack, E.Y., and Michiels, C.W. 2003. Modelling inactivation of *Staphylococcus aureus* and *Yersinia enterocolitica* by high-pressure homogenisation at different temperatures. International Journal of Food Microbiology 87, 55-62.

Doulah, M.S., Hammond, T.H., and Brookman, J.S.G. 1975. A hydrodynamic mechanism for the disintegration of *Saccharomyces cerevisiae* in an industrial homogeniser. Biotechnology and Bioengineering 17, 845-858.

Engler, C. R., and Asenjo, J. A. 1990. Cell disruption by homogenizer. Separation processes in biotechnology. Marcel Dekker, New York

Engler, C.R., and Robinson, C.W. 1981. Disruption of *Candida utilis* cells in high-pressure flow devices. Biotechnology and Bioengineering 23, 765-780.

FDA. 2001. Hazard analysis and critical control point (HACCP); procedures for the safe and sanitary processing and importing of juice: final rule (21 CFR Part 120), vol. 66. Fed. Reg., Washington, D.C. 6137-6202.

Feijoo, S. C., Hayes, W. W., Watson, C. E. and Martin, J. H. 1997. Effect of microfluidizer technology on Bacillus licheniformis spores in ice cream mix. J. Dairy Sci. 80: 2184-2187.

Galvez, A., Abriouela, H., Lopeza, R.L., and Omar, N.B. 2007. Bacteriocin-based strategies for food biopreservation. Int. J. Food Microbiol. 120: 51-70.

Gandhia, M., and Chikindas, M.L. 2007. *Listeria*: A foodborne pathogen that knows how to survive. Int. J. Food Microbiol. 113: 1-15.

Garcia-Graells, C., Masschalck, B. and Michiels, C. W. 1999. Inactivation of Escherichia coli in milk by high-hydrostatic pressure treatment in combination with antimicrobial peptides. J. Food Prot. 62: 1248-1254.

Geciova, J., Bury, D. and Jelen, P. 2002. Methods for disruption of microbial cells for potential use in the dairy industry—a review. Int. Dairy J. 12: 541-553.

Giffard, C.J., Dodd, H.M., Horn, N., Ladha, S., Mackie, A.R., Parr, A., Gasson, M.J., and Sanders, D. 1997. Structure-function relations of variant and fragment nisins studied with model membrane systems. Biochemistry 36: 3802-3810.

Giffard, C.J., Ladha, S., Mackie, A.R., Clark, D.C., and Sanders, D. 1996. Interaction of nisin with planar lipid bilayers monitored by fluorescence recovery after photobleaching. J. Membr. Biol. 151: 293-300.

Glaser, P., Frangeul, L., Buchrieser, C., et al. 2001. Comparative genomics of *Listeria* species. *Science* 294: 849-852.

Glass, K.A., Loeffelholz, J.M., Ford, J.P., and Doyle, M.P. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Applied and Environmental Microbiology 58: 2513-2516.

Guiotto, A., Pozzobon, M., Canevari, M., Manganelli, R., Scarin, M., and Veronese, F.M. 2003. PEGylation of the antimicrobial peptide nisin A: problems and perspectives. Il Farmaco 58: 45-50.

Hain, T., Steinweg, C., and Chakraborty, T. 2006. Comparative and functional genomics of *Listeria* spp. J. Biotechnol. 20: 37-51.

Harrison, S.T.L., Chase, H.A., and Dennis, J.S. 1991. The disruption of *Alcaligenes eutrophus* by high pressure homogenization: key factors involved in the process. Bioseparation 2, 155-166.

Hawley, H.B. 1957. Nisin in food technology. Food Manuf. 32: 270-376, 430-434.

Helander, I.M., and Mattila-Sandholm, T. 2000. Permeability barrier of the Gram-negative bacterial outer membrane with special reference to nisin. Int. J. Food Microbiol. 60: 153-161.

Hetherington, P. J., Follows, M., Dunnill, P. and Lilly, M. D. 1971. Release of protein from bakers' yeast (*Saccharomyces cerevisiae*) by disruption in an industrial homogenizer. Chemical engineering Research and Design: Trans. I. Chem E. 49: 142-148.

Hurst, A. 1981. Nisin. Adv. Appl. Microbiol. 27: 85-123.

Jack, R.W., Tagg, J.R., and Ray, B. 1995. Bacteriocins of Gram-positive bacteria. Microbiol. Rev. 59: 171-200.

Jay, J.M., Loessner, M.J., and Golden, D.A. 2005. Modern food microbiology. 7th ed. Springer Science Business Media, New York

Jean, J., Blais, B., Darveau, A. and Fliss, I. 2001. Detection of Hepatitis A Virus by the Nucleic Acid Sequence-Based Amplification Technique and Comparison with Reverse Transcription-PCR. Appl. Environ. Microbiol. 67: 5593-5600.

Jones, D. 1988. Composition and properties of the family Enterobacteriaceae. The society for applied bacteriology symposium series no. 17. J. Applied Bacteriology Symposium Supplement. Blackwell Scientific Publications, London

Jordan, S.L., Pascual, C., Bracey, E., and Mackey, B.M. 2001. Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. Journal of Applied Microbiology 91: 463-469.

Kauffmann, F. 1947. Review, the serology of the coli group. J. Immunol. 57: 71-100.
Kelemen, M. V. and Sharpe, J. E. E. 1979. Controlled cell disruption: a comparison of the forces required to disrupt different microorganisms. J. Cell Sci. 35: 431-441.

Keshavarz Moore, E., Hoare, M., and Dunnill, P. 1990. Disruption of baker's yeast in a high-pressure homogenizer: new evidence on mechanism. Enzyme and Microbial Technology 12, 764-770.

Kheadr, E. E., Vachon, J. F., Paquin, P. and Fliss, I. 2002. Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. Int. Dairy J. 12: 435-446.

Kisko, G., and Roller, S. 2005. Carvacrol and *p*-cymene inactivate *Escherichia coli* O157:H7 in apple juice. BMC Microbiology 5, 36-44.

Komitopoulou, E., Boziaris, I.S., Davies, E.A., Delves-Broughton, J., and Adams, M.R. 1999. *Alicyclobacillus acidoterrestris* in fruit juices and its control by nisin. International Journal of Food Science and Technology 34, 81-85.

Kozak, J., Balmer, T., and Byrne, R. 1996. Prevalence of *Listeria monocytogenes* in foods: incidence in dairy products. Food Control 7: 215-221.
Krause, G., Terzagian, R., and Hammond, R. 2001. Outbreak of *Salmonella* serotype Anatum infection associated with unpasteurized orange juice. Southern Medical Journal 94: 1168-1172.

Lanciotti, R., Sinigaglia, M., Angelini, P. and Guerzoni, M. E. 1994. Effects of homogenization pressure on the survival and growth of some food spoilage and pathogenic microorganisms. Lett. Appl. Microbiol. 18: 319-322.

Lanciotti, R., Gardini, F., Sinigaglia, M. and Guerzoni, M. E. 1996. Effects of growth conditions on the resistance of some pathogenic and spoilage species to high pressure homogenization. Lett. Appl. Microbiol. 22: 165-168.

Liang, Z., Mittal, G.S., and Griffiths, M.W. 2002. Inactivation of *Salmonella* Typhimurium in orange juice containing antimicrobial agents by pulsed electric fields. J. Food Prot. 65: 1081-1087.

Lins, L., Ducarme, P., Breukink, E., and Brasseur, R. 1999. Computational study of nisin interaction with model membrane. Biochimica et Biophysica Acta 1420: 111-120.

Lengeler, W. D., Drews, G. and Schlegel, H. G. 1999. Biology of the Prokaryotes Georg Thieme , Stuttgart

Lopez-Pedemonte, T., Brinez, W.J., Roig-Sagues, A.X., and Guamis, B. 2006. Fate of *Staphylococcus aureus* in cheese treated by ultrahigh pressure homogenization and high hydrostatic pressure. Journal of Dairy Science 89, 4536-4544.

Lu, J., Mittal, G.S., and Griffiths, M.W. 2001. Reduction in levels of *Escherichia coli* O157:H7 in apple cider by pulsed electric fields. J. Food Prot. 64: 964-969.

Lubelski, J., Rink, R., Khusainov, R., Moll, G.N., and Kuipers, O.P. 2008. Biosynthesis, immunity, regulation, mode of action and engineering of the model lantibiotic nisin. Cellular and Molecular Life Sciences 65, 455-476.

Luck, E., and Jager, M. 1995. Antimicrobial food additives: characteristics, uses, effects. 2nd ed. Springer-Verlag Berlin Heidelberg New York

MacDonald, F. and Sutherland, A. D. 1993. Effect of heat treatment on Listeria monocytogenes and gram-negative bacteria in sheep, cow and goat milks. J. Appl. Bacteriol. 75: 336-343.

Madigan, M.T., Martinko, J.M., and Parker, J. 2000. Brock's biology of microorganisms Prentice Hall, Inc., Upper Saddle River, NJ.

Mak, P.P., Ingham, B.H., and Ingham, S.C. 2001. Validation of apple cider pasteurization treatments against *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes*. Journal of Food Protection 64, 1679-1689.

Martin, I., Ruysschaert, J.M., Sanders, D., and Giffard, C.J. 1996. Interaction of the lantibiotic nisin with membranes revealed by fluorescence quenching of an introduced tryptophan. Eur. J. Biochem. 239: 156-164.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5: 607-625.

Miller, L.G., and Kaspar, C.W. 1994. *Escherichia coli* O157:H7 acid tolerance and survival in apple cider. J. Food Prot. 57: 460-464.

Mittal, G.S., and Griffiths, M.W. 2005. Pulsed electric field processing of liquid foods and beverages. In: Sun, D.W., (Ed.), Emerging Technologies for Food Processing. Academic Press, Boston, MA. 99-139.

Morris, C., Brody, A.L., and Wicker, L. 2007. Non-thermal food processing/preservation technotogies: A review with packaging implications. Packaging Technology and Science 20, 275-286.

Moroni, O., Jean, J., Autret, J. and Fliss, I. 2002. Inactivation of lactococcal bacteriophages in liquid media using dynamic high pressure. Int. Dairy J. 12: 907-913.

Mosqueda-Melgar, J., Raybaudi-Massilia, R.M., and Martin-Belloso, O. 2008. Non-thermal pasteurization of fruit juices by combining high-intensity pulsed electric fields with natural antimicrobials. Innovative Food Science and Emerging Technologies 9, 328-340.

Mutaku, I., Erku, W., and Ashenafi, M. 2005. Growth and survival of *Escherichia coli* O157:H7 in fresh tropical fruit juices at ambient and cold temperatures. International Journal of Food Science and Nutrition 56, 133-139.

Mulders, J.W.M., Boerrigter, I.J., Rollema, H.S., Siezen, R.J., de Vos, W.M. 1991. Identification and characterization of the lantibiotic nisin Z, a natural nisin variant. Eur. J. Biochem. 201: 581-584.

Olsen, S.J., Bishop, R., Brenner, F.W., Roels, T.H., Bean, N., Tauxe, R.V., and Slutsker, L. 2001. The changing epidemiology of *Salmonella*: trends in serotypes isolated from humans in the United States, 1987-1997. J. Infectious Diseases 183: 753-761.

Pandolf, W. D. 1998. High-pressure homogenization: Latest technology expands performance and product possibilities. Chem. Process. 61: 39-43.

Parish, M. 1997. Public health and nonpasteurized fruit juice. Crit. Rev. Microbiol. 23: 109-119.

Pena, W.E., and de Massaguer, P.R. 2006. Microbial modeling of *Alicyclobacillus acidoterrestris* CRA 7152 growth in orange juice with nisin added. J. Food Prot. **69**: 1904-1912.

Popper, L., and Knorr, D. 1990. Applications of high-pressure homogenization for food preservation. Food Technol. 44: 84-89.

Ratledge, C., and Wilkinson, S.G. 1988. Microbial lipids. Vol. 1. Academic Press,

New York, 117-201.

Riley, M., Abe, T., Arnaud, M.B., Berlyn, M.K.B., Blattner, F.R., Chaudhuri, R.R., Glasner, J.D., Horiuchi, T., Keseler, I.M., Kosuge, T., Mori, H., Perna, N.T., Plunkett, G., Rudd, K.E., Serres, M.H., Thomas, G.H., Thomson, N.R., Wishart, D., and Wanner, B.L. 2006. *Escherichia coli* K-12: a cooperatively developed annotation snapshot—2005. Nucleic Acids Res. 34: 1-9.

Rocourt, J. 1996. Risk factors of listeriosis. Food Control 7: 195-202.

Roering, A.M., Luchansky, J.B., Ihnot, A.M., Ansay, S.E., Kaspar, C.W., and Ingham, S.C. 1999. Comparative survival of *Salmonella typhimurium* DT 104, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in preservative-free apple cider and simulated gastric fluid. International Journal of Food Microbiology 46, 263-269.

Sado, P.N., Jinneman, K.C., Busby, G.J., Sorg, S.M., and Omiecinski, C.J. 1998. Identification of *Listeria monocytogenes* from unpasteurised apple juice using rapid test kits. J. Food Prot. 58: 1199-1202.

Sahl, H.G., and Bierbaum, G. 1998. Lantibiotics: biosynthesis and biological activities of uniquely modified peptides from gram-positive bacteria. Ann. Rev. Microbiol. 52: 41-79.

Sahl, H.G., Kordel, M., and Benz, R. 1987. Voltage-dependent depolarization of bacterial membranes and artificial lipid bilayers by the peptide antibiotic nisin. Arch. Microbiol. 149: 120-124.

Save, S.S., Pandit, A.B., and Joshi, J.B. 1994. Microbial cell disruption-role of cavitation. The Chemical Engineering Journal 55, B67-B72.

Shamlou, P.A., Siddiqi, S.F., and Titchener-Hooker, N.J. 1995. A physical model of high-pressure disruption of baker's yeast cells. Chemical Engineering and Science 50, 1383-1391.

Sauer, T., Robinson, C. W. and Glick, B. R. 1989. Disruption of native and recombinant Escherichia coli in a high-pressure homogenizer. Biotechnol. Bioeng. 33: 1330-1342.

Shiu, C., Zhang, Z., and Thomas, C.R. 2001. A comparison of the mechanical properties of different bacterial species. In: Durieux, A., Simon, J.P., (Eds.), Applied Microbiology, vol. 2. Springer, New York, NY. 155-162.

Sobrino-Lopez, A., and Martin-Belloso, O. 2008. Use of nisin and other bacteriocins for preservation of dairy products. Int. Dairy J. 18: 329-343.

Stevens, K.A., Sheldon, B.W., Klapes, N.A., and Klaenhammer, T.R. 1991. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl. Environ. Microbiol. **57**: 3613-3615.

Stevens, K.A., Sheldon, B.W., Klapes, N.A., and Klaenhammer, T.R. 1992. Effect of treatment conditions on nisin inactivation of gram-negative bacteria. J. Food Prot. **55**: 763-766.

Strachan, N.J.C., Dunn, G.M., Locking, M.E., Reid, T.M.S., and Ogden, I.D. 2006. *Escherichia coli* O157: Burger bug or environmental pathogen. Int. J. Food Microbiol. 112: 129-137.

Taylor, T.M., Roach, A., Black, D.G., Davidson, P.M., and Harte, F. 2007. Inactivation of *Escherichia coli* K12 exposed to pressures in excess of 300 MPa in a high-pressure homogenizer. Journal of Food Protection 70, 1007-1010.

ter Steeg, P. 1993. Interactions of nisin, lysozyme, and citrate in biopreservation. De Ware(n)-Chemicus 23: 183-190.

Vachon, J.F., Kheadr, E.E., Giasson, J., Paquin, P., and Fliss, I. 2002. Inactivation of foodborne pathogens in milk using dynamic high pressure. Journal of Food Protection 65, 345-352.

Van Den Hooven, H.W., Doeland, C.C.M., Van De Kamp, M., Konings, R.N.H., Hilbers, C.W., and Van De Ven, F.J.M. 1996. Three-dimensional structure of the lantibiotic nisin in the presence of membrane-mimetic micelles of dodecylphosphocholine and of sodium dodecylsulphate. Eur. J. Biochem. 235: 382-393.

Walker, M., and Phillips, C.A. 2008. The effect of preservatives on *Alicyclobacillus acidoterrestris* and *Propionibacterium cyclohexanicum* in fruit juice. Food Control 19: 974-981.

Willshaw, G.A., Cheasty, T., and Smith, H.R. 2000. *Escherichia coli*. Aspen Inc., Gathersburg, Maryland, USA

Wilson, G.S., and Miles, A.A. 1964. Topley and Wilson's Principles of Bacteriology and Immunity. 5th Ed., Edward Arnold, London 1: 806-826.

Wuytack, E.Y., Diels, A.M., and Michiels, C.W. 2002. Bacterial inactivation by high-pressure homogenisation and high hydrostatic pressure. International Journal of Food Microbiology 77, 205-212.

Yamazaki, K., Mukarami, M., Kawai, Y., Inoue, N., and Matsuda, T. 2000. Use of nisin for inhibition of *Alicylobacillus acidoterrestris* in acidic drinks. Food Microbiol. 17: 315-320.

Yuste, J., and Fung, D.Y. 2004. Inactivation of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 in apple juice by a combination of nisin and cinnamon. J. Food Prot. 67: 371-377.

Zapico, P., de Paz, M., Medina, M. and Nunez, M. 1999. The effect of homogenization of whole milk, skim milk and milk fat on nisin activity against Listeria innocua. Int. J. Food Microbiol. 46: 151-157.

Vita

Panchalee Pathanibul was born in Bangkok, Thailand in 1984. She attended Kasetsart University Laboratory School in Bangkok for all her elementary, middle, and high school for 12 years in Math and Science concentration. She also went for her undergraduate degree in Kasetsart University for another 4 years and received her Bachelor of Science degree in Food Science and Technology in 2006. During her undergraduate study, she had an opportunity to visit University of Tennessee, Knoxville, USA for a 1-month study tour under a joint program between Kasetsart University and University of Tennessee. She later decided to pursue her graduate study in University of Tennessee for a Master of Science degree in Food Science and Technology in Food Microbiology concentration since 2007.