

**ULTRA HIGH PRESSURE INACTIVATION OF *SACCHAROMYCES*
CEREVISIAE AND *LISTERIA INNOCUA* ON FRUIT**

By

MAITE ANDREA CHAUVIN

**A thesis submitted in partial fulfillment of
the requirements for the degree of**

MASTER OF SCIENCE IN FOOD SCIENCE

WASHINGTON STATE UNIVERSITY

Department of Food Science and Human Nutrition

DECEMBER 2004

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of MAITE
ANDREA CHAUVIN find it satisfactory and recommend that it be accepted.

Chair

ACKNOWLEDGMENTS

I would like to take the opportunity to acknowledge and thank all those who have helped me in any way through this research. I wish to thank my advisor, Dr. Barry G. Swanson and the other members of my committee Drs. Dong-Hyun Kang, and Joseph R. Powers for their time, support, encouragement, advice and confidence while I worked towards my masters degree.

In addition I would like to express my sincere appreciation to the following:

- My husband and parents for their support, encouragement and love
- Sun-Young Lee, Su-Sen Chang, and Peter Grey for your friendship, help and suggestions in my research. Thank you for always being there when I needed you
- Miyuki Miyawaki for your friendship, trust and advise
- Frank Younce for the endless high pressure sessions
- Dr Kang's lab team for your hand in the long experiments
- The office personnel for answering all my questions (Jodi, Richard, Carolee and Connie)

It has been fun working with all of you!

**ULTRA HIGH PRESSURE INACTIVATION OF *SACCHAROMYCES*
CEREVISIAE AND *LISTERIA INNOCUA* IN FRUIT**

Abstract

by Maite Andrea Chauvin, M.S.

Washington State University

December 2004

Chair: Barry G. Swanson

Shelf stable fruit preserves and pie fillings are traditionally stabilized with thermal processes to ensure food safety and prolonged shelf life. Fruit products exposed to high temperatures frequently present a cooked flavor, losing desirable texture, color, and nutrient quality. Nonthermal ultra high pressure processing exhibits the potential of achieving a “fresh like” quality fruit product desired by consumers while inactivating common pathogenic and spoilage microorganisms.

The objective of the current research was to investigate decimal reduction times obtained with selected ultra high pressure treatments. High pressure survivor curves for *Saccharomyces cerevisiae* and *Listeria innocua* inoculated on apples and blueberries were determined at 300 or 375 MPa, respectively. *Saccharomyces cerevisiae* D-values ranged from 19.7 s on apples to 22.4 s on blueberries. D-values for *Listeria innocua* ranged from 67.1 s on apples to 46.7 s on blueberries.

High pressure D-values of *Saccharomyces cerevisiae* and *Listeria innocua* were also evaluated in commercial apple sauce with adjusted soluble solids concentrations ranging from 13% to 60%. D-values for *Saccharomyces cerevisiae* in apple sauce treated with 300 MPa are 26.3, 29.9 and 46.5 s at 13, 20 and 30% of soluble solids, respectively. D-values for *Listeria innocua* in apple sauce treated with 375 MPa are 40.2, 55.2 and 120.6 s at 13, 20 and 30% of soluble solids, respectively. Ultra high pressure treatment of commercial apple sauce inoculated with *Listeria innocua* and *Saccharomyces cerevisiae* adjusted to 40, 50 or 60% sucrose concentrations reduced the initial inocula of 10^7 to 10^6 less than 1 log. Sucrose concentrations greater than 30% provide a baroprotective effect on microbial inhibition by ultra high pressure treatment.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
DEDICATION.....	xi

1. INTRODUCTION

LITERATURE REVIEW

1. Ultra High Pressure Preservation of Fruit.....	1
2. Ultra High Pressure Microbial Inactivation	
2.1 <i>Saccharomyces cerevisiae</i>	4
2.2 <i>Listeria monocytogenes</i>	6
2.3 Critical Process Factors in Ultra High Pressure Inactivation of Microorganisms.....	9
3. References.....	13

2. ULTRA HIGH PRESSURE INACTIVATION OF *SACCHAROMYCES*

CEREVISIAE AND *LISTERIA INNOCUA* ON APPLES AND

BLUEBERRIES

1. Abstract.....	18
2. Introduction.....	19
3. Materials and Methods.....	22

4. Results and Discussion.....	27
5. Conclusions.....	32
6. References.....	33
3. PROTECTIVE EFFECT OF SUCROSE FOR ULTRA HIGH PRESSURE INCUBATION OF <i>SACCHAROMYCES CEREVISIAE</i> AND <i>LISTERIA INNOCUA</i> IN COMMERCIAL APPLE SAUCE	
1. Abstract.....	50
2. Introduction.....	52
3. Materials and Methods.....	54
4. Results and Discussion.....	58
5. Conclusions.....	61
6. References.....	62
4. CONCLUSIONS AND FUTURE WORK	
1. Conclusions.....	69
2. Future Work.....	71

LIST OF TABLES

Chapter 1

1. Survival of *Listeria monocytogenes* following combined treatment of hydrostatic pressure, time and temperature.....9

Chapter 2

1. Specifications of apples and blueberries.....47
2. Ultra high pressure inactivation rates (k), D-values (D), and regression coefficients (R^2) of *Saccharomyces cerevisiae* and *Listeria innocua* on apples and blueberries at 21 °C.....48
3. Pressure come up time and inactivation of *Saccharomyces cerevisiae* and *Listeria innocua*.....49

Chapter 3

1. pH and water activity (a_w) of apple sauce at selected soluble solids concentrations67
2. Ultra high pressure decimal reduction times (D) of *Saccharomyces cerevisiae* and *Listeria innocua* in apple sauce at selected soluble solids concentrations.....68

LIST OF FIGURES

Chapter 2

1. High pressure (300, 450 and 600 MPa) inactivation of <i>Saccharomyces cerevisiae</i> on diced apples.....	37
2. High pressure (300, 450 and 600 MPa) inactivation of <i>Saccharomyces cerevisiae</i> on grapes.....	38
3. High pressure (300, 450 and 600 MPa) inactivation of <i>Saccharomyces cerevisiae</i> on strawberries.....	39
4. High pressure (300, 450 and 600 MPa) inactivation of <i>Saccharomyces cerevisiae</i> on blueberries.....	40
5. High pressure (300, 450 and 600 MPa) inactivation of <i>Listeria innocua</i> on diced apples.....	41
6. High pressure (300, 450 and 600 MPa) inactivation of <i>Listeria innocua</i> on grapes.....	42
7. High pressure (300, 450 and 600 MPa) inactivation of <i>Listeria innocua</i> on strawberries.....	43
8. High pressure (300, 450 and 600 MPa) inactivation of <i>Listeria innocua</i> on blueberries.....	44
9. High pressure (300 MPa) inactivation of <i>Saccharomyces cerevisiae</i> on diced apples and blueberries.....	45

10. High pressure (375 MPa) inactivation of *Listeria innocua* on diced apples
and blueberries.....46

Chapter 3

1. Ultra high pressure treatment (300 MPa, 150 s, 21°C) of *Saccharomyces cerevisiae*
apples sauce at selected soluble solids concentrations.....65

2. Ultra high pressure treatment (375 MPa, 300 s, 21°C) of *Listeria innocua* apple sauce
at selected soluble solids concentrations66

DEDICATION

This work is dedicated to my husband Fariss for his patience, love, and for always putting a smile on my face. I also dedicate my work to my parents and brother for their encouragement and endless support.

Be Happy.

It's one way of being wise.

~Colette

Chapter One

INTRODUCTION

LITERATURE REVIEW

1. Ultra High Pressure Preservation of Fruit

Food processors are constantly seeking preservation techniques that deliver convenient products that are safe and offer high quality characteristics (Hendrickx et al., 1995). Fruit products such as purees, pie fillings, preserves, and juices are widely consumed in the United States. Cooling, freezing, pasteurization, and commercial sterilization are some of the chemical-free processes that can be used for fruit product preservation (Silva and Silva, 1997).

Shelf stable fruit preserves and pie fillings are traditionally treated by thermal processes to ensure food safety and prolonged shelf life. Fruit products exposed to high temperatures frequently present a cooked flavor, losing desirable texture, color, and nutrient quality. Nonthermal ultra high pressure processing exhibits the potential for achieving the “fresh-like” quality fruit products desired by consumers, while inactivating common pathogenic and spoilage microorganisms. Ultra high pressure is also capable of inactivating detrimental enzymes, such as polyphenoloxidase, pectin methylesterase, and polygalacturonase, which result in the browning or softening of fruit (Meyer et al., 2003).

Ultra high pressure processing was first used in 1899 to study the effect of pressure in the preservation of milk (Hite, 1899). Hite also reported the possibility of microbiological inactivation in fruit derivatives. Cruess (1924) referred to Hite’s experiments in his textbook on commercial fruit and vegetable products and stated that

high pressure could become a means of processing fruit juices. It took almost 70 years until Cruess' prediction was fulfilled and the first high pressure treated fruit products reached the Japanese market in 1990 (Palou et al., 1997 b).

In the past, the main limitation in the application of ultra high pressure technology was the excessive capital investment for the high pressure equipment. However, the present evolution of the high pressure technology together with the desire of the food industry for high quality products has renewed interest in the use of ultra high pressure technology as a food preservation alternative (Rovere et al., 1994). Ultra high pressure equipment can reliably deliver pressures of 600 MPa on a commercial basis. Consumers are eager for high quality foods and beverages with a nutrient content and sensory character closer to their fresh or raw counterparts (Hoover, 1989). Ultra high pressure processing of fruit not only retains flavor but also inhibits the growth of pathogenic bacteria, yeast, and molds due to the low pH of fruit products (Garcia-Graells et al., 1998).

Ultra high pressure acts instantaneously and uniformly throughout the mass of food independent of size, shape, and food composition. Foods are compressed by a uniform pressure from every direction throughout the whole. The behavior of foods during treatment with high hydrostatic pressure is governed by the basic principle of Le Chatelier which states that phase transition, chemical reaction or molecular conformations accompanied by a decrease in volume will be enhanced by an increase in pressure. The phenomena involving an increase in volume will be inhibited (Palou et al., 1997 b).

During pressurization, adiabatic heating occurs leading an increase in the temperature of foods by approximately 3 °C per 100 MPa. Foods cool down to their original temperature on decompression if no heat is lost to or gained from the walls of the pressure vessel during the hold time at pressure. In general, the adiabatic heating depends on the pressure, the specific heat, and the compressibility of the food (Farkas and Hoover, 2000).

In 1990, Japan was the first market to introduce fruit-based foods preserved by ultra high pressure. The Japanese market includes several fruit jams and jellies, orange and grapefruit juices, salad dressings, fruit yogurts and fruit sauces (Aleman et al., 1998). High pressure processing reached a commercial reality in the United States in 1993. The first high pressure processed food marketed in the U.S. was Classic Guacamole®, a refrigerated fresh guacamole manufactured by Avomex (Mermelstein, 1998). Avomex, Inc. is a successful model of ultra high pressure technology achievement in marketing and sales with current gross sales in excess of \$60,000,000 annually (Meyer, 2003).

Safe and highly nutritious foods with fresh sensory characteristics are in demand in the 21st century. Ultra high pressure offers an alternative to thermal processes for low acid foods. This study was undertaken to determine the pressure and time required to inactivate *Listeria innocua* and *Saccharomyces cerevisiae* while maintaining the “fresh like” fruit texture and color of apple and blueberry preserves and pie fillings.

2. Ultra High Pressure Microbial Inactivation

2.1 *Saccharomyces cerevisiae*

Food spoilage is a complex process that results in discarding of excessive amounts of foods every year. Spoilage microorganisms must be controlled to guarantee high quality foods and extended shelf lives. Yeasts are an important group of spoilage microorganisms and represent a substantial economic threat to the food industry. Yeasts are sensitive to ultra high pressure inactivation (Basak et al., 1992; Hashizume et al., 1995; Palou et al., 1998). Ultra high pressure offers an alternative to traditional thermal treatments for eliminating yeasts while retaining acceptable quality standards (Zook et al., 1999).

Yeasts are unicellular fungi that exist throughout nature. Microbial fermentation and spoilage of fruit juices and other fruit products are most frequently associated with *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* growth results in ethanolic spoilage, carbonation, and production of hydrogen sulfide as well as other off-odors (Parish, 1991).

There are many hypothetical mechanisms for inactivating *Saccharomyces cerevisiae* with ultra high pressure. The primary sites of pressure damage are cell membranes. Scanning electron micrograph exhibited that yeast cells treated with a pressure of 400 MPa for 10 min at room temperature showed slight alterations in outer membrane shapes. Transmission electron micrograph exhibited inner structures of the cells began to decompose, especially the nuclear membrane, even when treated with a hydrostatic pressure of 100 MPa for 10 min at room temperature, at 400 MPa, most of the intracellular organelles in the cell such as the nucleus, mitochondria, endoplasmic

reticulum, and vacuole were deformed or disrupted. Furthermore at pressures greater than 500 MPa, neither the nuclei nor any of these intracellular organelles were recognizable in 100 % of cells (Shimada et al., 1993).

Ultra high pressure treatments greater than 300 MPa may partially inactivate selected enzymes. For instance, glyceraldehyde-3-phosphate dehydrogenase in yeast can be inactivated at 320 MP for 10 min and room temperature. A portion of the microbial inactivation mechanism during ultra high pressure treatments can be attributed to enzyme denaturation (Jaenicke, 1981). Microbial inactivation can also be attributed to the pressure effects on microbial ATPase that may disturb proton efflux from the cell interior (Smelt, 1995).

Zook et al. (1999) reported the ultra high pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juice and in a model juice buffer at pH 3.5, 4.0, 4.5 and 5.0. Approximately 0.5 to 1.0×10^6 ascospores/ml in juice or buffer were treated under pressures of 300 to 500 MPa during times ranging between 1 and 30 min and room temperature. D-values ranged from 10.8 min at 300 MPa to 8 s at 500 MPa. The range of z-values required to change the D-value by a factor of 10 was 115 to 121 MPa. No differences in D-values or z-values among buffers or juices at any pH were observed, indicating little influence of pH in the inactivation of *Saccharomyces cerevisiae*. Ultra high pressure was recommended for inactivation of *Saccharomyces cerevisiae* ascospores in acidic fruit juice systems.

Basak et al. (2002) reported that the resistance of *Saccharomyces cerevisiae* to ultra high pressure is greater in orange juice with large concentrations of soluble solids (42°Brix). The approximate inactivation for *Saccharomyces cerevisiae* in spaghetti

sauce at 253 MPa for 10 min and 25 °C was three log cycles, and at 253 MPa for 7 min and 45 °C seven log inactivation were observed (San Martin et al., 2002). Pandya et al. (1995) reported that six log cycles of *Saccharomyces cerevisiae* inoculated in citrate buffer at pH 4.0 were inactivated by an ultra high pressure treatment of 250 MPa for 30 min at 45 °C.

Ultra high pressure is an effective method for inactivating *Saccharomyces cerevisiae* due to the sensitivity of yeast species to pressure treatments. If the intensity of pressure increases the inactivation of *Saccharomyces cerevisiae* increases (Shimada et al., 1993). The extent of yeast inactivation can also be attributed to several parameters such as time, process temperature and composition of the media or food (Palou et al., 1997 a).

2.2 *Listeria monocytogenes*

The *Listeria* genus consists of small, non spore forming Gram positive rods. *Listeria monocytogenes* is omnipresent in the environment. Therefore, food and human exposure to *Listeria* species are very common. *Listeria* species are soil borne and survive in the soil for extended periods of time. *Listeria monocytogenes* is highly capable of persisting in the food processing environment and is difficult to control (Donnelly, 2001). Many *Listeria monocytogenes* strains are considered human pathogens. *Listeria monocytogenes* is implicated in several fatal outbreaks of foodborne illness and is a primary concern to the food industry (Barnes et al., 1989). Listeriosis, the name of the general group of disorders caused by *L. monocytogenes*, is a severe disease with a high fatality rate (20-30%) observed primarily in industrialized countries.

The 1987 incidence data collected by the CDC suggests that there are at least 1600 cases of listeriosis with 415 deaths per year in the U.S.

Listeria monocytogenes is more resistant to heat than many other non-spore forming foodborne pathogens and the potential survival of *Listeria monocytogenes* in foods subjected to mild heat treatment is of great concern (Farber and Peterkin, 1991). *L. monocytogenes* will grow at refrigeration temperatures and at temperatures as high as 140 to 150 °F (CDC, 2004).

High hydrostatic pressure is attracting much interest as an alternative to heat as a means of inactivating *Listeria monocytogenes* in food (Hoover et al., 1989). Alpas and Bozoglu (2003) reported that a pressure treatment of 350 MPa for 5 min at 40 °C will potentially inactivate *Listeria monocytogenes* strains inoculated in pasteurized apple, apricot, cherry and orange juices. Arroyo et al. (1999) reported that the application of high pressure (200, 300, 350 and 400 MPa) treatments for 30 min at 5 °C to baby lettuce, tomatoes, spinach, asparagus, onions, and cauliflowers inactivates *Listeria monocytogenes*. Arroyo et al. (1999) confirmed that *Listeria monocytogenes* was completely inactivated (7 log reduction) at 350 MPa for 30 min and 5 °C.

Erkmen and Dogan (2004) reported the effects of ultra high hydrostatic pressure on *Listeria monocytogenes* in raw milk, peach and orange juice. *Listeria monocytogenes* in raw milk, peach and orange juice subjected to ultra high pressure treatments from 200 to 700 MPa for 1 to 80 min at 25 °C resulted in survivor curves demonstrating that cell inactivation increased as pressure and time increased. Log reductions of *Listeria monocytogenes* were greater in orange juice, followed by peach juice, and milk. The low pH (3.55) of orange juice exhibited a synergistic effect with the pressure treatment in the

inactivation of bacteria. The pH reduction of the medium resulted in progressive increase in *Listeria* sensitivity cell to pressure. Erkmen and Dogan (2004) concluded that the rate of *Listeria monocytogenes* inactivation during ultra high pressure treatment depends on pressure, maturity of cell, composition of medium, and pressurization time.

Simpson and Gilmour (1997) studied the resistance to pressure of three strains of *Listeria monocytogenes* in 10 mmol l⁻¹ phosphate buffered saline (PBS) at pH 7.0 and model food systems each containing one of three main food constituents: protein (1, 2, 5 and 8% w/v bovine serum albumin in PBS), carbohydrate (1, 2, 5 and 10% w/v glucose in PBS) and lipid (olive oil 30% v/v in PBS emulsion). Both the PBS and the food models were exposed to 375 MPa for 5, 10, 15, 25 or 30 min at room temperature. In general, increasing the concentrations of bovine serum albumin (BSA) and glucose resulted in decreasing levels of inactivation of the three strains of *L. monocytogenes*. Survival of *L. monocytogenes* was greater in the olive oil/PBS emulsion than in the control PBS at all treatment times.

Ultra high pressure is an effective method for inactivating *Listeria monocytogenes* in 0.1 % peptone solutions (Table 1).

TABLE 1 Survival of *Listeria monocytogenes* exposed to selected pressure, time and temperature treatments

	Mean log ¹⁰ CFU/ml following pressurization at					
	276 MPa			345 MPa		
	Time (min)					
	5	10	15	5	10	15
Initial Inoculation	8.0	8.0	8.0	9.0	9.0	9.0
Temperature °C						
25	7.3	7.2	7.1	6.0	3.7	2.9
35	7.1	6.1	5.6	1.7	ND	ND
45	3.0	2.8	1.6	ND	ND	ND
50	1.3	1.0	ND	ND	ND	ND

ND, No CFU detected in 0.4 ml of *Listeria monocytogenes* cell suspensions. Each value is the mean of duplicate samples. (Kalchayanand et al., 1998)

2.3 Critical Process Factors in Ultra High Pressure Inactivation of Microorganisms

Pressure, time, temperature, type and growth phase of microorganism, pH and water activity are critical factors in the inactivation of pathogenic and spoilage microorganisms treated with ultra high pressure. An increase in pressure generally increases rate and the number of microorganisms inactivated (except with bacterial endospores). However, increasing the duration of the pressure treatment does not necessarily increase the inactivation of microorganisms with ultra high pressure. There is a minimum critical pressure which is required for microbial inactivation. At pressures less than the minimum critical pressure microbial inactivation will not occur regardless of the process time (Palou et al., 1998).

The ultra high pressure inactivation of bacteria, yeasts and molds is dependent upon temperature. The resistance to pressure of an endogenous or inoculated microbial strain is maximal at normal temperatures (15-30 °C) and decreases significantly at higher or lower temperatures. Temperatures ranging between 45 °C and 50 °C increase the rate of inactivation of food pathogens and spoilage microbes (Palou, 1997 b). Hashizume et al. (1995) studied the inactivation of *Saccharomyces cerevisiae* at a pressure range of 120 to 300 MPa at -20 to 50 °C for 20 min. Hashizume et al. (1995) reported that a similar degree of inactivation was achieved at high pressures (300 MPa) and high temperatures (50 °C) when compared with low pressures (120 MPa) and low temperatures (-20 °C). Hashizume et al. (1995) concluded that high pressure treatment applied at sub-zero temperatures requires smaller pressures than high pressure treatment at high temperatures to achieve equivalent microbial inactivation. Freezing temperatures (-20 °C) in treatments ranging from 100 to 400 MPa for 20 min on Satsuma mandarin juice enhance microbial inactivation when compared with ultra high pressure treatments at 20 °C. The decrease in pressure resistance of vegetative cells at low temperatures (< 5 °C) may be due to changes in the membrane structure and fluidity, weakening of hydrophobic interactions, and crystallization of phospholipids (Palou et al., 1997 b).

The pressure sensitivity of microorganisms also varies within species and within strains of the same genus, and with the stage of growth cycle the organisms have reached prior to inoculation and exposure to pressure. Cells in the exponential phase are more sensitive to pressure treatment than cells in the stationary phase of growth. (Palou et al., 1997 b). One of the most heat and pressure resistant pathogenic spore formers and lethal

to human beings is *C. botulinum*. Spore suspensions of strains 17B and Cap 9B tolerated exposures to 827 MPa for 30 min at 75 °C.

Gram positive vegetative bacteria are more resistant to pressure than vegetative cells of Gram negative bacteria. The more developed the life form, the greater its sensitivity to pressure (Farkas and Hoover, 2000). Gram positive pathogens, such as *Listeria monocytogenes* and *Staphylococcus aureus*, require treatments of 340 and 400 MPa for 20 min at 21 °C, respectively, to achieve 6 log inactivation (Patterson et al., 1995). Among Gram negative bacteria, *Vibrio parahaemolyticus* is one of the most pressure sensitive microorganisms, exhibiting 6 log inactivation in the initial population following treatment of 200 MPa for 20 min at room temperature (Palou et al., 1997 b).

Pressure inactivation rates will be enhanced by exposure to acidic pH. Ultra high pressure of foods may shift the pH of the food as a function of imposed pressure. During pressurization a decrease in the pKa of the acids and pH reduction is expected, a temporary reduction in pH and an increase in the dissociated form of the acids can be present during pressurization (Palou et al., 1997 b). The direction of the pH shift and its magnitude must be determined for each food treatment process. As pH is lowered many microbes become more sensitive to pressure inactivation, and recovery of sublethally injured cells is reduced (Farkas and Hoover, 2000). The effect of pH on the pressure resistance of *Bacillus coagulans* in peptone water are reported by Roberts and Hoover (1996). Spores are more sensitive to inactivation by pressure at lower pH. A decrease of 1.5 log of *Bacillus coagulans* was observed as the pH was reduced (adjusted with McIlvaine citrate phosphate buffer) from 7.0 to 4.0 during pressurization at 400 MPa for 30 min at 45 °C.

Oxen and Knorr (1993) observed that the pressure resistance of fungi increases as sucrose, fructose, or glucose concentrations in deionized water increased. Seven log reductions were observed in *Rhodotorula rubra* at water activities greater than 0.96 when treated at 400 MPa for 15 min and 25 °C. No inactivation of *Rhodotorula rubra* was observed when the water activities were lower than 0.91. Reducing the water activity protects microorganisms against inactivation by ultra high pressure. Palou et al. (1997 b) reported a reduction in the inactivation of *Zygosaccharomyces bailii* with reducing water activities. *Zygosaccharomyces bailii* in model systems (Sabouraud glucose 2% broth) was treated by ultra high pressure at 345 MPa for 5 min at 21 °C. Greater counts of *Zygosaccharomyces bailii* were observed in model systems with water activities of 0.92, 0.91, and 0.90. In model systems with water activities greater than 0.98 of a complete inactivation of *Zygosaccharomyces bailii* was observed. The observations of Oxen and Knorr (1993) and Palou et al.(1997 b) suggest that cell shrinkage at reduced water activities (or increasing soluble solids concentrations) caused thickening of the cell membrane, thereby reducing membrane permeability and protecting the cells from high pressure inactivation.

References

- Aleman, G.D., Ting, E.Y., Farkas, D.F., Mordre, S.C., Hawes, A.C., and Torres, J.A. 1998. Comparison of static and step-pulsed ultra-high pressure on the microbial stability of fresh cut pineapple. *J. Food Agric.* **76**: 383-388.
- Alpas, H., Kalchayanand, N., Bozoglu, F., and Ray, B. 1998. Interaction of pressure, time and temperature of pressurization on viability loss of *Listeria innocua*. *World J. Micro Biotechnol.* **14**: 251-253.
- Alpas, H. and Bozoglu, F. 2003. Efficiency of high pressure treatment for destruction of *Listeria monocytogenes* in fruit juices. *FEMS Immunol. Med. Micro.* **35**: 269-273.
- Arroyo, G., Sanz, P.D. and Préstamo, G. 1999. Response to high pressure, low temperature treatment in vegetables: determination of survival rates of microbial populations using flow cytometry and detection of peroxidase activity using cofocal microscopy. *J. Applied Micro.* **86**: 554-556.
- Barnes, R., Archer, P., Strack, J., and Ister, J.R. 1989. Listeriosis associated with consumption of turkey franks. *Morb. Mort. Weekly Rep.* **38**: 267-268.
- Basak, S., Ramaswamy, H.S., and Piette, J.P.G. 2002. High pressure destruction kinetics of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and concentrated orange juice. *Inn. Food Sci. Emerging Technol.* **3**: 223-231.
- Center for Disease Control. Listeriosis. Available at: <http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis>. Accessed March 31, 2004.

- Cruess, W.V. 1924. *Commercial fruit and vegetable products*. McGraw-Hill Book Co., Inc., New York.
- Donnelly, C.W. 2001. *Listeria monocytogenes*: A continuing challenge. *Nutr. Rev.* **59**: 183-194.
- Erkmen, O. and Dogan, C. 2004. Effects of ultra high hydrostatic pressure on *Listeria monocytogenes* and natural flora in broth, milk and fruit juices. *Inter. J. Food Sci. Technol.* **39**: 91-97.
- Farber, J.M. and Peterkin, P.I. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Micro. Rev.* **55**: 476-511.
- Farkas, D.F. and Hoover, D.G. 2000. High pressure processing. *J. Food Sci. Supplement* **64(4)**: 47-64.
- Garcia-Graells, C., Hauben, K.J., and Michiels, C.W. 1998. High pressure inactivation and sublethal injury of pressure-resistant *Escherichia coli* mutants in fruit juices. *Appl. Env. Micro.* **64(4)**: 1566-1568.
- Hashizume, C., Kimura, K., and Hayashi, R. 1995. Kinetic analysis of yeast inactivation by high pressure treatment at low temperatures. *Biosci. Biotechnol. Biochem.* **59**: 1455-1458.
- Hendrickx, M., Maesmans, G., De Cordt, S., Noronha, J., Van Loey, A., and Tobback, P. 1995. Evaluation of the integrated time-temperature effect in thermal processing of foods. *Crit. Rev. Food Sci. Nutr.* **35** (3): 231-262.
- Hite, B.H. 1899. The effect of pressure in the preservation of milk. *West Virginia Univ. Agric. Exp. St. Bull.* **58**: 15-35.

- Hoover, D.G., Metrick, C., Papineau, A., Farkas, D.F., and Knorr, D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. *Food Technol.* **43**(3): 99-107.
- Jaenicke, R. 1981. Enzymes under extreme conditions. *Ann. Rev. Biophys. Bioeng.* **10**: 1-67.
- Kalchayanand, N., Sikes, A., Dunne, C.P., and Ray, B. 1998. Interaction of hydrostatic pressure, time and temperature of pressurization and Pediocin AcH on inactivation of foodborne bacteria. *J. Food Prot.* **4**: 425-431.
- Mermelstein, N.M. 1998. High pressure processing begins. *Food Technol.* **52**(6): 104-108.
- Meyer, R., Kang, D., and Swanson, B.G. 2003. Ultra high pressure preservation of fruit preserves and pie fillings. *WTC Project No 504-A1*.
- Oxen, P. and Knorr, D. 1993. Baroprotective effects of high solute concentrations against inactivation of *Rhodotorula rubra*. *Lebens. Wissen. Technol.* **26**: 220-223.
- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., and Swanson, B.G. 1998. High hydrostatic pressure come-up time and yeast viability. *J. Food Prot.* **61**: 1657-1660.
- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J. and Swanson, B.G. 1997 a. Kinetic analysis of *Zygosaccharomyces bailii* inactivation by high hydrostatic pressure. *Lebens. Wissen Technol.* **30**: 703-708.

- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., and Swanson, B.G. 1997 b. High pressure treatment in food preservation. Chapter 22 In: Handbook of Food Preservation. Ed. by M. S. Rahman. Marcel Dekker, New York.
- Pandya, M.F., Jewett, F.F. and Hoover D.G. 1995. Concurrent effects of high hydrostatic pressure, acidity and heat on the destruction and injury of yeasts. *J. Food Prot.* **58**(3): 301-304.
- Parish, M.E. 1991. Microbial concerns in citrus juice processing. *Food Technol.* **45**(4): 128-132.
- Patterson, M.F., Quinn, M., Simpson, R., and Gilmour, A. 1995. Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. *J. Food Prot.* **58**(5): 524-529.
- Perrier-Cornet, J.P., Marechal, P.A., and Gervais, P. 1995. A new design intended to relate high pressure treatment to yeast cell mass transfer. *J. Biotechnol.* **41**: 49-58.
- Roberts, C.M. and Hoover, D.G. 1996 . Sensitivity of *Bacillus coagulans* spores to combination of high hydrostatic pressure, heat, acidity and nisin. *J. Appl. Bact.* **81**: 363.
- Rovere, P., Carpi, G., Maggi, A., Gola, S., and Dall'Aglio, G. 1994. Stabilization of apricot puree by means of high pressure treatments. *Prerhambeno-technol. Biotechnol. Rev.* **32**(4): 145-150.
- San Martin, M.F., Barbosa-Canovas, G.V., and B.G. Swanson. 2002. Food processing by high hydrostatic pressure. *Crit. Rev. Food Sci. Nutr.* **42** (6): 627-645.

- Shimada, S., Andou, M., Naito, N., Yamada, N., Osumi, M., and Hayashi, R. 1993. Effects of hydrostatic pressure on the ultrastructure and leakage of internal substances in the yeast *Saccharomyces cerevisiae*. *Appl. Micro. Biotechnol.* **40**: 123-131.
- Silva, F.V. and Silva, C.L. 1997. Quality optimization of hot filled pasteurized fruit purees: container characteristics and filling temperatures. *J. Food Eng.* **32**: 351-364.
- Simpson, R.K. and Gilmour, A. 1997. The effect of high hydrostatic pressure on *Listeria monocytogenes* in phosphate-buffered saline and model food systems. *J. Appl. Micro.* **83**: 181-188.
- Smelt, J.P. 1995. Some mechanistic aspects of inactivation of bacteria by high pressure. *In: Proceedings of the European Symposium – Effects of High Pressure on Foods*. France: University of Montpellier, February 16-17 (1995).
- Zook, C.D., Parish, M.E., Braddock, R.J., and Balaban, M.O. 1999. High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juice. *J. Food Sci.* **64**(3): 533-535.

Chapter Two

Ultra High Pressure Inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* on Apples and Blueberries

Abstract

This study was undertaken to determine the pressure and time required to inactivate *Saccharomyces cerevisiae* and *Listeria innocua* on apples and blueberries while maintaining a “fresh like” fruit texture and color. D-values for *Saccharomyces cerevisiae* and *Listeria innocua* inoculated on diced apples and blueberries following ultra high pressure treatment at 300 and 375 MPa at room temperature were determined. *Saccharomyces cerevisiae* D-values for 300 MPa ranged from 19.7 s on diced apples to 22.4 s on blueberries. *Listeria innocua* D-values for 375 MPa ranged from 67.1 s on diced apples to 46.7 s on blueberries. Ultra high pressure is an efficient and effective option for inactivating *Saccharomyces cerevisiae* and *Listeria innocua* on apples, blueberries or other fruit while preserving a fresh appearance and texture.

Key Words: Nonthermal processes, *Listeria innocua*, *Saccharomyces cerevisiae*, microbial inactivation, fruit.

Introduction

The idea of using pressure as a process variable in food processing is not new. The first report of ultra high pressure treatments inactivating bacteria was conducted by H. Roger in 1895. Inactivation studies of bacteria in food were conducted by Bert Hite (Hite, 1899). Hite demonstrated that the shelf life of milk was extended for four days after pressure treatment at 600 MPa for 1 h at room temperature. For the last 20 years, the use of ultra high pressure was extensively explored by the food industry and related research institutions due to the increased demand by consumers for improved nutritional and sensory characteristics of food without loss of "fresh" taste. In recent years, ultra high pressure was extensively used in Japan to stabilize a variety of food products like jams and fruit-juices (Tewari et al., 1999).

Fruit products treated with high temperatures frequently exhibit a cooked flavor, and a loss of texture, color, and nutrient quality. Nonthermal ultra high pressure processing exhibits the potential of achieving a "fresh like" fruit product desired by consumers while inactivating pathogenic and spoilage microorganisms. Other advantages of ultra high pressure treatment over traditional thermal processing of preserved fruit include reduced process times; minimal heat damage problems; retention of flavor; little vitamin C loss; few undesirable changes in fruit during pressure shift freezing due to reduced crystal size and multiple ice-phase forms; and minimal undesirable functionality alterations (Tewari et al., 1999). Kimura et al. (1994) compared the quality (flavor components, color, hue, and nutrients) of pressure treated (400-500 MPa, 10-30 min at room temperature) and heat treated (93 °C, 20 min) strawberry jams during storage at 5 and 25 °C for 1-3 months. Immediately after

processing, the pressure treated jams exhibited better fresh quality than heat treated jams. The quality of the heat treated jams after 3 months storage was maintained in both at low temperature and room temperature. The quality for pressure treated jams after 3 months storage was maintained at low temperatures, but not at room temperature. The deterioration of pressure treated jams held at ambient temperature was attributed to the presence of dissolved oxygen and enzymes. However, pressure treated jams were stored at refrigeration temperatures with minimal loss in sensory and nutritional characteristics for up to 3 months.

Gow and Hsin (1996) compared the quality and shelf life of pressure treated (600 MPa for 15 min at 25 °C) with thermally pasteurized (88-90 °C for 24 s) guava puree. At 600 MPa a substantial inactivation of microbes (6 log reduction) was observed with small color changes, degradation of pectin, cloud formation, and equivalent ascorbic acid content as the fresh guava puree. However, enzyme inactivation (polyphenoloxidase, pectin methylesterase, and polygalacturonase) was more pronounced in thermally treated guava puree. The pressure treated guava puree (600 MPa for 15 min at 25 °C) maintained quality similar to freshly extracted guava puree for 40 days when stored at 4 °C.

Successful inactivation of microorganisms in foods by ultra high pressure treatment is reported by numerous researchers (Alpas et al., 1998; 2003; Arroyo et al., 1999; Aleman et al., 1998, Palou et al; 1997; 1998). Farkas and Hoover (2000) reported that the effectiveness of microbial inactivation in foods under high pressure treatment depends on: pressure, time to achieve treatment pressure, time at pressure, decompression

time, food initial temperature, treatment temperature, food composition, food pH, food water activity, and packaging material integrity.

Kinetic parameters are necessary for the validation of high pressure food processing as a means of ensuring food safety. The inactivation of microorganisms assumes a first order linear relationship between compression time and microbial population. Several non-linear models have also been studied, but without strong evidence to support alternative needs of non linear assumptions, first order kinetics may be used (Farkas and Hoover, 2000). Parish (1998) studied ultra high pressure inactivation of *Saccharomyces cerevisiae* in unpasteurized orange juice (pH 3.7) treated at pressures between 350 and 500 MPa at selected times and room temperature. D-values varied from 1 to 38 s. Palou et al. (1997) investigated inactivation kinetics of *Zygosaccharomyces bailii*, a fruit juice spoilage yeast, in model food systems at pH 3.5 and water activities of 0.98 and 0.95 treated with pressures from 241 to 517 MPa for selected times at 21 °C. First order kinetics was observed, with inactivation rates and D-values that varied from 0.176 to 2.833 min⁻¹ and from 13.1 to 0.8 min, respectively. Zook (1999) determined the inactivation kinetics of *Saccharomyces cerevisiae* YM-147 in orange and apple juices as well as a model juice buffer which consisted of 8 g/L citric acid, 50 g/L sucrose, 25 g/L fructose and 2.5 g/L D-glucose at pH 3.5 to 5.0. Juices and model juice buffers were ultra high pressure treated at pressures ranging from 300 to 500 MPa for 1 s to 30 min at 34 to 43 °C. D-values varied from 10.8 min to 8 s, respectively. Mussa et al. (1999) observed first order inactivation kinetics for *Listeria monocytogenes* Scott A and the natural microflora of raw milk after treatments of 150 to 350 MPa for selected times at 18 °C. The D-values for *Listeria monocytogenes* and natural microflora

at 150, 250, 300, and 350 MPa were 84.4, 46.0, 26.6, 13.9 min, and 64.0, 25.6, 17.6, 9.19 min, respectively. The inactivation rates for *Listeria monocytogenes* varied from 0.027 to 0.165/min⁻¹ and for natural microflora from 0.036 to 0.251/min⁻¹.

Fruit products such as purees, pie fillings, preserves, and juices are widely consumed in the United States. Fruit products processed by ultra high pressure treatment must ensure a satisfactory reduction in the initial microbial counts to obtain extended shelf life and microbial safety. The objective of the current research was to investigate D-values obtained with selected ultra high pressure treatments to inactivate spoilage microorganisms such as *Saccharomyces cerevisiae* and pathogenic microorganisms such as *Listeria innocua* inoculated on diced apples and blueberries.

Materials and Methods

Preliminary Studies

Preliminary studies were carried out to estimate the pressure and time required to inactivate *Listeria innocua* and *Saccharomyces cerevisiae* on diced apples and whole blueberries, strawberries and grapes. Fruits were high pressure treated at 300, 450 and 600 MPa for 0.75, 1.5, or 3 min at 21 °C. *Listeria innocua* was plated on Oxford Agar (Difco) and *S. cerevisiae* was plated on Yeast and Mold Petrifilm (3M). Cell counts were conducted after 48 h of incubation at 37 °C or 32 °C, respectively.

Furthermore in the preliminary study, observation of the survivor curves of *Saccharomyces cerevisiae* and *Listeria innocua* on diced apples and blueberries were

designed. Pressures were selected to result in moderate destruction only. One pressure each for *Saccharomyces cerevisiae* and for *Listeria innocua* was chosen: 300 MPa and 375 MPa, respectively.

Preparation of Inocula and Inoculation of Apples and Blueberries

Four *Saccharomyces cerevisiae* cultures (ATTC 2601, ATTC 9763, UCD 522 and Pasteur Red) obtained from the Food Science and Human Nutrition yeast collection at Washington State University (Pullman, WA) were used to inoculate Granny Smith diced apples and blueberries. Specifications of apples and blueberries are presented in Table 1. *Saccharomyces cerevisiae* colonies were revived on Yeast Malt broth and incubated at 25 °C for 48 h to promote multiplication of cells. A sterile loop (5-10 µl) of Yeast Malt broth with each *Saccharomyces cerevisiae* strain was cultured into three 9 ml tubes of Yeast Malt broth at 32 °C for 24 h (stationary phase), harvested by centrifugation at 2,200 x g for 25 min at 4 °C (IEC Centa – CL2 Centrifuge, Needham, MA) and washed three times with buffered peptone water. The final pellet was resuspended in buffered peptone water, to give approximately to 10⁷⁻⁸ yeast cells per milliliter

Five to six grams of diced apples and blueberries were placed in a biosafety laminar flow hood with the fan on (22 ± 2 °C). One hundred microliters of *Saccharomyces cerevisiae* suspension were applied to the skin on top of each diced apple and blueberry by depositing droplets at ten locations (on each piece of fruit) with a micropipetor. Inoculated fruit were held in the hood for three hours with the fan on.

Listeria innocua ATTC 33090, ATTC 51742, SEA 15C19, and SEA 15C20 were used as surrogates of *Listeria monocytogenes*. *Listeria innocua* cultures were maintained on Brain-heart infusion agar slants (Difco Laboratories, Sparks, MD). A sterile loop of each *Listeria innocua* strain was transferred into three 9 ml tubes of Tryptose Broth (Difco Laboratories, Sparks, MD) with 1% yeast at 37 °C for 24 h (stationary phase), harvested by centrifugation at 2,200 x g for 25 min, and washed three times with buffered peptone water. The final pellet was resuspended in buffered peptone water, to give approximately 10⁷⁻⁸ viable cells per milliliter. Five to six grams of Granny Smith diced apples and blueberries were placed in a biosafety laminar flow hood with the fan on (22 ± 2 °C). One hundred microliters of *Listeria innocua* suspension were applied to the skin on top of each diced apple and blueberry by depositing droplets at ten locations (on each piece of fruit) with a micropipettor. Inoculated fruit were held in the hood for three hours with the fan on.

Ultra High Hydrostatic Pressure Treatment

Pressure treatments were applied with an adiabatic system (Engineering Pressure Systems, Inc. National Forge Co. Andover, MA), including a pressure vessel, pump system and heating control system. The pressure vessel (Engineering Pressure Systems, Inc. National Forge Co. Andover, MA) delivers a maximum pressure of 689 MPa. The hydrostatic pump (Hydro Pac Inc., Fairview, PA) provides 689 MPa within the vessel after a minimal compression time of approximately 5 min. An installed electric heater heats and maintains the vessel to selected temperatures with a maximum temperature of 90 °C ± 2.

Inoculated apples and blueberries were sealed in two ethylene-vinyl alcohol copolymer film (EVOH) bags. An inner bag was filled with 25 ml of peptone water and five to six grams of inoculated fruit, then double heat sealed. The outer bag was filled with 50 ml of deionized water and double heat sealed. Prior to sealing air in both bags was squeezed out to remove air bubbles.

After the chamber was loaded with inoculated fruit and closed, the vessel of the chamber was filled with pressure transmitting medium, 5% Mobil Hydrasol 78 water solution. Diced apples or blueberries inoculated with *Saccharomyces cerevisiae* were double sealed and subjected to ultra high hydrostatic pressure treatment at 300 MPa and 21 °C. Times selected were 0 (come up time of 2.8 min with no hold), 20, 40, 60, 80 and 100 s. Diced apples or blueberries inoculated with *Listeria innocua* were subjected to ultra high hydrostatic pressure treatment at 375 MPa and 21 °C. Times selected were 0 (come up time of 3.2 min with no hold), 30, 60, 90, 120, 150 and 180 s.

Each experiment was conducted in triplicate. The pressure treated inoculated fruits were immersed in iced water (2 °C) immediately after high pressure treatment and held for 2 h to promote recovery of the pressurized cells (Mussa et al., 1999).

Enumeration of Surviving Cells

After a 2 h resuscitation period, the bags containing inoculated fruit were aseptically opened and homogenized in a stomacher (Sewer LTD., Model 80, Norfolk, UK) for 2 min at medium speed. After homogenization, untreated (control) and pressure treated fruit inoculated with *Saccharomyces cerevisiae* were enumerated by spread plating 1 ml of homogenized fruit on Yeast and Mold Petrifilm (3M Microbiology

Products, St Paul, MN). A similar procedure was performed with *Listeria innocua* by spread plating 100 μ l of inoculated homogenized fruit on Oxford Agar (Difco Laboratories, Detroit, MI,). Inoculated fruit was serially 10-fold diluted with 9 ml sterile buffered peptone water. Cell counts were conducted after 48 h of incubation at 32 °C for *Saccharomyces cerevisiae* or 37 °C for *Listeria innocua*. The enumeration means from three independent replicates of *Saccharomyces cerevisiae* and *Listeria innocua* in each fruit were recorded and plotted to determine inactivation rates and D-values.

Mathematical Analysis

The inactivations of microorganisms during the pressure hold times were assumed to follow a first order kinetic process. Linear regression analyses were performed on the survival data for each microorganism using Excel Microsoft Office software (Microsoft Corporation, Version 2002, Seattle, WA. USA). The linear regression analyses provided a regression coefficient (R^2) and an estimate of the intercept and slope of a straight line for each survivor curve. Inactivation rates (k) were calculated from the reciprocal of the slope of the survival curves. The D-values (time required for 90% destruction of microorganisms) were estimated from the slope of the regression line obtained from the linear portion of the survival curve of the plot of log CFU/ml vs. pressurization time ($D = 2.303/k$).

Statistical Analysis

Data were analyzed with a computer package (Minitab Statistical Software, State College, PA) using analysis of variance to assess the mean and variability reported as

standard deviation, Fisher's least significant difference (LSD) was calculated to interpret the differences among fruits and microorganisms. The statistical significance was established at the 5% significance level ($p \leq 0.05$).

Results and Discussion

Preliminary studies were performed on apples, blueberries, strawberries and grapes with high pressure treatments of 300, 450 and 600 MPa for 0.75, 1.5, or 3 min at 21 °C (Figs 1-8). The effective parameters for *Saccharomyces cerevisiae* inactivation (6 log CFU/ml) were 300 MPa for 1.5 min at 21 °C on grapes and strawberries. *Saccharomyces cerevisiae* on apples required 300 MPa for 3 min at 21 °C to reach undetectable levels (< 1 Log CFU/ml). *Saccharomyces cerevisiae* was more resistant to inactivation on blueberries than other fruits. Undetectable levels (< 1 Log CFU/ml) of *Saccharomyces cerevisiae* on blueberries were reached with 450 MPa for 3 min at 21 °C. The effective parameters for *Listeria innocua* inactivation (7 Log CFU/ml) on strawberries, apples and blueberries were 450 MPa for 1.5 min at 21 °C. *L. innocua* on grapes required 450 MPa for 3 min at 21 °C for inactivation (< 1 Log CFU/ml).

The results of the preliminary studies demonstrated that *Saccharomyces cerevisiae* and *Listeria innocua* are inactivated with increasing pressure treatments. Ultra high pressure can induce tetraploidy in *Saccharomyces cerevisiae* (Hamada et al., 1992), indicating that ultra high pressure can interfere with replication of DNA. At a pressure of approximately 100 MPa the nuclear membrane shape of yeasts was altered, and at more than 400 MPa the mitochondria and the cytoplasm were deformed or

disrupted (Shimada et al., 1993). Isaacs et al. (1995) demonstrated that ribosomal destruction of *E. coli* and *L. monocytogenes* by electron microscopy in high pressure treated cells will result in the widespread impairment of cell functions, reduced growth rate and cell inactivation.

Furthermore in the preliminary study, observation of the survivor curves of *Saccharomyces cerevisiae* and *Listeria innocua* on apples and blueberries were plotted. Pressures were selected to result in moderate destruction only. One pressure each for *Saccharomyces cerevisiae* and for *Listeria innocua* was chosen: 300 MPa and 375 MPa, respectively.

The ultra high pressure survivor curves for both *Saccharomyces cerevisiae* and *Listeria innocua* on apples and blueberries were plotted as Log CFU/ml as a function of holding time at constant pressure, (Figs 9 and 10). Apparent first order kinetics for microbial inactivation was observed, and a first order kinetic model was used to calculate the rate of inactivation from this experiment. The inactivation rates (k) were calculated from the reciprocal of the slope of the survival curves. The D-values were estimated to assess the pressure resistance of microorganisms. The regression coefficient (R^2) measured the closeness of fit of the regression line to the survival curve. Table 2 presents k , D-values and R^2 .

Inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* on fruit during pressure hold time followed the first order model, as reflected in high (> 0.88) correlation values (R^2). A slight shouldering was observed in *Listeria innocua* survival curve on diced apples and blueberries after 90 s of ultra high pressure treatment. Slight shouldering in the survival curves may be attributed to microbial population

heterogeneity, adaptation and recovery of microorganisms during and after pressure treatments, or as a result of experimental errors (Palou et al. 1997 b). *Saccharomyces cerevisiae* D_{300} values at 21 °C ranged from 19.7 s on diced apples to 22.4 s on blueberries. *Listeria innocua* D_{375} values at 21 °C ranged from 67.1 s on diced apples to 46.7 s on blueberries. There were significant differences ($p \leq 0.05$) in D-values at constant pressures between both microorganisms. As expected, *Listeria innocua* is more pressure resistant than *Saccharomyces cerevisiae* on fresh fruit.

First order inactivation rates and decimal reduction times following ultra high pressure treatments are reported previously. For example, *Zygosaccharomyces bailii*, a fruit juice spoilage yeast, was studied in model food systems at pH 3.5 and water activities of 0.98 and 0.95 treated with pressures from 241 to 517 MPa for selected times at 21 °C. In this study, first order kinetics was observed, with k and D-values that varied from 0.176 to 2.833 min^{-1} and from 13.1 to 0.8 min, respectively. Hashizume et al. (1995) observed that pressure inactivation kinetics for *Saccharomyces cerevisiae* suspended in 8.5 g/l saline solutions at water activities of approximately 0.99 follows a first order kinetic model with a D-value of 12.3 min at 270 MPa and 25 °C. Parish (1998) studied ultra high pressure inactivation of *Saccharomyces cerevisiae* on unpasteurized orange juice (pH 3.7) treated at pressures between 350 and 500 MPa at selected times and room temperature. D-values ranged from 1 to 38 s. Zook (1999) determined the inactivation kinetics of *Saccharomyces cerevisiae* YM-147 in orange and apple juices as well as a model juice buffer which consisted of 8 g/L citric acid, 50 g/L sucrose, 25 g/L fructose and 2.5 g/L d-glucose at pH 3.5 to 5.0. All juice and model

juices were ultra high pressure treated at pressures ranging from 300 to 500 MPa for 1 s to 30 min at 34 to 43 °C. D-values varied from 10.8 min to 8 s, respectively.

Carlez et al. (1993) reported a first order kinetic model with D-values of 5 min at 360 MPa and 20 °C for *Listeria innocua* inoculated in minced meat. Mussa et al. (1999) observed first order inactivation kinetics of *Listeria monocytogenes* Scott A and the natural microflora of raw milk after treatments of 150 to 350 MPa for selected times at 18 °C. The D-values for *Listeria monocytogenes* and natural microflora at 150, 250, 300, and 350 MPa were 84.4, 46.0, 26.6, and 13.9 min, and 64.0, 25.6, 17.6, and 9.19 min, respectively. The inactivation rates ranged from 0.027 to 0.165 min⁻¹ for *Listeria monocytogenes* and from 0.036 to 0.251 min⁻¹ for natural microflora.

Table 3 presents come up times for pressure treatments. The pressure come up times exert an important effect on the survival fraction by reducing the number of microorganisms present on the fruits. *Saccharomyces cerevisiae* counts decreased 1.11 and 1.28 Log CFU/ml on diced apples and blueberries after 2.8 min of come up time. *Listeria innocua* counts decreased 0.36 and 0.28 Log CFU/ml on diced apples and blueberries after 3.2 min of come up time. Palou et al. (1997 a) reported 2 log cycle reductions of *Zygosaccharomyces bailii* after 3.7 min of come up time on model systems (a_w 0.95) treated at 431 MPa and 21 °C. Cheftel (1995) reported that the rate of pressure increase and decrease is often neglected as an experimental variable in ultra high pressure microbial inactivation studies, and the initial population can be notably reduced during come up time.

Saccharomyces cerevisiae inactivation rates were 0.12 s⁻¹ on diced apples and 0.10 s⁻¹ on blueberries. *Listeria innocua* inactivation rates were 0.04 s⁻¹ on diced apples

and 0.05 s^{-1} on blueberries. Although pressures are different (300 and 375 MPa, respectively) the calculated inactivation rates were similar for apples and blueberries, indicating that chemical composition of the fruits during ultra high pressure treatment does not affect microbial inactivation. D-values were not significantly different ($p \geq 0.05$) among diced apples and blueberries, suggesting that microorganisms on fruit will be effectively and similarly inactivated with ultra high pressure treatment. No protective or detrimental effects associated with fruit constituents were observed. Malic acid and citric acid are the major organic acids in apples and blueberries, respectively. No difference for ultra high pressure inactivation of *Saccharomyces bayanus* cells in de-acidified mandarin orange juice supplemented with 0.70 % citric acid or malic acid were observed (Ogawa et al. 1990). Our results on diced apples or blueberries were supported by previous experiments treating fruit with ultra high pressure.

Ultra high pressure inactivation of yeast and vegetative bacteria on fruit is effective because of fruit acidity and inherent low pH (< 4.0). As pH is lowered most microbes become more susceptible to ultra high pressure inactivation, and recovery of sublethally injured cells is reduced (Farkas and Hoover, 2000). When microorganisms are grown in an acid pH (< 4.6) environment, the flow of protons across the cell membrane regulates pH homeostasis of the cells. The flow of protons requires membrane bound (Na, K)-ATPase to transport protons across the membrane (Parkson et al., 1985). A decrease in volume of the cell membrane during ultra high pressure treatment changes the structural conformation of enzymes leading to inhibition of the proton transport system (Parkson et al., 1985). Enhanced permeability of cell membranes following ultra

high pressure treatment also leads to ATP dissipation and inability of cells to maintain pH homeostasis (Parkson et al., 1985).

The factors that affect the pressure sensitivity of microorganisms on foods include the type and growth phase of microorganism, composition of the dispersion medium, and rate of pressurization (Farkas and Hoover, 2000). Furthermore, it is generally recognized by process microbiologists that microbial destruction kinetics results are often influenced by the manner in which the cells are grown and handled, the method of inoculum preparation, and handling procedures.

Conclusions

The D-values of *Saccharomyces cerevisiae* or *Listeria innocua* on fresh fruit can be obtained with pressure treatments at pressures of 300 or 375 MPa at 21 °C respectively, over short times of 22 to 67 s. *Saccharomyces cerevisiae* D-values for 300 MPa were 19.7 s on diced apples and 22.4 s on blueberries. *Listeria innocua* D-values for 375 MPa were 67.1 s on diced apples and 46.7 s on blueberries. The pressure come-up times exert an important effect on the survival fraction by reducing the number of microorganisms present on the fruits. There were no significant differences in D-values ($p \geq 0.05$) among inoculated fruits. *Saccharomyces cerevisiae* or *Listeria innocua* on diced apples and blueberries presented similar D- values. Ultra high pressure is suitable for the inactivation of *Saccharomyces cerevisiae* or *Listeria innocua* on fresh fruit.

Acknowledgment

Funding for this research was provided by the Washington Technology Center and Washington Farms, Inc. TM 3813 East 80th Street, Tacoma, WA.

References

- Aleman, G.D., Ting, E.Y., Farkas, D.F., Mordre, S.C., Hawes, A.C., and Torres, J.A. 1998. Comparison of static and step-pulsed ultra-high pressure on the microbial stability of fresh cut pineapple. *J. Food Agric.* **76**: 383-388.
- Alpas, H., Kalchayanand, N., Bozoglu, F., and Ray, B. 1998. Interaction of pressure, time and temperature of pressurization on viability loss of *Listeria innocua*. *World J. Micro. Biotechnol.* **14**: 251-253.
- Alpas, H. and Bozoglu, F. 2003. Efficiency of high pressure treatment for destruction of *Listeria monocytogenes* in fruit juices. *FEMS Immunol. Med. Micro.* **35**: 269-273.
- Arroyo, G., Sanz, P.D., and Préstamo, G. 1999. Response to high pressure, low temperature treatment in vegetables: determination of survival rates of microbial populations using flow cytometry and detection of peroxidase activity using cofocal microscopy. *J. Applied Micro.* **86**: 554-556.
- Carlez, A., Rosec, J.P., Richard, N., and Cheftel, J.C. 1993. High pressure inactivation of *Citobacter freundii*, *Pseudomonas fluorescens* and *Listeria innocua* in inoculated minced beef muscle, *Lebensm. Wiss. Technol.* **26**:357-363.
- Cheftel, J.C. 1995. High pressure, microbial inactivation and food preservation. *Food Sci. Technol. Inter.*, **1**: 75-90.

- Farkas, D.F. and Hoover, D.G. 2000. High pressure processing. *J. Food Sci. Suppl.* 64(4): 47-64.
- Gow, C.Y. and Hsin, T.L. 1996. Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf life of guava puree. *Int. J. Food Sci.* 31: 205-213.
- Hamada, K., Nakatomi, Y., and Shimada, S. 1992. Direct induction of tetraploids or homozygous diploids in the industrial yeast *Saccharomyces cerevisiae* by hydrostatic pressure. *Curr. Genet.* 22: 371-376.
- Hashizume, C., Kimura, K., and Hayashi, R. 1995. Kinetic analysis of yeast inactivation by high pressure treatment at low temperatures, *Biosci. Biotechnol. Biochem.* 59: 1455-1458.
- Hite, B.H. 1899. The effect of pressure in the preservation of milk. *West Virginia Univ. Agric. Exp. St. Bull.* 58: 15-35.
- Isaacs, N.S., Chilton, P., and Mackey, B. 1995. Studies on the inactivation of microorganisms by high pressure. In: High Pressure Food Processing. Ed. by D.A. Ledward, D.E. Johnston, R.G. Earnshaw and A. Hasting, Nottingham University Press.
- Kimura, K., Yosida, Y., Ohki, K., Fukumoto, T., and Sakui, N. 1994. Comparison of keeping quality between pressure-processed jam and heat-processed jam: changes in flavor components, hue, and nutrients during storage. *Biosci. Biotech. Biochem.* 58: 1386-1391.
- Ogawa, H., Fukuhisa, K., Kubo, Y., and Fukumoto, H. 1990. Pressure inactivation of yeasts, molds, and pectinesterase in Satsuma mandarin juice:

- Effects of juice concentration, pH, and organic acids, and comparison with heat sanitation. *Agric. Biol. Chem.* **54**(5):1219-1225.
- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., and Swanson, B.G. 1998. High hydrostatic pressure come-up time and yeast viability. *J. Food Prot.* **61**: 1657-1660.
 - Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J., and Swanson, B.G. 1997 a. Kinetic analysis of *Zygosaccharomyces bailii* inactivation by high hydrostatic pressure. *Lebens. Wissen Technol.* **30**: 703-708.
 - Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., and Swanson, B.G. 1997 b. High pressure treatment in food preservation. Chapter 22 In: Handbook of Food Preservation. Ed. by M.S.Rahman. Marcel Dekker, New York.
 - Parish, M.E. 1991. Microbial concerns in citrus juice processing. *Food Technol.* **45**(4): 128-132.
 - Parkson, L., Chong, G., Fortes, P.A.G., and Jamenson, D.M. 1985. Mechanisms of inhibition of (Na, K)-ATPase by hydrostatic pressure studied with fluorescent probes. *J. Biol. Chem.* **260**(27), 14484-14490.
 - Mussa, D.M., Ramaswamy, H.S., and Smith J.P. 1999. High pressure destruction kinetics of *Listeria monocytogenes* in milk. *Food Res. Int.* **31**:343-350.
 - Shimada, S., Andou, M., Naito, N., Yamada, N., Osumi, M. and Hayashi, R. 1993. Effects of hydrostatic pressure on the ultra structure of internal substances in the yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **40**: 123-131.
 - Tewari, G., Jayas, D.S., and Holley, R.A. 1999. High pressure processing of foods: an overview. *Sci. des Aliments.* **19**: 619-661.

- Zook, C.D., Parish, M.E. Braddock, R.J., and Balaban, M.O. 1999. High pressure inactivation kinetics of *Saccharomyces cerevisiae* ascospores in orange and apple juices. *J. Food Sci.* **64**(3): 533-535.

Fig. 1 High pressure (300, 450 and 600 MPa) inactivation of *Saccharomyces cerevisiae* on diced apples

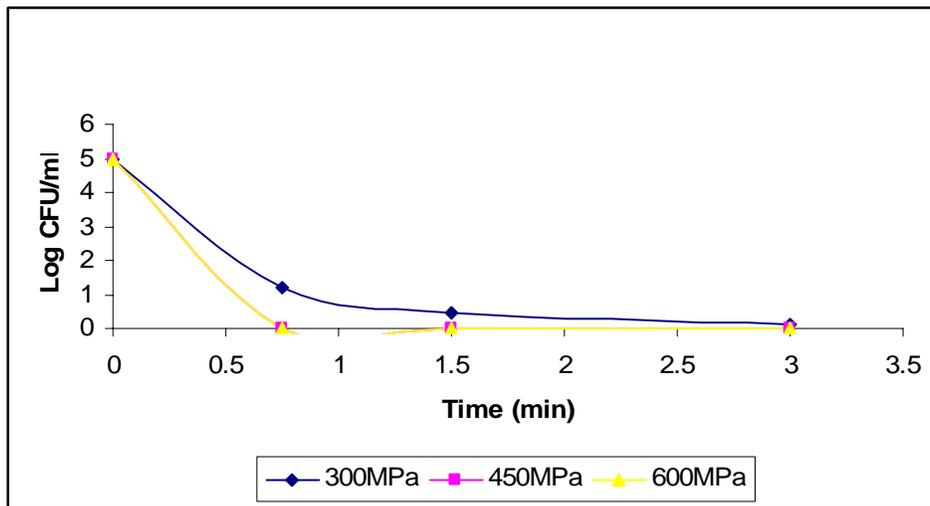


Fig. 2 High pressure (300, 450 and 600 MPa) inactivation of *Saccharomyces cerevisiae* on grapes

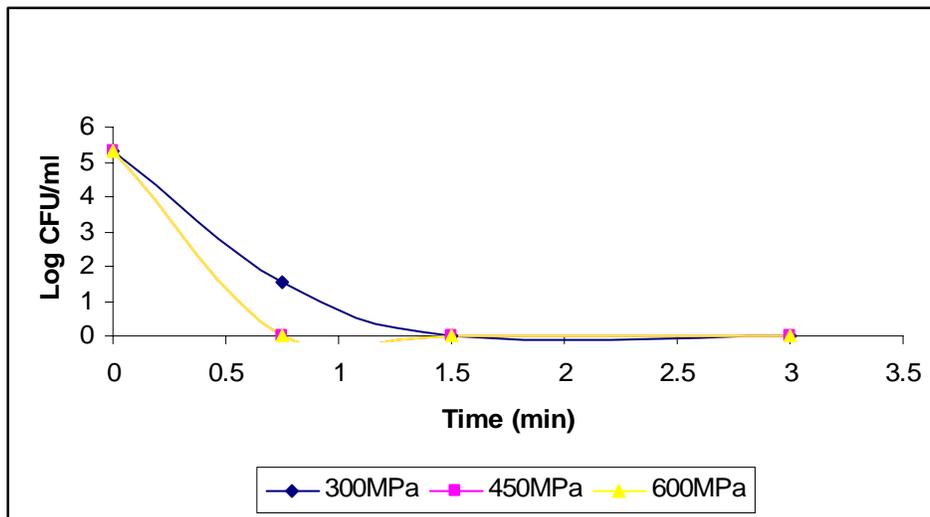


Fig. 3 High pressure (300, 450 and 600 MPa) inactivation of *Saccharomyces cerevisiae* on strawberries

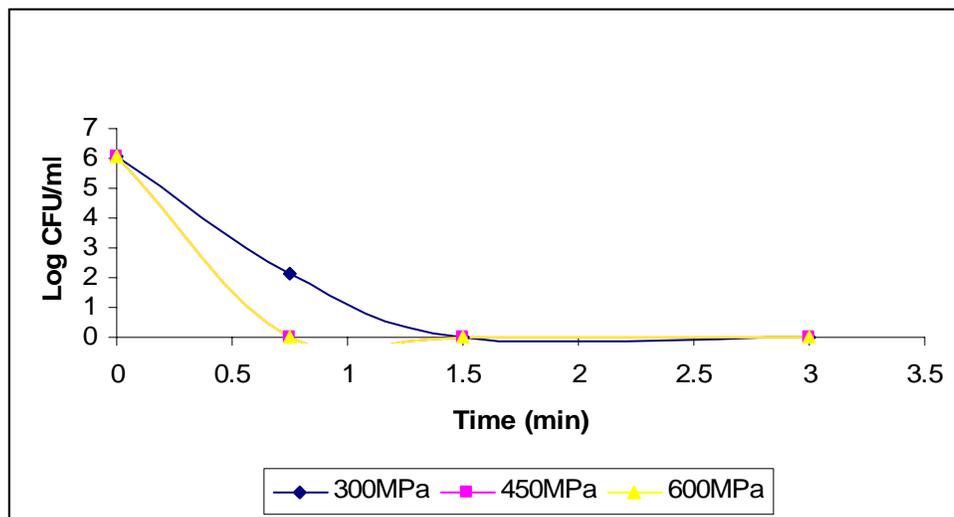


Fig. 4 High pressure (300, 450 and 600 MPa) inactivation of *Saccharomyces cerevisiae* on blueberries

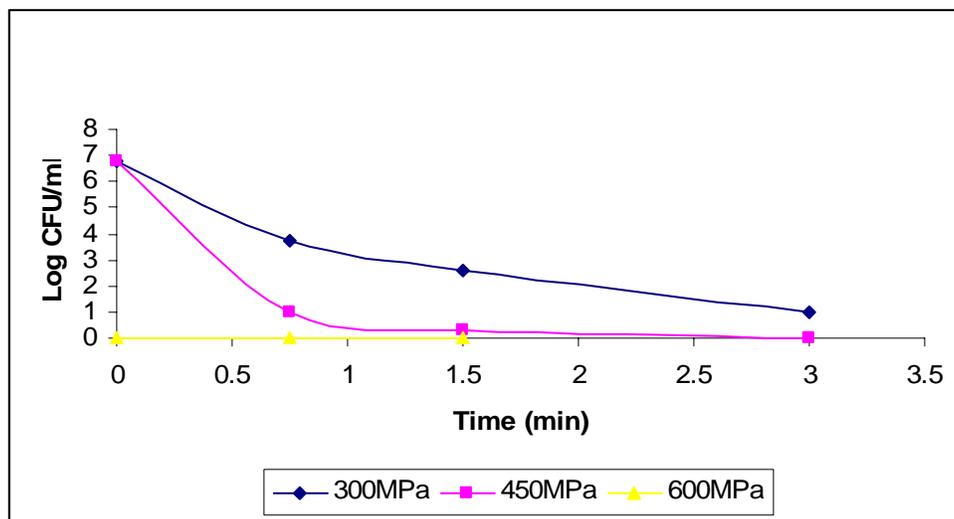


Fig. 5 High pressure (300, 450 and 600 MPa) inactivation of *Listeria innocua* on diced apples

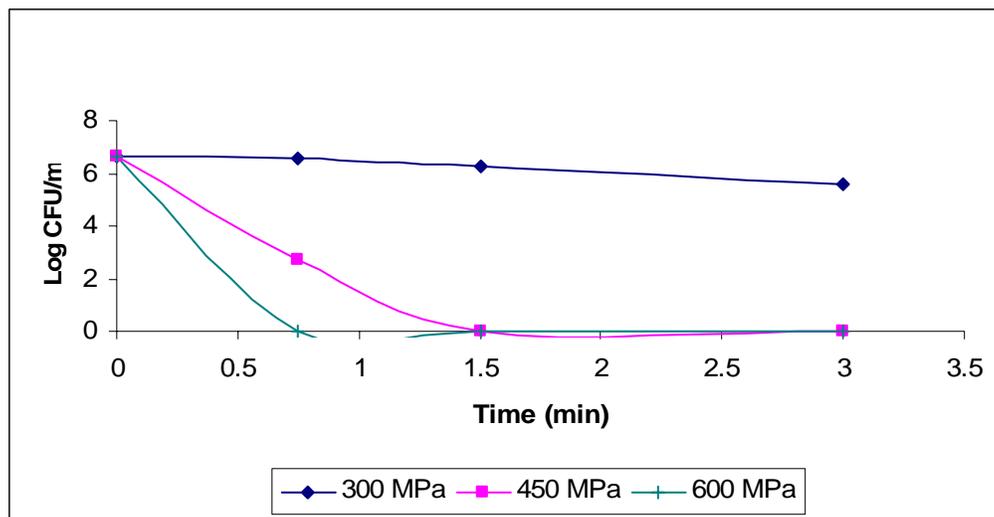


Fig. 6 High pressure (300, 450 and 600 MPa) inactivation of *Listeria innocua* on grapes

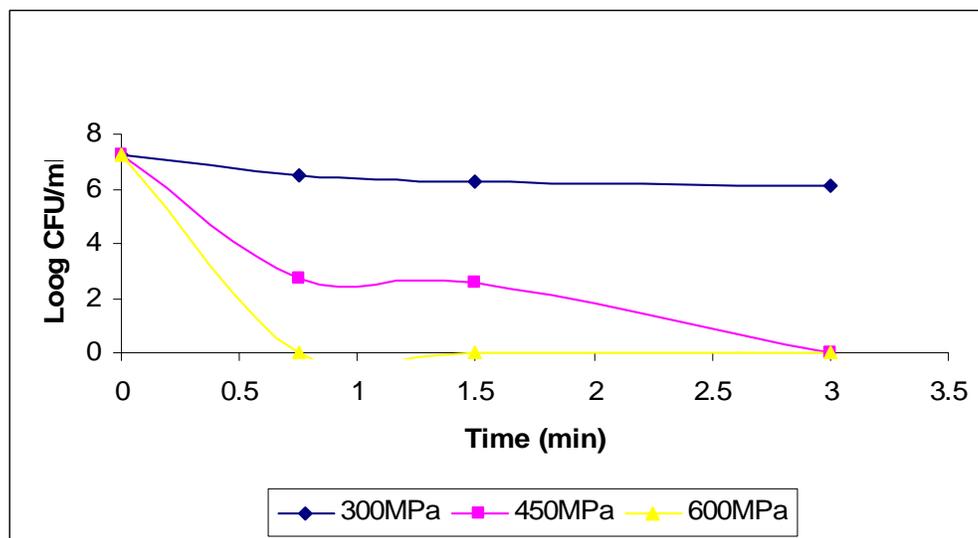


Fig. 7 High pressure (300, 450 and 600 MPa) inactivation of *Listeria innocua* on strawberries

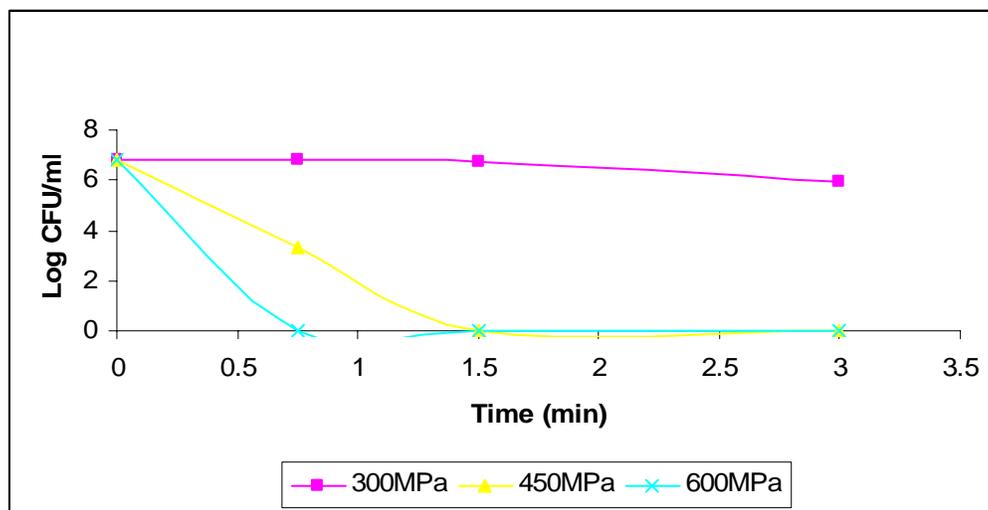


Fig. 8 High pressure (300, 450 and 600 MPa) inactivation of *Listeria innocua* on blueberries

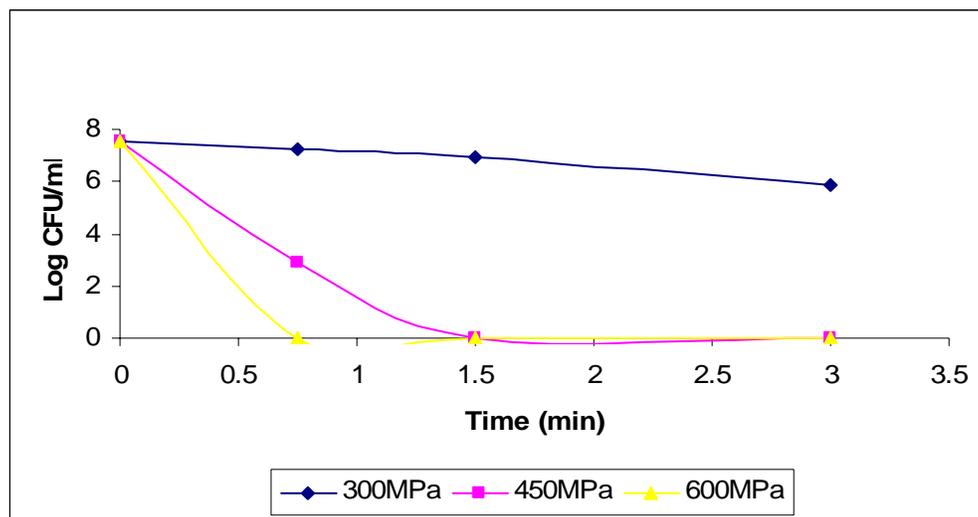


Fig. 9 High pressure (300 MPa) inactivation of *Saccharomyces cerevisiae* on diced apples and blueberries

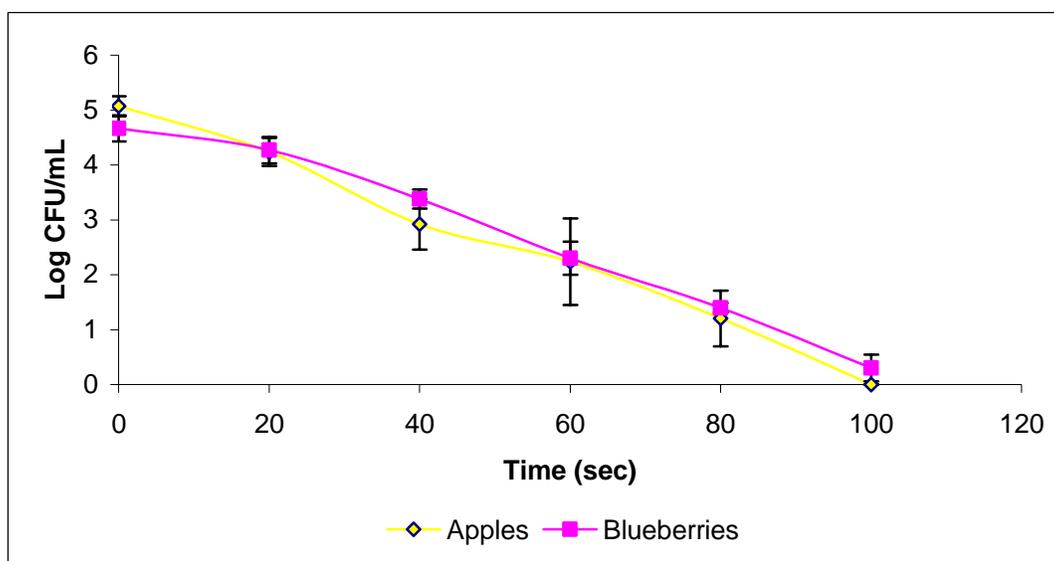


Fig. 10 High pressure (375 MPa) inactivation of *Listeria innocua* on diced apples and blueberries

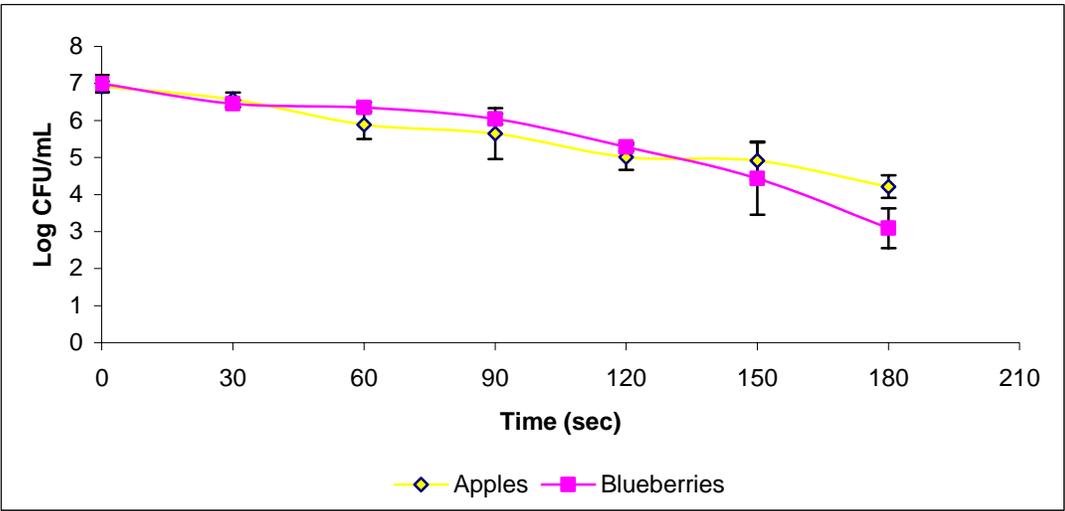


Table 1 Specifications of apples and blueberries

	Apples	Blueberries
pH	3.20	3.00
% Brix	12.58	11.60
% Total Solids	20.95	19.00
% Moisture	79.00	81.00

Table 2 Ultra high pressure inactivation rates (k), D-values (D), and regression coefficients (R²) of *Saccharomyces cerevisiae* and *Listeria innocua* on apples and blueberries

	Pressure (MPa)	k (s ⁻¹) ^c		D (s) ^c		R ²
		\bar{X}	S _x	\bar{X}	S _x	
<i>Saccharomyces cerevisiae</i>	300 MPa					
Apples		0.12	0.00	19.74 ^a	0.38	0.98
Blueberries		0.10	0.00	22.36 ^a	0.86	0.97
<i>Listeria innocua</i>	375 MPa					
Apples		0.04	0.01	67.05 ^b	10.96	0.93
Blueberries		0.05	0.01	46.71 ^b	9.04	0.88

^c Means (\bar{X}) and standard deviations (S_x) of three determinations.

R² Regression coefficient from survival curves of three determinations

D-values with different superscript letter are significantly different (p ≤ 0.05)

Table 3 Pressure come up time and inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* (N_0/N_i)

	Pressure (MPa)	Come up Time (min)	Log (N_0/N_i)
<i>Saccharomyces cerevisiae</i>	375 MPa		
Apples		2.8	-1.11
Blueberries		2.8	-1.28
<i>Listeria innocua</i>	300 MPa		
Apples		3.2	-0.36
Blueberries		3.2	-0.28

N_0 = microorganism count (CFU/mL) after the come up time to selected pressures

N_i = microorganism initial population (CFU/mL)

Chapter 3

Sucrose and Ultra High Pressure Inactivation of *Saccharomyces cerevisiae* and *Listeria innocua*

Abstract

D-values for ultra high pressure inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* were evaluated in commercial apple sauce with selected concentrations of soluble solids ranging from 13% to 60%. Apple sauce with *Saccharomyces cerevisiae* was treated at 300 MPa for 0 to 150 s at room temperature. Apple sauce with *Listeria innocua* was treated at 375 MPa for 0 to 300 s at room temperature. D-values for both microorganisms at soluble solids concentrations of 13, 20, and 30% were calculated.

D-values for inactivation of *Saccharomyces cerevisiae* were 26.3, 29.9, and 46.5 s in apple sauce adjusted with sucrose to 13, 20 and 30% soluble solids, respectively. D-values for inactivation of *Listeria innocua* were 40.2, 55.2 and 120.6 s in apple sauce also adjusted with sucrose to 13, 20 and 30% of soluble solids. Ultra high pressure treatment of commercial apple sauce inoculated with *Saccharomyces cerevisiae* or *Listeria innocua* adjusted to 40, 50, or 60% soluble solids reduced an initial inocula of 10^7 to 10^6 by less than 1 log. Soluble solids concentrations greater than 30% provide a baroprotective effect on inactivation by high pressure treatment.

The use of ultra high pressure technology by the food industry to inactivate microorganisms may be limited in food models with high soluble solids concentrations. Soluble solids adjusted with sucrose concentrations greater than 30% protect against the destruction of microorganisms. An effective pressure treatment that ensures the

microbial stability of foods depends on an understanding of the relationship between microorganisms and food components.

Key Words: Nonthermal processes, *Listeria innocua*, *Saccharomyces cerevisiae*, water activity, inactivation.

Introduction

Food preservation by ultra high hydrostatic pressure is used as a commercial processing method in many parts of the world. The Japanese market, for example, offers ultra high pressure treated fruit preserves, orange and grapefruit juices, salad dressings, fruit yogurts, and fruit sauces (Aleman et al., 1998). The degree of microbial inactivation achieved by ultra high pressure treatment of acid foods depends on a number of interacting factors, including the composition of the medium or food, the magnitude and extent of the pressure treatment, the type and number of microorganisms, and the treatment temperature (Palou et al., 1997 b). Food constituents such as sucrose, glucose, fructose, or sodium chloride appear to protect microorganisms from the effects of ultra high pressure (Oxen and Knorr, 1993). Even with fruit preserves with a pH of less than 4.6, considerations must be taken to ensure the adequacy of the ultra high pressure treatment in providing microbial stability.

Hashizume et al. (1995) reported a reduction in the inactivation of *Saccharomyces cerevisiae* with increasing sucrose concentrations (0-30% w/w) when treated with ultra high pressures of 260 MPa for 20 min at 25 °C. Oxen and Knorr (1993) observed that the pressure resistance of fungi increases as sucrose, fructose, or glucose concentrations in deionized water increased. Seven log reductions were observed in *Rhodotorula rubra* at water activities greater than 0.96 when treated at 400 MPa for 15 min at 25 °C. No inactivation of *Rhodotorula rubra* was observed when the water activities were lower than 0.91 when treated at 400 MPa for 15 min at 25 °C. Palou et al. (1997 b) reported a reduction in the inactivation of *Zygosaccharomyces bailii* with increasing soluble solids concentration. *Zygosaccharomyces bailii* in model systems (Sabouraud glucose 2%

broth) was treated by ultra high pressure at 345 MPa for 5 min at 21 °C. Reduced inactivation of *Zygosaccharomyces bailii* was observed in model systems with sugar concentrations greater than 40% (400 MPa for 15 min at 25 °C). In model systems with sugar concentrations of less than 20% soluble solids, a complete inactivation of *Zygosaccharomyces bailii* was observed (400 MPa for 15 min at 25 °C).

Many food constituents such as sucrose, glucose, fructose, sodium chloride, provide a baroprotective effect on inactivation by high pressure treatment. Cell shrinkage at reduced water activities or increased soluble solids concentrations result in thickening of the cell membrane, thereby reducing membrane permeability and protecting the cells from high pressure inactivation (Oxen and Knorr (1993) and Palou et al. (1997 a).

The objective of this investigation was to evaluate the baroprotective effect of soluble solids adjusted with sucrose in commercial apple sauce. Ultra high pressure treated apple sauce inoculated with *Saccharomyces cerevisiae* or *Listeria innocua* at selected soluble solids concentrations of 13% to 60% were evaluated to determine the baroprotective effect of soluble solids on microbial inactivation during ultra high pressure treatment.

Material and Methods

Preparation of inocula

Four *Saccharomyces cerevisiae* cultures (ATTC 2601, ATTC 9763, UCD 522 and Pasteur Red) obtained from the Food Science and Human Nutrition yeast collection at Washington State University (Pullman, WA), were used in this study. *Saccharomyces cerevisiae* colonies were revived on Yeast Malt broth and incubated at 25 °C for 48 h to promote cell multiplication. A sterile loop (5-10 µl) of Yeast Malt broth with each *Saccharomyces cerevisiae* strain was cultured into three 9 ml test tubes of Yeast Malt broth incubated at 32 °C for 24 h, harvested by centrifugation at 2,200 x g for 25 min at 4 °C (IEC Centa – CL2 Centrifuge, Needham, MA), and washed three times with buffered peptone water. The final pellet was resuspended in buffered peptone water, corresponding approximately to 10^7 - 10^8 mixed culture of *Saccharomyces cerevisiae* cells per milliliter.

Listeria innocua cultures (ATTC 33090, ATTC 51742, SEA 15C19, and SEA 15C20) were used as surrogates of *Listeria monocytogenes*. *Listeria innocua* colonies were maintained on Brain-Heart Infusion agar slants (BHI) until use (Difco Laboratories, Sparks, MD). A sterile loop of each *Listeria innocua* strain was transferred into three 9 ml test tubes of Tryptose Broth (Difco Laboratories, Sparks, MD) with 1% yeast extract, incubated at 37 °C for 24 h, harvested by centrifugation at 2,200 x g for 25 min and washed three times with buffered peptone water. The final pellet was resuspended in buffered peptone water, corresponding to approximately 10^7 - 10^8 mixed culture of *Listeria innocua* cells per milliliter.

Apples Sauce Preparation and Ultra High Pressure Treatment

Commercial apple sauce (Tree Top, Inc, Selah, WA) was purchased from a local supermarket. Apple sauce were prepared by transferring 100 µl of the mixed *Saccharomyces cerevisiae* culture or 100 µl of the mixed *Listeria innocua* culture into 5 ml of apple sauce containing a standard soluble solids concentration of 13%, or following adjustment of sucrose to obtain soluble solids concentrations of 20, 30, 40, 50 and 60%. The required quantity of sucrose needed to reach the selected soluble solid concentration in 100 g of apple sauce sample calculated with the following equation:

$$x = \frac{ab + ac - b}{1 - a}$$

a= % Desired soluble solids

b= % Initial soluble solids/100 (standard)

c= % Water concentration (standard)

x= quantity of sucrose added (g)

For example, the amount of sucrose (g) needed to reach 20% of soluble solids in 100 g of apple sauce is demonstrated in the following calculation:

$$\text{Sucrose (g)} = (0.20 \times 13) + (0.20 \times 87) - 13 / (1 - 0.20)$$

$$\text{Sucrose (g)} = 8.75 \text{ g}$$

The soluble solids concentration (%) was determined after each adjustment with an ABBE refractometer (Milton Roy Co., NY) at 25 °C.

After inoculation, initial counts of 1×10^6 *Saccharomyces cerevisiae* CFU/ ml and 1×10^7 *Listeria innocua* CFU/ml were observed. The 5 ml of apple sauce containing the microbial suspensions were placed in 25 ml ethylene-vinyl alcohol copolymer film (EVOH) sterile bags and double heat sealed. The sealed bags were overwrapped in an

outer polyethylene bag containing water to exclude any contact between the pressure medium and the apple sauce, and double heat sealed. Prior to heat sealing, air in both bags was manually pressed out to prevent bubbles.

Saccharomyces cerevisiae was high pressure treated at 300 MPa for a come up time of 2.8 min with zero hold (denoted as 0 time), 30, 60, 90, 120, and 150 s at room temperature. *Listeria innocua* was treated at 375 MPa for a come up time of 3.2 min with zero hold (denoted as 0 time), 60, 120, 180, 240 and 300 s at room temperature. For each inoculated apple sauce, a control apple sauce receiving no ultra high hydrostatic pressure treatment was prepared. The ultra high pressure treatment was applied in a warm isostatic pressure system (Engineered Pressure Systems, Inc., Andover, MA) with a cylindrical pressure chamber (height: .25 m, diam. 0.10 m). A 5% Mobil Hydrasol 78 water solution was used as the pressure medium. Each experiment was carried out in triplicate.

The pH, soluble solids concentration (%), and water activity in each apple sauce were assayed before inoculation. The pH was determined with an Orion pH model 420A (Orion Research, Inc., Boston, MA). Water activity values were determined with the water activity meter Aqua Lab CX-2 (Decagon Devices, Inc., Pullman, WA). The soluble solids concentration (%) was determined with an ABBE refractometer (Milton Roy Co., NY) at 25 °C. Analyses were performed in triplicate.

Enumeration of Surviving Cells

After 2 h of resuscitation, the control and pressure treated apple sauces inoculated with *Saccharomyces cerevisiae* were enumerated by spread plating 1 ml of apple sauce

on Yeast and Mold Petrifilm (3M Microbiology Products, St Paul, MN). Apple sauces inoculated with *Listeria innocua* were enumerated by spread plating 100 µl of apple sauce on Oxford Agar (Difco Laboratories, Detroit, MI.). All inoculated apple sauces were serially diluted 10-fold with 9 ml sterile buffered peptone water. Cell counts were conducted after 48 h of incubation at 32 °C for *Saccharomyces cerevisiae* or 37 °C for *Listeria innocua*. The mean of the enumeration results was calculated from three independent replicates of *Saccharomyces cerevisiae* and *Listeria innocua* for each inoculated apple sauce at selected soluble solids concentrations.

Mathematical Analysis

Ultra high pressure inactivation of microorganisms during pressure hold time was assumed to follow a first order kinetic process. Linear regression analyses were performed on the survival data for each microorganism using Excel Microsoft Office software (Microsoft Corporation, Version 2002, Seattle, WA, USA). The linear regression analyses provided a regression coefficient (r^2) and an estimate of the intercept and slope of a straight line for each survivor curve. The D-values (time required for 90% inactivation of microorganisms) were estimated from the slope of the regression line obtained from the linear portion of the survival curve of the plot log CFU/ml vs. pressurization time ($D = 2.303/k$).

Statistical Analysis:

Data were analyzed with a computer package (Minitab Statistical Software, State College, PA) using analysis of variance to assess the mean and variability reported as

standard deviation, Fisher's least significant difference (LSD) was calculated to interpret the differences among selected soluble solids concentrations. The statistical significance was established at the 5% significance level ($p \leq 0.05$).

Results and Discussion

Inactivation data for *Saccharomyces cerevisiae* and *Listeria innocua* by ultra high pressure treatments in commercial apple sauce prepared with the standard 13% soluble solids and adjusted with sucrose to soluble solid concentrations of 20, 30, 40, 50, and 60% are presented in Figs. 1 and 2. Initial inoculated populations of 1×10^6 *Saccharomyces cerevisiae* CFU/ml and 1×10^7 *Listeria innocua* CFU/ml were observed.

The soluble solids concentrations influenced the inactivation of microorganisms during high pressure treatment. As the soluble solids concentrations of the apple sauces increased, the inactivation of *Saccharomyces cerevisiae* decreased. Six, five, and three log cycle inactivation of *Saccharomyces cerevisiae* in apple sauce were observed at 13, 20, and 30% soluble solids concentrations with an ultra high pressure treatment of 300 MPa for 150 s and room temperature. Less than one log cycle inactivation was observed at 40, 50, and 60% soluble solids concentrations with an ultra high pressure treatment of 300 MPa for 150 s and room temperature. *Saccharomyces cerevisiae* inactivation decreased as the apple sauce soluble solids concentration increased.

A similar response to *Saccharomyces cerevisiae* was observed for *Listeria innocua*. Seven, six, and three log cycle inactivation of *Listeria innocua* were observed at 13, 20, or 30 % soluble solids concentrations with an ultra high pressure treatment of

375 MPa for 300 s and room temperature. Less than one log cycle inactivation was observed at 40, 50, and 60% soluble solids concentration with ultra high pressure treatment of 375 MPa for 300 s and room temperature. *Listeria innocua* inactivation decreased as the apple sauce soluble solids concentration increased.

This study confirms the observations of Ogawa et al. (1992), Oxen and Knorr (1993), Hashizume et al. (1995), Palou et al. (1997 a) that high soluble solids concentrations in foods have a protective effect against pressure inactivation. The observations of Oxen and Knorr (1993) and Palou et al. (1997 a) suggest that cell shrinkage at reduced water activities or increased soluble solids concentrations resulted in thickening of the cell membrane, thereby reducing membrane permeability and protecting the cells from high pressure inactivation.

D-values for ultra high pressure inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* were evaluated in commercial apple sauce with selected concentrations of soluble solids ranging from 13% to 60%. D-value is defined as the time needed to inactivate 90% of the initial population of microorganisms and is calculated as $D = 2.303/k$. The calculated regression coefficient (R^2) represents the closeness of fit of the regression line to the survivor curve. Calculated D-values presenting the inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* as well as regression coefficients are presented in Table 2.

Inactivation of *Saccharomyces cerevisiae* and *Listeria innocua* on apple sauces during pressure hold time followed the first order model, as reflected in high (> 0.9) correlation values (R^2). D-values for inactivation of *Saccharomyces cerevisiae* at 300 MPa for 150 s at room temperature were 26.3, 29.9, and 46.5 s in apple sauce containing

soluble solids of 13, 20 and 30%. D-values for inactivation of *Listeria innocua* at 375 MPa for 300 s at room temperature were 40.2, 55.2 and 120.6 s in apple sauce containing soluble solids of 13, 20, and 30%. Ultra high pressure treatment of apple sauce containing sucrose to adjust soluble solids to 40, 50 and 60% reduced the initial inocula of *Saccharomyces cerevisiae* and *Listeria innocua* by less than one log cycle, and therefore D-values were not calculated for those soluble solids concentrations. Larger D-values demonstrate the protective effect of increased soluble solids concentrations pressure inactivation of *Saccharomyces cerevisiae* (300 MPa at 21 °C) and *Listeria innocua* (375 MPa at 21 °C). *Saccharomyces cerevisiae* and *Listeria innocua* D-values increased as the apple sauce soluble solids concentration increased.

We can also observe that there were significant differences ($p \leq 0.05$) in D-values at constant pressures between both microorganisms. As expected, *Listeria innocua* is more pressure resistant than *Saccharomyces cerevisiae* on apples sauces adjusted with sucrose to 13, 20, and 30% soluble solids.

Water activities and pH values of apple sauce with selected soluble solids concentrations are presented in Table 1. pH values do not vary significantly among apple sauces adjusted with selected soluble solids concentration, suggesting that little pH effect could be identified in this experiment. At water activities less than 0.95 little inactivation of *Saccharomyces cerevisiae* (300 MPa, 150 s and room temperature) and *Listeria innocua* (375 MPa, 300 s and room temperature) was observed. While at water activities greater than 0.98, inactivation of *Saccharomyces cerevisiae* (300 MPa, 150 s and room temperature) and *Listeria innocua* (375 MPa, 300 s and room temperature) in apple sauces were six and seven log cycles, respectively. The relatively narrow water

activity range responsible for the different rates of inactivation described as a protective effect is attributed to osmotic effects of high soluble solids in the apple sauces.

When the osmotic pressure of the medium is increased, the tolerance of microorganisms to ultra high pressure is enhanced. Baroprotective effects of sodium chlorides or sugars have been observed in studies with *Escherichia coli*, *Saccharomyces cerevisiae*, *Zygosaccharomyces spp.*, and *Rhodoturula rubra* (Knorr, D. 1994, Oxen and Knorr, 1993, Palou et al., 1997 b, Takahashi et al., 1993). The influence of salts and sugars on the water activity of foods or suspension media does not explain the baroprotective effects of these solutes and it has been suggested that specific interactions between sugars and biological macromolecules contribute to their baroprotective effects. Furthermore, ionic and nonionic solutes had different effects on physiological properties of pressure treated cells. Sucrose preserved the metabolic activity and membrane integrity of cells during ultra high pressure treatment, whereas salt preserved the membrane integrity but not the metabolic activity (Molina-Hoppner, 2004).

Conclusions

D_{300} values for inactivation of *Saccharomyces cerevisiae* were 26.3, 29.9, and 46.5 s in apple sauce adjusted with sucrose to 13, 20 and 30% soluble solids, respectively. D_{375} values for inactivation of *Listeria innocua* were 40.2, 55.2 and 120.6 s in apple sauce also adjusted with sucrose to 13, 20 and 30% of soluble solids. Ultra high pressure treatment of apple sauce inoculated with *Saccharomyces cerevisiae* or *Listeria innocua* adjusted to 40, 50, or 60% soluble solids reduced an initial inocula of 10^7 to 10^6 by less than 1 log. The baroprotective effect of increased soluble solids concentrations

suggests that inactivation by ultra high pressure is dependent not only on pressure, time and temperature, but also on food composition. *Saccharomyces cerevisiae* and *Listeria innocua* D-values increased as the apple sauce soluble solids concentration increased. We can also conclude that there were significant differences ($p \leq 0.05$) in D-values at constant pressures between both microorganisms. As expected, *Listeria innocua* is more pressure resistant than *Saccharomyces cerevisiae* on apples sauces adjusted with sucrose to 13, 20, and 30% soluble solids.

Acknowledgment

Funding for this research was provided by the Washington Technology Center and Washington Farms, Inc. TM 3813 East 80th Street, Tacoma, WA.

References

- Aleman, G.D., Ting, E.Y., Farkas, D.F., Mordre, S.C., Hawes, A.C., and Torres, J.A. 1998. Comparison of static and step-pulsed ultra-high pressure on the microbial stability of fresh cut pineapple. *J. Food Agric.* **76**: 383-388.
- Hashizume, C., Kimura, K., and Hayashi, R. 1995. Kinetic analysis of yeast inactivation by high pressure treatment at low temperatures. *Biosci. Biotechnol. Biochem.* **59**: 1455-1458.
- Hoover, D.G., Metrick, C., Papineau, A., Farkas, D.F., and Knorr, D. 1989. Biological effects of high hydrostatic pressure on food microorganisms. *Food Technol.* **43**(3): 99-107.

- Knorr, D. 1994. Hydrostatic pressure treatment of food microbiology. In: New Methods of Food Preservation. Ed by G.W. Gould, p. 159-175. Blackie, London, United Kingdom.
- Molina-Hoppner, A., Doster, W., Vogel, R.F., and Ganzle, M.G. 2004. Protective effect of sucrose and sodium chloride for *Lactococcus lactis* during sublethal and lethal high pressure treatments. *Appl. Environ Micro.* **70**(4): 2013-2020.
- Ogawa, H., Fukuhisa, K., and Fukumoto, H. 1992. Effect of hydrostatic pressure on sterilization and preservation of citrus juice. In: High Pressure and Biotechnology. Ed by C. Balny., R. Hayashi., K. Hermans., and P. Masson, p. 269-278. John Libbey and Co. Ltd., London.
- Oxen, P. and Knorr, D. 1993. Baroprotective effects of high solute concentrations against inactivation of *Rhodotorula rubra*. *Lebens. Wissen. Technol.* **26**: 220-223.
- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., Welti-Chanes, J., and Swanson, B.G. 1997 a. Combined effect of high hydrostatic pressure and water activity on *Zygosaccharomyces bailii* inhibition. *Letters Appl. Microbiol.* **24**: 417-420.
- Palou, E., Lopez-Malo, A., Barbosa-Canovas, G.V., and Swanson, B.G. 1997 b. High pressure treatment in food preservation. Chapter 22 In: Handbook of Food Preservation. Ed. by M.S. Rahman. Marcel Dekker, p. 533-576. New York.
- Takahashi, K., Ishii, H., and Ishikawa, H. 1993. Sterilization of bacteria and yeast by hydrostatic pressurization at low temperature: effect of temperature, pH

and the concentration of proteins, carbohydrates and lipids. In: High Pressure Bioscience and Food Science. Ed. by R. Hayashi, p. 244-249. San-Ei Publishing Co., Kyoto, Japan.

Fig. 1 Ultra High Pressure Treatment (300 MPa, 150 s, room temperature) of *Saccharomyces cerevisiae* at selected soluble solids concentrations.

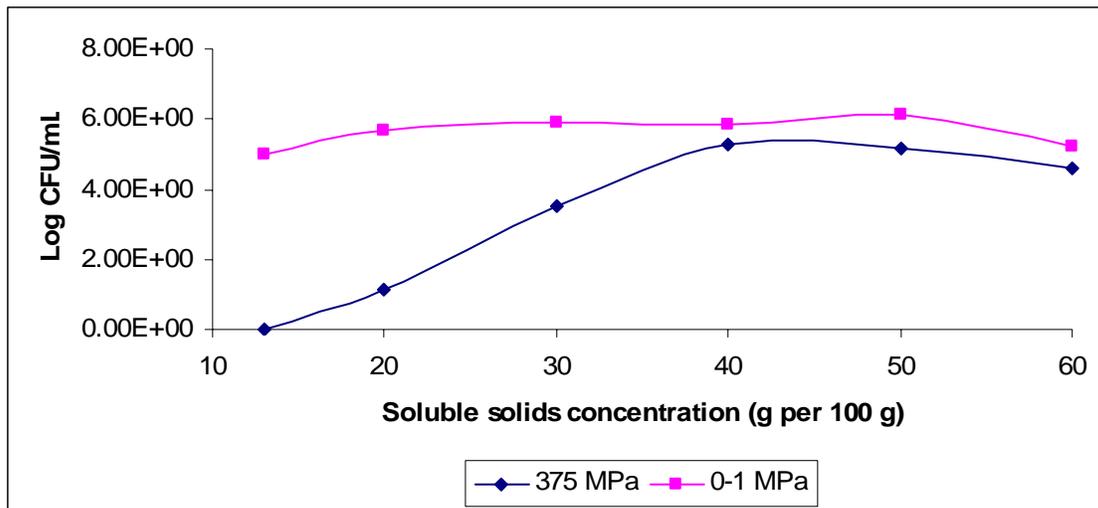


Fig. 2 Ultra High Pressure Treatment (375 MPa, 300 s, and room temperature) of *Listeria innocua* apple sauce at selected soluble solids concentrations.

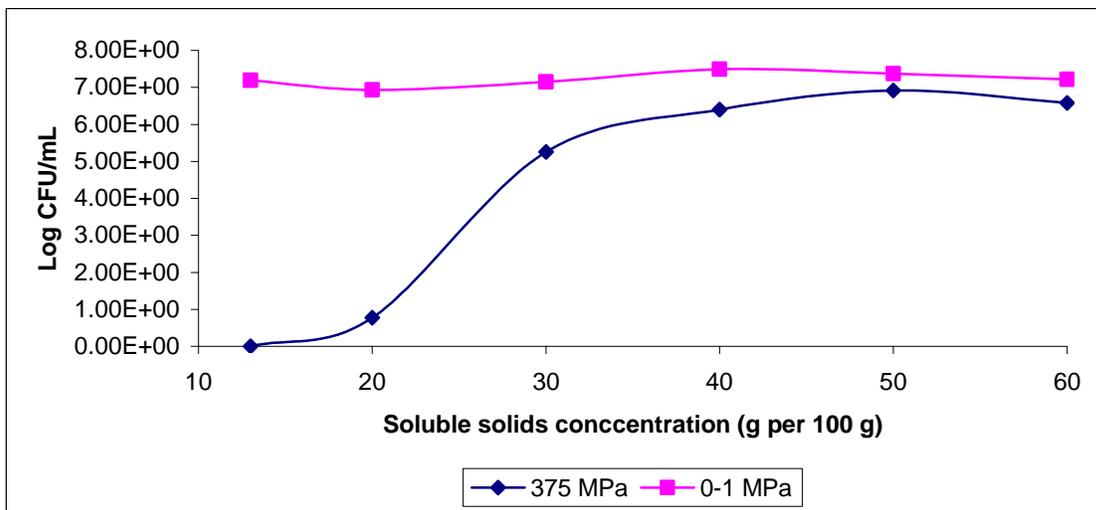


Table 1 pH and water activity (a_w) of apple sauce at selected soluble solids concentrations

% Soluble Solids	pH	a_w
13	3.72	0.987
20	3.64	0.979
30	3.62	0.972
40	3.65	0.954
50	3.67	0.934
60	3.70	0.880

Table 2 Ultra high pressure decimal reduction times (D) of *Saccharomyces cerevisiae* and *Listeria innocua* in apple sauce at selected soluble solids concentrations.

Sucrose concentration	Pressure (MPa)	D (s) ^z		
		\bar{X}	Sx	R ²
<i>Saccharomyces cerevisiae</i>	300 MPa			
13		26.31 ^a	2.46	0.94
20		29.91 ^b	4.43	0.95
30		46.45 ^c	1.74	0.92
<i>Listeria innocua</i>	375 MPa			
13		40.16 ^d	3.20	0.97
20		55.18 ^e	16.47	0.93
30		120.62 ^f	17.53	0.90

^z Means (\bar{X}) and standard deviations (S_x) of three determinations.

R² Regression coefficient from survival curves of three determinations

D-values with different superscript letter are significant different ($p \leq 0.05$)

Chapter Four

Conclusions and Future Work

1. Conclusions

- Ensuring the safety and extending the shelf life of fruit products is possible by utilizing ultra high pressure treatments.
- *Saccharomyces cerevisiae* D₃₀₀ values ranged from 19.7 s on diced apples to 22.4 s on blueberries at room temperature.
- *Listeria innocua* D₃₇₅ values ranged from 67.1 s on diced apples to 46.7 s on blueberries at room temperature.
- Pressure come up times exert an important effect on the survival fraction by reducing the number of microorganisms present on the fruit. *Saccharomyces cerevisiae* counts decreased 1.11 and 1.28 Log CFU/ml on diced apples and blueberries after 2.8 min of come up time. *Listeria innocua* counts decreased 0.36 and 0.28 Log CFU/ml on diced apples and blueberries after 3.2 min of come up time.
- The chemical composition of apples or blueberries did not affect microbial response during ultra high pressure. D-values at 300 (*Saccharomyces cerevisiae*) and 375 (*Listeria innocua*) MPa were not significantly different ($p \geq 0.05$) among diced apples and blueberries inoculated with *Saccharomyces cerevisiae* and *Listeria innocua*.
- Fruit products with a pH lower than 4.0 can be successfully processed with high pressure technology. Ultra high pressure preserved fruit products combine safety and extended shelf life stability.

- The baroprotective effect of sugar concentrations greater than 30 % suggest that inhibition of microorganisms by ultra high pressure is dependent not only on time, pressure, and temperature, but also on food composition.

2. Future Work

- Further research to completely inactivate detrimental enzymes in fruit using ultra high pressure, minimal heating temperatures and short time treatments will potentially result in fruit products that retain a fresh-like texture and appearance desired by consumers.
- Subjective sensory observations are needed to support meaningful interpretation of the quality of ultra high pressure processed fruit.
- Interactions between pressure, food constituents and storage dependent changes of foods are needed. Research and development work should include shelf-life testing.
- Ultra high pressure processed foods have the potential to replace a portion of the thermal food processed foods market as consumer demands for fresh-like nutritious products continue to grow. Identifying commercially feasible applications may be the most difficult challenge for the use of high pressure technology.
- More systematic work is needed for understanding the interactions between food ingredients and high pressure inactivation of microorganisms.