AN ABSTRACT OF THE THESIS OF

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Static ultra-high pressure (200, 270, and 340 MPa) and sinusoidal and step-pressure (0-270 MPa) pulses were compared by determining their effects on bacteria and yeast survival counts. Samples were commercial pineapple chunks used as is and fresh cut pineapple and pineapple juice inoculated with *Saccharomyces cerevisiae* 2407-1a. Surviving bacteria and yeast counts were determined using plate count agar (PCA), acidified potato dextrose agar (PDA) and yeast extract dextrose agar (YEPD).

Commercially acceptable reduction of microflora for pineapple (greater than 2.5 log cycles) were observed at static pressure of 340 MPa for 15 min at

three temperatures (~4, 21, and 38°C). Longer process times (40 and 60 min) at this pressure did not result in further significant count reductions. Lower pressure treatments, 270 and 200 MPa for 15 min, at these temperatures reduced the microbial population of pineapple by 1-2 log and less than one log, respectively. Static pressures treatments of 270 and 340 MPa at 21°C were found to reduce bacterial and yeast counts by 5-log cycles on inoculated fresh cut pineapple. Total bacteria and yeast counts remained <25CFU/plate after a 1/10 sample dilution during 60 days of storage at 4°C.

Sinusoidal and step-pressure pulses were compared with static pressure treatments for the inactivation of *S. cerevisiae* 2407-1a in pineapple juice. No inactivation was observed after 40-4,000 fast sinusoidal pulses (10 cycles/s) at 4-400 s in the 235-270 MPa range. Static 270 MPa treatments at 40 and 400 s resulted in 0.7 and 5.1 decimal reductions, respectively. Slower 0-270 MPa step pulses at 0.1 (10 pulses), 1 (100 pulses) and 2 (200 pulses) cycles/s with 100 s total on-pressure time resulted in 3.3, 3.5, and 3.3 decimal reductions, respectively. A comparable static pressure treatment resulted in 2.5 decimal reductions. Changing the on-pressure/off-pressure time ratio showed that treatments with longer on-pressure time were more effective.

Step pressure pulses (0-270 MPa) on inoculated pineapple chunks for 100s at 0.1 (10 pulses, T3) and 2 (200 pulses, T5) cycles/s were more effective than a 100 s static 270 MPa (T1). After 30 days of refrigerated storage T1 samples showed $>10^3$ CFU/g in bacteria and yeast counts, while T3 and T5 had

< 10CFU/plate after a 1/10 sample dilution. Both 5 and 10 min step pulsed 0-270 MPa treatments were as effective as 15 min static 270 and 340 MPa. Treatments T3 and T5 for 5 min yielded 0-4 CFU/plate after a 1/10 sample dilution during 30 days of refrigerated storage and recovery of pressure-injured cells was not observed. Static 15 min pressure at 270 MPa and 340 MPa resulted in 0-10 and 0-8 CFU/plate after a 1/10 sample dilution during the same 30 days of refrigerated storage. Step pressure treatment seems to be more effective than static pressure for inactivating bacteria and yeast in pineapple; recovery of pressure-injured cells was not observed under refrigerated storage conditions.

MICROBIAL STABILITY OF FRESH CUT PINEAPPLE AND PINEAPPLE JUICE PASTEURIZED BY STEP-PULSED AND STATIC ULTRA HIGH PRESSURE TREATMENTS

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Giovanna Dominick Alemán-Ordoñez, Author

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INTRODUCTION

Food processing and preservation may be defined as any method of treating food to ensure its safety and prolong the time for which the acceptable attributes of quality, including color, texture and flavor, and nutritional value are retained. Microorganisms, such as bacteria and fungi rapidly spoil food. Enzymes present in all raw foods, promote degradation and chemical changes affecting especially texture and flavor. Foods must be of the highest quality to ensure a quality product.

Conventional food preservation methods are classified as chemical or physical methods. Chemical methods include control of water activity, salting, fermentation, food additives, and food preservatives. Some additives, used to preserve foods (such as nitrites and sulfites) may have adverse health effects.

Physical methods are the most widely used food preservation methods and include heat pasteurization and sterilization, irradiation, low temperature processing, and dehydration. The main objective of these methods is to create a hostile physical environment for food microorganisms while minimizing changes in chemical composition and physical structure.

HEAT PASTEURIZATION AND STERILIZATION

This method depends upon the selective destruction of microbes by controlled heating of foods. Sterilization requires temperatures higher than 100°C and this high temperature requirement often brings undesirable changes in color, texture, flavor, and nutritional value.

IRRADIATION

This is a process similar to thermal processing but with severe consumer acceptance limitations in spite of superior product quality.

LOW TEMPERATURE PROCESSING

This method preserves food by preventing microorganisms from multiplying. Like heat sterilization, low temperature processing has undesirable side-effects upon texture and appearance of food. Freezing of poor quality foods will encourage spoilage just as heat treatment exacerbates initial quality defects.

DRYING AND DEHYDRATION

Drying is the earliest known method of food preservation. Drying does not kill bacteria; it only inhibits growth by taking away moisture. Dehydration affects the cellular structure of the product profoundly and reconstitution rarely returns the food to anything like its original state. Deterioration of dried foods is caused by lipid oxidation and nonenzymatic browning.

NEW METHODS

The demand for high quality foods with extended shelf life, minimally processed and free of additives has increased greatly. Conventional food preservation methods do not meet these requirements due to alterations of food texture, flavor, and nutritional value.

Ultra high pressure (UHP) is an emerging physical method of food preservation effective for sterilizing and preserving a wide range of foods. Unlike thermal processing, hydrostatic pressure treatment is uniform and instantaneous throughout a food (Farkas, 1986). The pressure application process is thus independent of the volume of the sample. Furthermore, high pressure treatments do not seem to affect covalent bonds; hence, flavor and appearance are usually superior to comparable products preserved by heat.

UHP processing is carried out in low compressibility liquids such as water or oil. The pressure is generated by a hydraulic pump, and the pressurized liquid is enclosed in a steel cylinder of great resistance. Holding the sample under pressure for an extended time does not require extra energy.

The effect of high pressure can be explained by the principle of Le Chatelier, which indicates that any phenomenon accompanied by a decrease in volume is enhanced by an increase in pressure. High pressure places a constraint on volume and favors reactions decreasing volume. These reactions can cause biopolymers to unfold and alter their functional behavior, as in enzyme activity and microbial membrane permeability changes leading to cell death (Johnston, 1994).

Evidence of the advantages of ultra high pressure preservation was demonstrated almost a century ago by Hite et al. (1899, 1914). However, several decades passed before commercial products reached consumers in the early 90's. The advantages of ultra high pressure have been demonstrated in the inactivation of microorganisms and enzymes with processed foods having improved quality, particularly flavor and color.

The first part of this research was to show the effect of static UHP on the inactivation of bacteria, yeast and mold present on fresh cut pineapple processed under commercial conditions (Alemán et al., 1994). The second part of this research compared the effect of pulse and static UHP as preservation method for pineapple juice (Alemán et al., 1996). Samples were inoculated with *Saccharomyces cerevisiae* and the parameters studied were pulsation rate, number and type of pressure pulses, and storage time. Finally, we compared the effect of static and pulsed ultra high pressure on the microbial stability of fresh cut pineapple inoculated with *Saccharomyces cerevisiae* stored refrigerated.

TABLE 1

Preservation methods

PHYSICAL METHODS	
DESTRUCTION OF MICROORGANISMS	
Sterilization	Destruction of microbes by heating above 100°C
Pasteurization	Heating to 55° - 70°C to destroy pathogenic bacteria
CONTROL OF MICROORGANISMS	
Freezing	Freezing and storage below -15°C
Refrigeration	Chilling and storage around 0 - 5°C
Modified atmosphere	Storing (CA) or packaging (MAP) of foods in a controlled atmosphere
Dehydration	Removal of water without necessary destruction of microbes
CHEMICAL METHODS	
CONTROL OF MICROORGANISMS	
Additives	Use of chemicals like potassium benzoate and phosphoric acid.
Fermentation	Chemical changes in organic substances produced by organisms such as bacteria, yeast and molds.
NEW METHODS	
DESTRUCTION OF MICROORGANISMS	
Ultra high pressure	Application of pressures up to 100,000 psi to destroy microorganisms.
Others	Irradiation, high-electric field pulses, oscillating magnetic field pulses, microwave effects, and other new methods are under investigation

LITERATURE REVIEW

HISTORICAL BACKGROUND

Bert Hite, a chemist at the Agricultural Experiment Station in Morgantown, W. VA., was first in using high pressure as a method for food preservation. He assembled a pressure unit machine which could reach 600 MPa (100,000 psi). He and his coworkers used ultra high pressure (UHP) to tests milk, meat, fruits and beverages (Hite, 1899; Hite et al., 1914). After decades of slow progress, major efforts have been achieved in Europe, the U.S. and Japan since the 1980s with pressure treated foods reaching the Japanese market in the early 1990s. These products included a range of jams, sauces, salad dressings, ready-to-eat desserts, grapefruit and mandarin juices.

The study of UHP effects has focused in five main areas: (1) the alteration of foaming, gelling or emulsifying behavior of proteins, (2) the alteration of enzyme activity, (3) the control of phase change such as fat solidification or ice formation and melting, (4) modification of food microstructure, and (5) the inactivation of microorganisms. UHP food applications were largely ignored through most of this century and only a few early papers reported the effects of UHP on foods and food constituents. In addition to the work by Hite et al. (1899, 1914), Bridgman (1914) studied the pressure denaturation of egg white proteins. Timson and Short (1965) evaluated the effect of pressure on raw milk microflora while Wilson (1974) combined pressure and heating for the pasteurization of low acid foods. Charm et al. (1977) suggested the use of high pressure (20 MPa) for

the long term refrigerated storage of foods. In 1980, Elgasim and Kennick evaluated the effect of UHP on beef protein quality. Pressures up to 450 MPa have been used to inactivate vegetative forms of microorganisms (Hoover et al., 1989; Metrick et al., 1989; Styles et al., 1991; Alemán et al., 1994, 1996). UHP is effective at ambient temperatures and retains food flavor, aroma and color better than thermal processing (Farkas, 1986; Hayashi, 1989). Ogawa et al. (1989) proposed UHP for the sterilization of citrus and other juices. Higher pressures (450-800 MPa) are needed to eliminate sporeformers (Hayakawa et al., 1994). Finally, UHP can also activate and inactivate enzymes (Morild, 1981), promote gel formation (Cheftel, 1991) and milk curdling by rennet (Ohmiya et al., 1987), accelerate starch digestion (Hayashi and Hayashida, 1989) and alter food microstructures (Torres et al., 1996).

UHP studies also include the kinetics of pressure inactivation of bacteria, yeast, molds, bacterial and mold spores (Butz et al., 1986, 1990, 1991) and viruses (Brauch et al., 1990; Carl and Ludwig, 1991). These studies showed that the inactivation of most vegetative bacteria follows first order reactions while other microorganisms and viruses follow complicated kinetic behaviors. This complication has expanded the need to evaluate the effect of UHP in various food systems using a wide range of spoilage and pathogenic organisms.

EFFECTS ON PROTEINS AND ENZYMES

High temperature treatment usually lead to the irreversible denaturation of proteins; in contrast, the effect of applying hydrostatic pressure to proteins is

often reversible and it is probably due to conformational changes and subunit dissociation/association processes (Morild, 1981). Bridgman (1914) first reported the denaturation of proteins by UHP when he subjected egg white to high pressure. Irreversible denaturation was observed above 300 MPa and intensified by increasing pressure and time. High pressure can induce protein denaturation by altering the weak interactions that stabilize the folded conformations (Hermans, 1982). Suzuki and Suzuki (1963) concluded that the two primary means by which enzymes are inactivated are alteration of intramolecular structures and conformational changes at the active site. Ogawa et al. (1990a,b) reported that in citrus juice pectinesterase and peroxidase activities were markedly reduced by pressurization. Böttcher and Knorr (1991) showed that tyrosinase activity is affected by temperature and pressure resulting in synergistic combined treatments. These pressure induced effects are affected by pH (Suzuki and Kitamura, 1963), substrate concentration (Marquis and Matsumura, 1978; Heremans, 1978), subunit structure of enzyme (Penniston, 1971), and temperature (Laidler, 1951). Hayashi et al. (1989) found the textural properties of pressure and heat induced gels of eggs to be different. Pressure induced gels were softer and resisted. Ohmori et al. (1991) found that the exposure of beef extracts to UHP increased their free amino acid content and trypsin digestibility. They also concluded that the overall quality of meat was improved.

EFFECT OF HYDROSTATIC PRESSURE ON MICROORGANISMS

Effect on microorganisms is one of the main aspects of UHP; but not all high hydrostatic pressure applications are related to shelf life extension. Watanabe et al. (1991) used pressure to inactivate *Erwinia ananas* so that pressure treated cells could be safely added to foods as effective ice nuclei for freeze drying, freeze concentration, and frozen texture modification of foods. Styles et al., 1991) studied the effect of UHP on bacterial food pathogens showing that *L. monocytogenes* Scott A could be destroyed by exposure to 340 MPa within 80 minutes at 23°C in UHT milk. Leakage of UV-absorbing substances from *S. cerevisiae* were observed after pressure treatment at 400 MPa and room temperature (Shimada et al., 1990). Freshly squeezed orange juice and other juices inoculated with yeast and molds were treated for 10 minutes at 400 MPa and room temperature. After 17 months of storage, samples showed no increase in total counts (Ogawa et al., 1989, 1990a,b). The effect of UHP on microorganism depends also on the magnitude and duration of compression, the stage of growth of the organisms, testing media, and parameters such as temperature and pH during treatment (ZoBell, 1970; Dring, 1976).

Spores are not extensively inactivated by UHP and represent a challenge to this new technology. Spores of bacteria like *Bacillus subtilis* are known to survive up to 1200 MPa, other non spore-forming bacteria such as *Microbacterium* species seem resistant to pressures up to 800 MPa (Timson and Short, 1965). Information on the effect of UHP treatment on microorganisms is

very variable. Gram positive bacteria are more resistant than gram negative ones. Yeasts and molds seem to be very sensitive, on the other hand spores and viruses are the most resistant to pressure treatment.

Research on barophilic microorganisms, i.e. pressure tolerant organisms usually observed in deep sea environments, is directly applicable to foods. For example, Schwarz and Colwell (1974) found *V. parahaemolyticus* to be more sensitive to pressure than *V. bathycetes*. Later work showed that *V. parahaemolyticus* is one of the most pressure-sensitive food pathogens (Styles et al., 1991; Metrick et al., 1989).

MICROBIAL INACTIVATION MECHANISMS

Several mechanisms for microbial inactivation by high hydrostatic pressure have been proposed but few details are known. The cell membrane seems to be a primary site of pressure damage (Morita, 1975). Changes in cell morphology can be induced by UHP that are reversible at low pressures but irreversible at high pressures (Farr, 1990). It has been shown in *Saccharomyces cerevisiae* that at pressures up to 400 MPa large amounts of intracellular material leaks from the cell and at 500 MPa the nucleus can no longer be recognized (Shimada et al., 1990). Other possible mechanism of cell inactivation include the binding of amino acyl-tRNA to ribosome and mRNA (Hardon and Albright, 1974) and the inactivation of important intracellular enzymes (Jaenicke, 1981). Perrier-Cornet et al. (1995) constructed a micro-reactor equipped with a light microscope and a precise pressure generator to study the effect of pressure on the growth and

viability of *Saccharomyces cerevisiae* and *Saccharomycopsis fibuligera*. Cell volume variations were measured using a computerized image analysis system. A shrinkage in average cell volume was observed at 250 MPa (25%) which was not fully recovered after cell decompression to atmospheric pressure. The 10% permanent shrinkage was associated with an irreversible mass transfer between the cell and the pressure medium caused by a modification of cell membrane properties, i.e., disruption or increase in membrane permeability. They did not report data on the kinetics of the irreversible mass transfer.

HIGH PRESSURE PULSES

Information on UHP pulses on foods is limited to a report by Hayakawa et al. (1994) who showed that pressure pulses accelerate the inactivation of spores. Six 0-600 MPa cycles of 5 min at 70°C (30 min total) completely sterilized a solution containing 10^6 spores/mL of *Bacillus stearothermophilus*. Static application at 600 and 800 MPa for 60 min could only reduce the initial number of viable spores to 200 and 30 spores/mL, respectively. They also suggested that the adiabatic expansion velocity of pressurized water was responsible for spore inactivation. Water phase and hydrophobicity changes with pressures >400 MPa have been associated with protein denaturation (Hayakawa et al., 1994). Pressure pulsing, a novel and effective means to increase the effectiveness of UHP runs, has been found effective in modifying cheese microstructures (Torres et al., 1996).

SENSORIAL AND NUTRITIONAL CHARACTERISTICS OF FOODS

Fresh fruit purees, different kinds of juices, various flavor extracts and raw fish, can be processed by UHP without any alteration of their raw or fresh taste and flavor characteristics (Shimada et al., 1990) although food color may be affected by UHP. Pigments such as carotenoids, chlorophyll and anthocyanins appear to resist pressurization while myoglobin seems more sensitive. Fresh meat may lose its bright red color when treated above 300 MPa. Maillard reactions are slowed down under pressure but the opposite effect appears to take place with reactions catalyzed by polyphenol oxidase (Asaka and Hayashi 1991).

Texture is more sensitive to pressure, with detrimental or beneficial changes (Shimada et al., 1990). Products such as fruits that have internal gas vacuoles are severely and irreversibly compressed due to the high compressibility of gases. This compressibility may cause morphological alterations and enhancement of enzymatic reactions but these effects are less pronounced in fruit fragments. Meat tenderization is a positive effect of pressure with some beef muscles markedly tenderized by applying 100 MPa for 4 min at 35°C (Beilken et al., 1990). Tenderization probably takes place through fragmentation of myofibrils by the action of cathepsins. These proteolytic enzymes are released in the cytoplasm when the lysosomal membrane is altered by pressure (Ohmori et al., 1991). Pressure applications to gels and emulsions may bring desirables textures, in addition to lower bacterial counts and extended shelf-life. Starch gelatinization and protein unfolding enhanced by pressure application may result in higher digestibility and nutrient availability (Cheftel,

1991). Pressurized fruit juices retained most (>95%) of their initial vitamin content.

TABLE 2

Potential food applications of UHP processing

Field	Application Description
Gels	Texture modification of various gelled or emulsified foods including surimi and polysaccharide gels. Also used to facilitate cold temperature gelation.
Proteins and enzymes	Increased binding of ligands to proteins and denaturation caused by compressibility and structural changes are effects used in meat tenderization and enzyme activity modification. Other effects studied include peptide hydrolysis and synthesis by proteases, enzymatic digestion of soy proteins, lipase reaction, muscle proteases, inactivation of oxidases, enzyme or toxin inactivation, and decoloration of hemoglobin.
Starches	Gelatinization at reduced temperature to enhance the cooking properties of pressurized rice, softening of legume seeds and cereal grains. Also used to facilitate starch hydrolysis in sake brewing.
Change in phase transitions	The reversible decrease in the melting point of ice allows the cold storage of foods without freezing. The increase in the melting point of lipids has been applied to the tempering of chocolates by controlling crystal polymorphism.
Microstructure	Modification of cheese microstructure to modify cheese texture and enhance moisture control.
Pasteurization, sterilization	Inactivation and destruction of pathogenic microbes e.g., *Salmonella*species in egg white or minced meat and *Vibrio parahaemolyticus* and viruses in seafood.

III. ULTRA-HIGH PRESSURE PASTEURIZATION OF FRESH CUT PINEAPPLE

ABSTRACT

Ultra high pressure (200, 270, and 340 MPa), temperature (~4, 21 and 38°C), and time (5, 15, 40, and 60 min) combinations were evaluated as a means to extend the shelf life of fresh cut pineapple chunks. Cut pineapple obtained from an industrial processor was packed in heat-sealed polyethylene pouches. Triplicate samples were temperature adjusted and treated in an Autoclave Engineers IP 2-22-60 isostatic press. Surviving bacteria and total yeast and mold counts were determined using plate count agar (PCA) and acidified potato dextrose agar (PDA), respectively. Commercially acceptable reductions of microflora, 3.0 (~4°C), 3.1 (21°C) and >2.5 (38°C) decimal reductions as measured by growth on PCA, were found after 15 min at the highest pressure used (340 MPa). Pressure treated pineapple pieces had PCA counts < 50 CFU/g. At 270 MPa and 15 min, greater than 2 decimal reductions were observed only at 38°C. Exposure to pressures of 200 MPa resulted in about one decimal reduction in PCA counts for all temperature and time combinations tested. PDA counts followed a similar behavior for all pressure treatments.

INTRODUCTION

In general, high or ultra high pressure treatment refers to a process that subjects foods sealed in flexible packages, to high hydrostatic pressures with or without additives or mild heating. Water is normally the pressure transmitting medium. Unlike thermal processing, hydrostatic pressure treatment is uniform and instantaneous throughout a food and the effects do not follow a concentration gradient (Farkas, 1986). Inactivation of microorganisms is one of the most important aspects of high hydrostatic pressure. Ludwig et al. (1992) showed that the inactivation rate of *E. coli* is strongly dependent on pressure.

Early studies on the effect of high hydrostatic pressure on foods and food microorganisms showed that subjecting milk to a pressure of 680 MPa for 10 min reduced the bacterial count from 10^7 cells/mL to 10^1 - 10^2 cells/mL (Hite, 1899). Fruits, such as peaches and pears, were shown to remain preserved for five years after pressure treatment (Hite et al., 1914). Stability was maintained because spoilage organisms, yeasts and lactic acid bacteria, are barosensitive and the low pH of fruit did not support spore outgrowth (Hoover et al., 1989). More recent examples of hydrostatic pressure applications include meat tenderization and protein quality modifications (Elgasim and Kennick, 1980). A particularly interesting application is the modification of enzyme functionality, e.g. casein degradation (Ohmiya et al., 1989), starch digestion (Hayashi and Hayashida, 1989), and milk curdling by rennet (Ohmiya et al., 1987). The sterilization of citrus juices (Ogawa et al., 1989, 1992), the formation of gels,

preservation of egg products (Hayashi et al., 1989), and the inactivation of bacterial food pathogens (Styles et al., 1991) by hydrostatic pressure have been examined also.

Pressures exceeding 300 MPa (45,000 psi) have been reported ineffective in destroying bacterial spores (Sale et al., 1970). Treatment of *Bacillus* spp. spores at pressures of 100 to 300 MPa were found to be more lethal than higher pressures up to 800 MPa. It has been speculated that lower pressures (100-300 MPa) may induce spore germination and the outgrowth stage may be more sensitive to pressure (Hoover et al., 1989).

High pressure processing may satisfy the demand from consumers in developed countries for high quality foods with extended shelf life that are minimally processed and additive free. Furthermore, high pressure treatments do not affect covalent bonds; hence, flavor and appearance are usually superior to comparable products preserved by heat. Hydrostatic pressure has been found to be effective for sterilizing and preserving citrus juice without changing its original flavor and taste (Ogawa et al., 1989, 1992). Finally, this process is isostatic, that is, every food particle receives the same pressure, and the process pressure is achieved virtually instantaneously.

The objective of this study was to investigate the effect of high hydrostatic pressure on the inactivation of bacteria, yeast and mold present on fresh cut pineapple that was processed under commercial conditions.

MATERIALS & METHODS

Sample treatment and source

Bags of fresh cut royal Hawaiian pineapple (480 g) were obtained and transported under refrigeration from Dole Packaged Foods Co. (Honolulu, Hawaii). Upon arrival, the fruit was stored at 1°C for approximately one week. One bag of pineapple was used for each treatment and three replicates plus one control were obtained from the same bag. Each replicate sample was analyzed microbiologically in triplicate. Samples were subjected to combinations of pressures (200, 270 or 340 MPa, 30,000, 40,000 or 50,000 psi), temperatures (~4, 21 and 38°C), and times (5, 15, 40, and 60 min).

Pressurization

Two or three pineapple pieces were placed in a polyethylene bag and heat-sealed with elimination of as much entrapped air as possible. This bag was then placed in an outer bag which had been filled with distilled water and was then heat sealed after careful elimination of air bubbles. These samples were temperature adjusted for 5 to 10 minutes prior to pressure treatment. The water in the outer bag facilitated an even distribution of pressure on the pineapple pieces. Pressurization of samples was achieved using an Autoclave Engineers (Erie, PA) isostatic pressure model #IP2-22-60 with a cylindrical pressure chamber (height = 22 inches, diameter = 2 inches). Bagged samples were submerged in water containing 2% hydraulic fluid (Hydrolubic 142™, Houghton

and Co., Valley Forge, PA) which acted as the hydrostatic fluid medium in the press. Pressure treated samples were plated for microflora counts immediately after treatment. Controls were secured from untreated samples obtained from the same commercial bag.

Microbiological assays

Appropriate dilutions from both control and treated samples were made in 0.1% peptone water with aliquots plated using the pour plate technique. The media were plate count agar (PCA, pH = 7; Difco, Detroit, MI) for bacteria, and potato dextrose agar acidified with 10% tartaric acid (PDA, pH = 3.5; Difco) for yeast and mold. Triplicate PCA plates were incubated at 37°C for 48 hours while PDA plates were incubated at room temperature for 5 days (20-25°C).

Statistical design

Statistical designs provided by Dole Packaged Foods Co. were computer-generated (S-Matrix, Company, City, State) and included a full design, screening design A, screening design B and comprehensive design (Table 3). The r* value defined the boundaries of the confidence level interval (90% for N > 16). Thisted's index is a measure of the amount of multicollinearity of the experimental design matrix. The statistical procedure selected was the comprehensive design protocol (30 cells) which provided minimal overkill and intrinsic overlap, with enough data can be collected to produce a clear analysis and conclusions.

TABLE 3

Statistical experimental design for the pressure treatment of fresh-cut pineapple

Design Description	No. of cells	r*	Thisted's index
Full design	50	0.2353	5.1159
Screening design A	20	0.3784	1.1136
Screening design B	22	0.3599	2.1521
Comprehensive design	30	0.3061	2.1497

RESULTS & DISCUSSION

The purpose of this study was to evaluate the effect of high pressure treatment on viable bacteria, yeast and mold present in fresh pineapple. Fresh cut pineapple chunks were pressure treated at different combinations of pressure, temperature and time (24 combinations + 6 duplicates). The decimal reduction (DR) values from the pressurization treatments reflect the inactivation effect of pressure on bacteria (Table 4) and yeasts and molds (Table 5). A commercially acceptable reduction of microflora was observed at 340 MPa for 15 min at all three temperatures used (Tables 4a, 5a). Following this treatment < 50 CFU/g survived. Longer process times (40 and 60 min) at this pressure did not result in further significant count reductions. At 270 MPa for 15 min, the population was reduced by 1-2 decimal reductions. Again an extension of the treatment time did

not increase significantly the inactivation of bacteria, yeast, and mold (Tables 4b, 5b). A pressure of 200 MPa resulted in less than one decimal reduction at all temperatures and times tested except for the 40 min-38°C treatment, which resulted in almost three decimal reductions in bacterial counts. This may reflect the case of a sample with a high initial count (Tables 4c, 5c). From these data, it appears that there is an apparent processing time threshold after which no further significant microbial inactivation occurs. The increased inactivation of bacteria at higher pressure previously reported (Styles et al., 1991) was also observed.

There are several possible mechanisms by which microorganisms are inactivated by high hydrostatic pressure, but few details are known although the cell membrane is a primary site of pressure damage (Morita, 1975). At higher pressures, microbial death is considered to be due to permeabilization of cell membranes. Pressure-induced changes in cell morphology are reversible at low pressures, but may be irreversible at high pressures (Farr, 1990). At pressures of 400 to 500 MPa, the structure of the nucleus and cytoplasmic organelles of *Saccharomyces cerevisiae* could no longer be recognized and were completely deformed. Pressures up to 400 MPa at room temperature resulted in the leakage of large amounts of intracellular material (Hayashi, 1989). Information on the inactivation mechanisms of microorganisms was not an objective of this study, but experimental results showed that high pressure treatment would be an effective treatment for fresh pineapple.

TABLE 4

Time, temperature and pressure effects on pasteurization of fresh cut pineapple, total plate counts[a]

a. 370 MPa

		Time (min)					
T, °C		5	15	40		60	
~4	N_o=	$4.8x10^3$	$4.5x10^4$	$3.9x10^3$	$7.2x10^3$	$7.8x10^3$	$6.0x10^3$
	N=	60[b]	50[b]	<10	<10	10[b]	<10
	DR=	1.9	3.0	>2.1	>2.9	>2.9	>2.5
21	N_o=	$9.7x10^3$	$1.3x10^4$	$8.9x10^4$	$5.1x10^3$	$3.7x10^6$	$1.1x10^4$
	N=	210[b]	10[b]	10[b]	13[b]	50[b]	<10
	DR=	1.7	3.1	4.0	2.6	4.9	>2.6
38	N_o=	$8.0x10^3$	$2.8x10^3$	n.t.[c]		n.t.	
	N=	23[b]	<10				
	DR=	2.5	>2.5				

TABLE 4 - continued

b. 270 MPa

T, °C		Time (min) 5		15	40	60
~4	N_o=	$1.9x10^4$		$1.1x10^4$	n.t.	n.t.
	N=	$3.1x10^2$		$2.9x10^2$		
	DR=	1.8		1.6		
21	N_o=	n.t.		n.t.	n.t.	n.t.
	N=					
	DR=					
38	N_o=	$4.2x10^5$	$1.9x10^3$	$2.9x10^3$	$2.9x10^3$	$2.9x10^3$
	N=	90[b]	$3.4x10^2$	30[b]	20[b]	<10
	DR=	4.0	0.7	2.0	2.2	>2.5

TABLE 4 - continued

c. 200 MPa

T, °C		Time (min) 5	15		40	60
~4	N_o=	$3.5x10^3$	n.t.		$7.0x10^3$	$3.2x10^4$
	N=	$9.2x10^2$			$1.2x10^3$	$9.3x10^2$
	DR=	0.6			0.8	1.5
21	N_o=	n.t.	n.t.		n.t.	$1.2x10^3$
	N=					$1.6x10^2$
	DR=					0.9
38	N_o=	$9.1x10^3$	$3.7x10^4$	$9.0x10^3$	$2.4x10^5$	$5.6x10^3$
	N=	$2.6x10^3$	$6.3x10^3$	$2.6x10^3$	$2.7x10^2$	$5.1x10^2$
	DR=	0.5	0.8	0.5	3.0	1.0

[a] DR = log N_o - log N, where N_o = initial total plate counts, N = total plate counts after treatment, and DR = decimal reductions

[b] estimated values, plate counts <25 CFU/plate

[c] not tested, condition not included in statistical design

TABLE 5

Time, temperature and pressure effects on pasteurization of fresh cut pineapple, total yeast and mold counts[a]

a. 370 MPa

T, °C		Time (min) 5	15	40		60	
~4	N_o=	$4.8x10^3$	$3.8x10^3$	$3.5x10^3$	$8.8x10^3$	$1.5x10^4$	$8.5x10^3$
	N=	60[b]	<10	<10	<10	<10	<10
	DR=	1.9	>2.6	>2.5	>2.0	>3.2	>2.9
21	N_o=	$3.5x10^3$	$1.8x10^4$	$4.8x10^3$	$4.2x10^3$	$1.4x10^5$	$1.1x10^4$
	N=	140[b]	<10	<10	<10	<10	<10
	DR=	1.4	>3.3	>2.7	>2.6	>4.0	>2.5
38	N_o=	$4.9x10^3$	$2.6x10^3$	n.t.[c]		n.t.	
	N=	<10	<10				
	DR=	>2.7	>2.4				

TABLE 5 - continued

b. 270 MPa

		Time (min)				
T, °C		5		15	40	60
~4	N_o=	$2.0x10^3$		$9.1x10^3$	n.t.	n.t.
	N=	$9.0x10^2$		$5.4x10^2$		
	DR=	0.3		1.2		
21	N_o=	n.t.		n.t.	n.t.	n.t.
	N=					
	DR=					
38	N_o=	$5.8x10^3$	$2.7x10^3$	$3.4x10^3$	$3.6x10^3$	$3.4x10^3$
	N=	30[b]	$3.8x10^2$	30[b]	20[b]	<10
	DR=	2.3	0.9	2.0	2.3	2.1

TABLE 5 - continued

c. 200 MPa

T, °C		Time (min)				
		5	15		40	60
~4	N_o=	$3.2x10^3$	n.t.		$5.2x10^3$	$4.5x10^4$
	N=	$1.2x10^2$			$2.8x10^3$	$1.1x10^3$
	DR=	0.4			0.3	0.6
21	N_o=	n.t.	n.t.		n.t.	$3.9x10^3$
	N=					$6.5x10^2$
	DR=					0.8
38	N_o=	$8.6x10^3$	$4.5x10^3$	$1.1x10^4$	$3.3x10^3$	$5.8x10^3$
	N=	$2.9x10^3$	$2.0x10^3$	$1.0x10^3$	$2.1x10^2$	$6.4x10^2$
	DR=	0.5	0.4	1.1	1.2	1.0

[a] DR = log N_o - log N, where N_o = initial total plate counts, N = total plate counts after treatment, and DR = decimal reductions

[b] estimated values, plate counts <25 CFU/plate

[c] not tested, condition not included in statistical design

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IV. PULSED ULTRA HIGH PRESSURE TREATMENTS FOR PASTEURIZATION OF PINEAPPLE JUICE

ABSTRACT

Sinusoidal and step-pressure pulses were compared with static pressure treatments for the inactivation of *Saccharomyces cerevisiae* 2407-1a in pineapple juice. No inactivation was observed after 40-4,000 fast sinusoidal pulses (10 cycles/s) at 4-400 s in the 235-270 MPa range. Static 270 MPa treatments at 40 and 400 s resulted in 0.7 and 5.1 decimal reductions, respectively. Slower 0-270 MPa step pulses at 0.1 (10 pulses), 1 (100 pulses) and 2 (200 pulses) cycles/s with 100 s total on-pressure time resulted in 3.3, 3.5, and 3.3 decimal reductions, respectively. A comparable static pressure treatment resulted in 2.5 decimal reductions. Changing the on-pressure/off-pressure time ratio (3:1, 3:3 and 1:3) showed that treatments with longer on-pressure time, e.g., 0.6 s on-pressure and 0.2 s off-pressure, were significantly more effective showing nearly a 4 decimal reduction in 100 s.

INTRODUCTION

Hydrostatic pressure is a food preservation method with excellent quality retention which could reduce the need for additives and high-temperature treatments (Farkas, 1986). Applications of ultra-high pressure (UHP) processing were reported almost a century ago and the first commercial products appeared in Japan (Knorr, 1993). Unlike thermal processing, hydrostatic pressure treatments are independent of product size and geometry, and their effect is uniform and instantaneous.

UHP is effective at ambient temperatures reducing thermal effects on food quality. Pressures up to 450 MPa around 20-25°C have been used to inactivate vegetative forms of microorganisms (Hoover et al., 1989; Metrick et al., 1989; Styles et al., 1991; Alemán et al., 1994). As compared to thermal processing, UHP retains food flavor, aroma and color (Farkas, 1986; Hayashi, 1989) and has been proposed for the sterilization of citrus and other juices (Ogawa et al., 1989). Higher pressures (450-800 MPa) are needed to eliminate sporeformers (Hayakawa et al., 1994). UHP can also activate and inactivate enzymes (Morild, 1981), promote gel formation (Cheftel, 1991) and milk curdling by rennet (Ohmiya et al., 1987), accelerate starch digestion (Hayashi and Hayashida, 1989), and alter food microstructures (Torres et al., 1996).

Shimada et al. (1993) have shown that cell walls and inner structures of pressure-treated *Saccharomyces cerevisiae* were markedly disrupted by UHP. Many intracellular cell organelles (nucleus, mitochondria, endoplasmic reticulum,

and vacuoles) showed deformation and disorganization. UHP kills microorganisms by inducing physiological imbalances caused by internal and external structural damage (ZoBell, 1970; Marquis, 1976).

Information on pulsed UHP use in food processing is generally unavailable. Hayakawa et al. (1994) showed that pressure pulses accelerated the inactivation of spores. Complete sterilization of a solution containing 10^6 spores/mL of *Bacillus stearothermophilus* was achieved after six 0-600 MPa cycles of 5 min at 70°C (30 min total). Static application at 600 and 800 MPa for 60 min could only reduce the initial number of viable spores to 200 and 30 spores/mL, respectively. They also suggested that the adiabatic expansion velocity of pressurized water was responsible for spore inactivation. Water phase and hydrophobicity changes with pressures >400 MPa have been associated with protein denaturation (Hayakawa et al., 1994). Pressure pulsing, a novel and effective means to increase the effectiveness of UHP runs, has been found effective in modifying cheese microstructures (Torres et al., 1996).

Perrier-Cornet et al. (1995) constructed a 25 µL micro-reactor equipped with a light microscope and precise pressure generator to study the effect of pressure on the growth and viability of *Saccharomyces cerevisiae* and *Saccharomycopsis fibuligera*. Cell volume variations were measured using a computerized image analysis system. A shrinkage in average cell volume was observed at 250 MPa (25%) which was not fully recovered after cell decompression to atmospheric pressure. The 10% permanent shrinkage was associated with an irreversible mass transfer between the cell and the pressure

medium caused by a modification of cell membrane properties, i.e., disruption or increase in membrane permeability. They did not report on the kinetics of the irreversible mass transfer, all samples were held for 15 min at 250 MPa.

Our objective was to compare pulsed and static UHP as preservation methods for fruit juice. Parameters studied were pulsation rate and number and type of pressure pulses.

MATERIALS AND METHODS

Sample preparation and viability determinations

Unsweetened pineapple juice (500 mL, Dole Packaged Foods Co., San José, CA) inoculated with 100 µL of *S. cerevisiae* 2407-la obtained from the Genetics Program at Oregon State University (≈10^7 counts/mL) was incubated 4 h at 30°C. Juice samples (5 mL) in polyethylene bags (1x7 cm), heat-sealed with elimination of entrapped air, were stored refrigerated for use the next day. All tests were conducted at 270 MPa and room temperature (≈23°C). Yeast counts on UHP-treated samples and untreated controls were estimated immediately after treatment using yeast extract peptone dextrose (YEPD) agar plates containing 1% yeast extract, 2% peptone, 1% dextrose and 1.5% agar incubated 48 h at 30°C. Least significant differences in numbers of yeast decimal reductions by UHP were determined using the SAS Statistical Package (Version 6.04, SAS Institute, Cary, NC).

Pressure treatments

Static pressure tests were conducted using an isostatic pressure unit (Model #IP2-22-60, Autoclave Engineers, Erie, PA). This unit had a cylindrical pressure chamber (height = 0.56 m, diameter = 0.05 m) and operated with 2% hydraulic fluid (Hydrolubic 142™, Houghton and Co., Valley Forge, PA) in distilled water as the hydrostatic fluid. Juice sample bags were overwrapped in an outer bag containing water and submerged in the chamber filled with the hydrostatic fluid.

Pressure-pulsing experiments were conducted at the R&D facilities of Flow International Corporation (Kent, WA). All experiments were conducted using the same yeast-inoculated juice batch. Each treatment consisted of two UHP runs with 2 samples/run. Values reported were averages of the 2 samples for each run. Juice samples were exposed to sinusoidal pressure pulses (Fig. 1) using an UHP direct-drive triplex pump. Step-pressure pulses were tested using an UHP intensifier pump (Fig. 2). In both pumps the hydrostatic fluid was water at room temperature. An oscilloscope and pressure transducers were used to record the pressure-time profile for each treatment with an estimated ±1% precision at 270 MPa. Digital oscilloscope pressure readings were calibrated against a precision bourdon tube test gauge.

Digital oscilloscope recordings were used to determine the time during each sinusoidal pressure cycle that juice was exposed to pressures expected to reduce microbial counts (17.2 ms @ 235-270 MPa, Fig. 1). The experimental design included 3 levels of accumulated time in this pressure range (4, 40 and

400 s) which was then divided by 17.2 ms to generate the number of sinusoidal pressure pulses tested (S3-S5, Table 6). Static-pressure tests (40 and 400 s) were used to evaluate the relative effectiveness of sinusoidal pressure pulses (S1 and S2).

Step-pressure pulses can be characterized by a maximum pressure and 4 time parameters (Fig. 3a). The experimental design (Table 6) included single pressure steps of 300 and 100 s (R1 and R2) and tests on pressure-pulsing rate (R3-5). In runs R1 and R2 the pressure come-up (T_1) and come-down (T_3) times were 0.45 and 0.2 s, respectively. In runs R3-R5, the pressure come-up, come-down, and off-pressure times (T_4) were 0.5, 0.2 and 0.02 s, respectively. The effect of on-pressure/off-pressure time ratio (T_2/T_4, R6-R9) were tested using the same pressure come-up and come-down times (Fig. 3b-e).

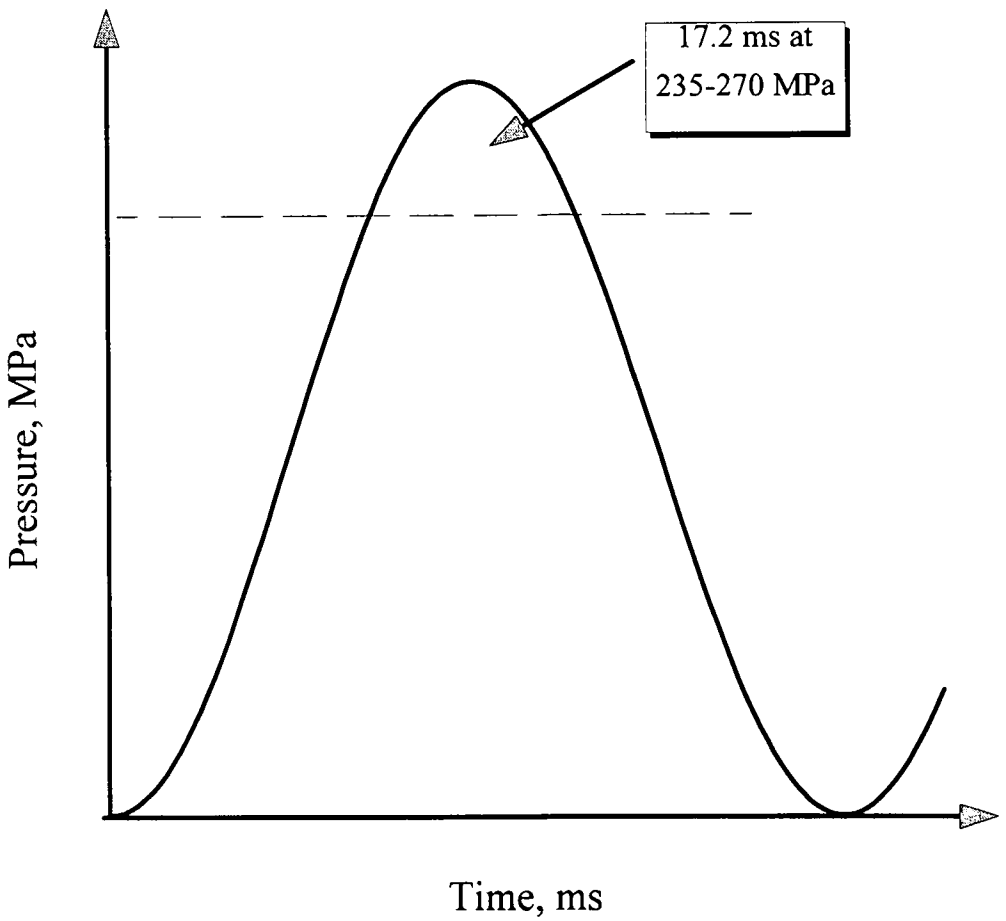

FIGURE 1

Sinusoidal UHP cycles with effective pressure identified as time at 235-270 MPa

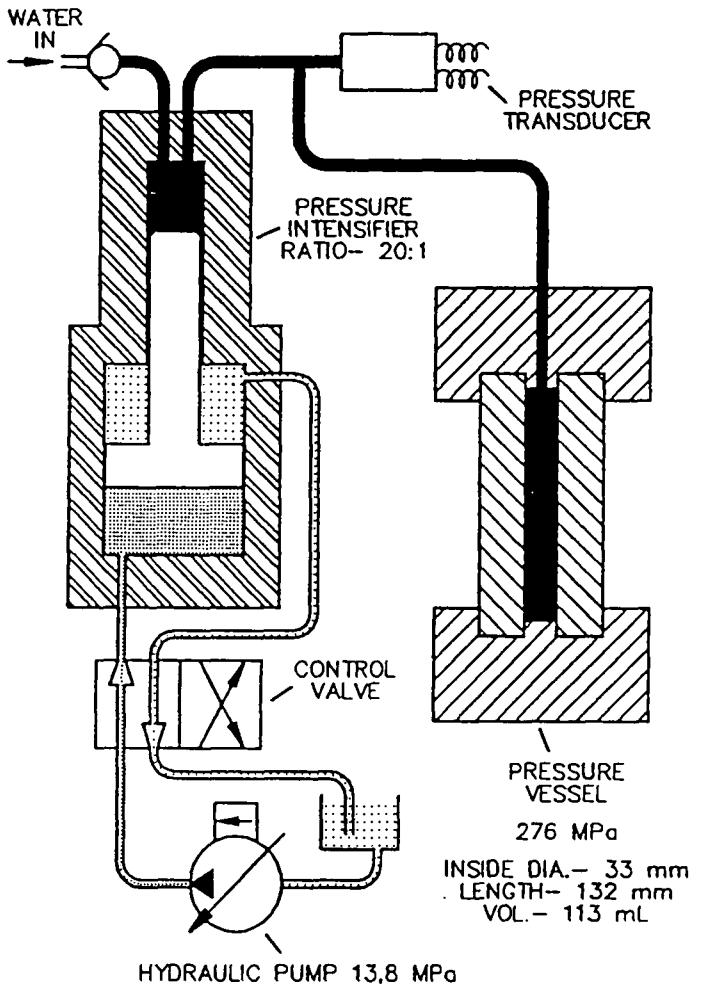

FIGURE 2

Step-pressure pulse processing using an UHP intensifier pump

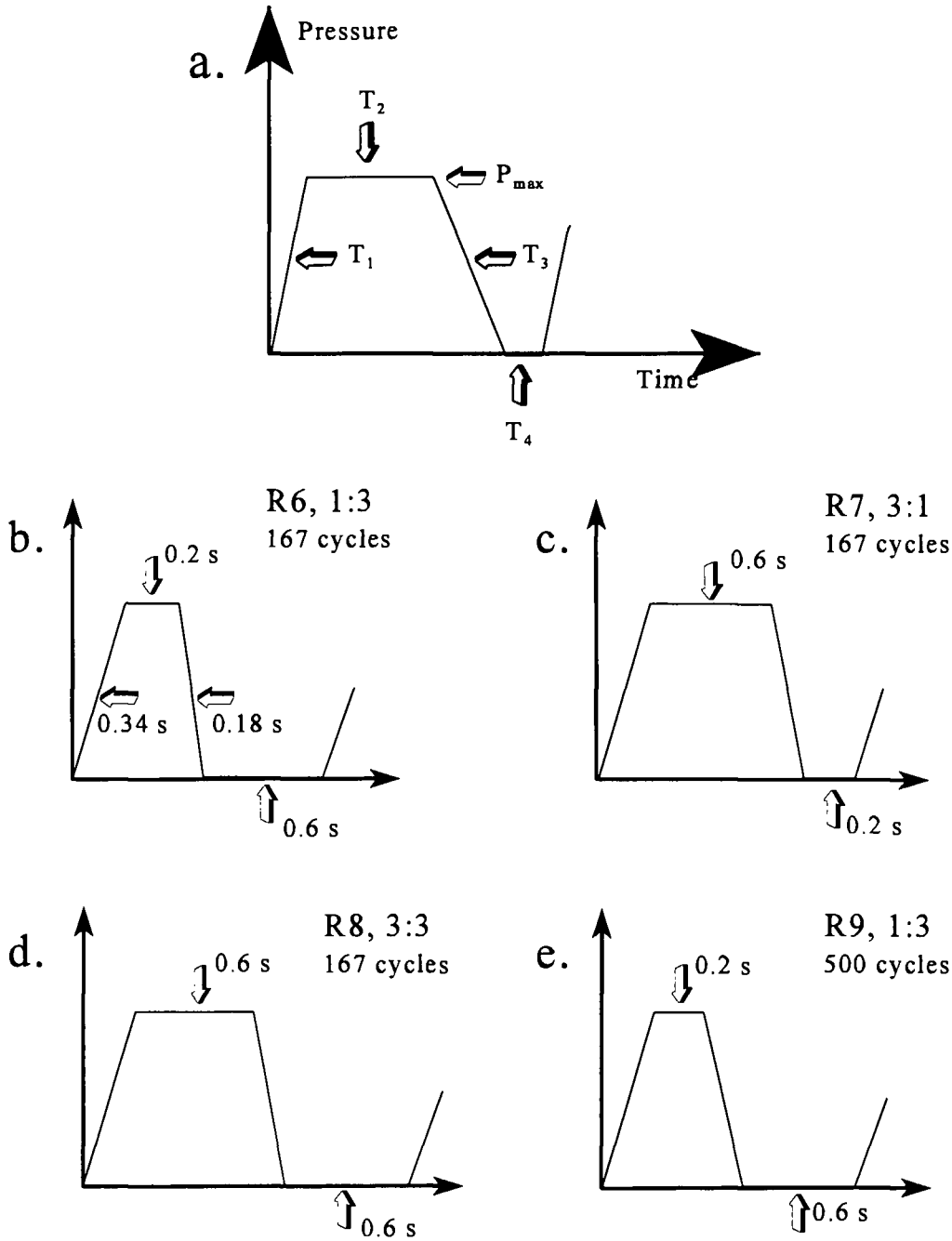

FIGURE 3

Evaluation of step-pressure pulses
(a) Step-pressure characteristics, maximum pressure (P_{max}) and pressure come up (T_1), on-pressure (T_2), pressure come down (T_3) and off-pressure (T_4) times
(b-e) Effect of on-pressure/off-pressure time ratio (T_2/T_4) with constant pressure come-up and come-down time

TABLE 6

Ultra high pressure processing of pineapple juice to evaluate the effectiveness of step and sinusoidal pressure pulses[1]

Run	UHP time[2]	Pressure condition
C	0	Untreated control
Fast sinusoidal pulses reaching 270 MPa (40,000 psi)		
S1	≈40 s	Static
S2	≈400 s	Static
S3	≈4 s	40 cycles, 10 cycles/s, 0.39 min process time
S4	≈40 s	400 cycles, 10 cycles/s 3.9 min process time
S5	≈400 s	4,000 cycles, 10 cycles/s, 39 min process time
Step-pressure pulses reaching 270 MPa		
R1	≈300 s	Static
R2	≈100 s	Static
R3	≈100 s	10 cycles with 10 s @270 MPa
R4	≈100 s	100 cycles with 1 s @270 MPa
R5	≈100 s	200 cycles with 0.5 s @270 MPa
R6	≈33 s	167 cycles, on-pressure:off-pressure time (T_2/T_4) = 1:3
R7	≈100 s	167 cycles, on-pressure:off-pressure time (T_2/T_4) = 3:1
R8	≈100 s	167 cycles, on-pressure:off-pressure time (T_2/T_4) = 3:3
R9	≈100 s	500 cycles, on-pressure:off-pressure time (T_2/T_4) = 1:3

[1] All conditions were evaluated at Flow International Corporation (Kent, WA) except runs S1 and S2 which were evaluated at Oregon State University

[2] Process time at 270 MPa (step pulses) or in the 235-270 MPa pressure range (sinusoidal pulses)

RESULTS AND DISCUSSION

Sinusoidal pressure pulses

Fast sinusoidal pressure pulses (10 cycles/s, S3-S5) were ineffective treatments (Table 7). Runs S4 (≈40 s) and S5 (≈400 s) resulted in no detectable lowering of microbial counts and comparable static pressure treatments (S1 and S2) achieved 0.7 and 5.1 decimal reductions, respectively. This unexpected result was confirmed in 3 additional runs with similar results (data not shown).

Step-pressure treatments: pulsing rate

Slower UHP step-pressure cycles were more effective than static-pressure treatments. However, pulsing rate had no effect on surviving yeast counts (Table 7). Single 300 and 100 s static-pressure pulses resulted in 3 (R1) and 2.5 (R2) decimal reductions, respectively. Pressure pulses at 0.1 (10 pulses), 1 (100 pulses) and 2 (200 pulses) cycles/s with a total UHP-treatment time of 100 s resulted in 3.3, 3.5, and 3.3 decimal reductions, respectively. These reductions were larger than the comparable static-pressure test (R2) and higher than the value observed for run R1 with a UHP-processing time 3 times longer.

Step-pressure treatments: on-pressure/off-pressure time ratio

In runs R3-R5, the off-pressure time was kept at a minimum (T_4 = 0.02 s). Tests on the effects of on-pressure/off-pressure time ratio (T_2/T_4) on microbial inactivation while maintaining the step-pressure time parameters T_1 and T_3

constant were also determined (Table 7, R6-9). A run with short on-pressure time compared to off-pressure time (R9) was equally effective to a single pressure pulse with the same on-pressure time (R2). An equal on-pressure and off-pressure time (R8) was equally effective to the single pulse (R2). However, a run with longer on-pressure time compared to off-pressure time (R7) was more effective than all other runs showing nearly 4 decimal reductions in 100 s.

The complete survival of *S. cerevisiae* after up to 39 min of fast sinusoidal pressure pulses reaching 270 MPa was unexpected. Studies on the physicochemistry of biological systems, particularly of internal and external cellular membranes, at high pressure are needed. The physical changes of water from low to high pressure (Hayakawa et al., 1994) and an irreversible mass transfer from the cell (Perrier-Cornet et al., 1995) have been suggested as mechanisms for the UHP inactivation of microorganisms.

TABLE 7

Ultra high pressure processing of pineapple juice inoculated with *Saccharomyces cerevisiae* 2407-1a to evaluate the effectiveness of step pulses at 270 MPa (40,000 psi) at room temperature

Run	Yeast counts (CFU/mL)		Decimal reductions
	A	B	
A. Sinusoidal pressure pulses			
C		$1.3x10^7$	---
S1	$2x10^6$	$3x10^6$	0.7
S2	$7x10^1$	$1.2x10^2$	5.1
S3	$1.3x10^7$	$1.3x10^7$	≈0
S4	$1.3x10^7$	$1.3x10^7$	≈0
S5	$1.1x10^7$	$1.2x10^7$	≈0
B. Step-pressure pulses[1]			
C	$5.4x10^4$	$3.5x10^4$	---
a. Effect of pulsing rate			
R1	$1.1x10^1$	$1.3x10^1$	3.0^a
	$1.0x10^2$	$6.5x10^1$	
R2	$1.7x10^2$	$1.5x10^2$	2.5^b
	$1.2x10^2$	$1.9x10^2$	
R3	$2.4x10^1$	$1.9x10^1$	3.3^c
	$2.1x10^1$	$2.2x10^1$	
R4	$1.7x10^1$	$2.4x10^1$	3.5^c
	$1.0x10^1$	$6.5x10^0$	
R5	$1.9x10^1$	$8.0x10^0$	3.3^c
	$2.7x10^1$	$2.8x10^1$	
b. Effect of on-pressure/off-pressure time ratio (T_2/T_4)			
R6	$4.6x10^3$	$5.8x10^3$	0.9^d
R7	$4.0x10^0$	$6.5x10^0$	3.9^e
R8	$1.3x10^2$	$1.7x10^2$	2.5^b
R9	$1.6x10^2$	$1.8x10^2$	2.4^b

[1]Means for treatments with different superscript on the decimal reduction values are significantly different ($P<0.05$).

CONCLUSIONS

Slower step-pressure pulses increased sharply the effectiveness of UHP processing. Compared to static-pressure processing, step-pressure pulsing achieved a larger reduction in yeast counts in less time (100 vs 300 s). The characteristics of the step-pressure pulses need to be further optimized and it would be advantageous to conduct in-depth studies to determine the kinetics of the UHP effect on microbial cells.

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V. COMPARISON OF STATIC AND STEP-PULSED ULTRA-HIGH PRESSURE ON THE MICROBIAL STABILITY OF FRESH CUT PINEAPPLE

ABSTRACT

Peeled fresh pineapple cut into 2.5 cm cubes and inoculated with 10^{4-5} *Saccharomyces cerevisiae*/g was packed in heat-sealed polyethylene pouches. Pineapple pouches were subjected at ambient temperature to static and step-pulsed ultra high pressure (UHP) treatments. Static treatments included 100 s and 15 min at 270 MPa and 15 min at 340 MPa. Step-pulsed pressure treatments included 100 s, 300 s, and 600 s at 0-270 MPa using pulse frequencies of 0.1 and 2 cycles/s. Inoculated treated and untreated samples were held at 4°C for 60 days. The number of bacteria were counted using plate count agar (PCA) and yeast growth was followed using yeast extract peptone dextrose agar (YEPD). Static treatment at 270 MPa for 15 min resulted in a few counts at 30 days and detectable counts at day 60. Static treatment at 340 MPa for 15 min generated samples with at most 1-2 colonies per plate at day 60. Step-pulsed pressure treatments for 100 s at 0-270 MPa using pulses of 0.1 (10 pulses) and 2 (200 pulses) pulses/s were more effective than a 100 s static 270 MPa treatment. Step-pulsed pressure treatments (300 and 600 s) at 0.1 (30 and 60 pulses) and 2 (600 and 1200 pulses) pulses/s were as effective as 15 min static pressure treatments at 270 and 340 MPa. This storage study confirmed the superiority of step-pulsed over static pressure treatments. Recovery of pressure-injured cells was not observed in either treatment.

INTRODUCTION

Ultra high pressure (UHP) treatments were first used in the late 1800s by Bert Hite in an attempt to increase the shelf life of milk and meats without using heat (Hite, 1899). His later work included fruits and vegetables at pressures up to 650 MPa (96,000 psi). Pressure treated peaches and pears remained commercially sterile for at least five years after treatment (Hite et al., 1914), suggesting that UHP could be an ideal food preserving method. Its effect on food and food ingredients can be related to the fact that mainly non-covalent bonds are affected (Cheftel, 1992). Microorganisms associated with sweet, ripe fruits, e.g., yeasts and lactic acid bacteria, were more easily inactivated by pressure treatments than organisms associated with vegetables (Hoover et al., 1989). Interest in commercial high-pressure-treated foods begun in the early 1990s with minimally processed, additive free, and fresh-like high-acid foods preserved by UHP marketed successfully in Japan. The Japanese market includes several fruit jams and jellies, orange and grapefruit juice, salad dressings, fruit yogurt and fruit sauces.

High hydrostatic pressure is a physical process that acts instantaneously and independently of product size and geometry. It is effective with mild heating and even at ambient temperature reducing thermal energy consumption when compared with conventional sterilization processes (Oxen and Knorr, 1993; Farr, 1990). The effect of UHP treatment on microorganisms varies with Gram positive more resistant than Gram negative bacteria (Hoover et al., 1989). Yeasts and

molds are very sensitive while spores and viruses are the most resistant to high pressure. Orange and mandarin juices pressure-treated at 400 MPa for 10 minutes can be stored at room temperature for 5 months retaining fresh taste and flavor (Ogawa et al., 1989). Styles et al. (1991) investigated hydrostatic pressure effects on pathogens showing that *Listeria monocytogenes* Scott A strains in ultra-high temperature-processed (UHT) milk could be inactivated within 80 min at 340 MPa. *Vibrio parahaemolyticus* T-3765-1 in clam juice could be destroyed within 10 min at 170 MPa. UHP effects include also protein denaturation (Balny and Masson, 1993) and enzyme inactivation (Seyderhelm et al., 1996; Morild, 1981). Model studies in a phosphate buffer showed the following enzyme inactivation resistance: lipoxygenase < lactoperoxidase < pectinesterase < lipase < phosphatase < catalase < polyphenol oxidase < peroxidase. Other high pressure applications include improving the texture, emulsifying, whipping, and dough-forming properties of food and food ingredients (Hoover, 1989; Torres et al., 1996), lipid phase-change (MacDonald, 1992), genetic mechanisms (Welch et al., 1993) and biochemical reactions (Heremans, 1982).

Information on pulsed UHP treatments on foods is very limited. Hayakawa et al. (1994) reported that step-pulsed pressure treatments accelerated the inactivation of spores. Complete sterilization of a solution containing 10^6 spores/mL of *Bacillus stearothermophilus* was achieved after six 0-600 MPa cycles of 5 min at 70°C (30 min total). Static treatment at 600 and 800 MPa for 60 min could only reduce the initial number of viable spores to 200 and 30 spores/mL, respectively. Pulsed treatments, a novel and effective means to

increase the effectiveness of UHP, has been found effective in modifying cheese microstructures (Torres et al., 1996). The pasteurization of fruit juices showed that step-pulsed pressure (0.1 and 2 pulses/s) achieved a larger reduction in yeast counts in less time than comparable static pressure and fast sinusoidal pulses (10 pulses/s) treatments (Alemán et al., 1996). The purpose of this study was to evaluate and compare the effect of these static and step-pulsed UHP treatments and refrigerated storage on the microbial stability of fresh cut pineapple inoculated with *Saccharomyces cerevisiae*.

MATERIALS & METHODS

Sample preparation

One tenth mL YEPD broth containing 10^{5-6}cells/mL of *S. cerevisiae* strain 2407-Ia was added to 500 mL of canned unsweetened pineapple juice (Dole Packaged Foods Co., San José, CA) and incubated for 3 hours at 32°C to yield juice having 10^{4-5}cells/mL. Fresh pineapple was obtained from a local supermarket, peeled and cut into 2.5 cm cubes. Cubes were held in inoculated juice for 1 hour at 32°C to yield inoculated pineapple having 10^{3-4} cells/g. Pineapple cubes for static and pulsed experiments were placed into polyethylene bags and heat-sealed with elimination of entrapped air. Samples for static pressure treatments were placed in a heat-sealed outer bag filled with distilled water to prevent sample contact with the hydraulic fluid in the isostatic press. All packaged cubes were then held overnight at 4°C before treatment. After

treatment, static and pulsed samples were stored at refrigeration temperature for up to 60 and 30 days, respectively.

Pressure treatments

Static pressure treatments were achieved using an Autoclave Engineers (Erie, PA) isostatic press model No. IP2-22-60 with a cylindrical pressure chamber (height = 56 cm, diameter = 5 cm). Bagged samples were submerged in the chamber, which contained water and 2% Hydrolubic 142™ (Houghton & Co., Valley Forge, PA) as the pressure medium. Samples were subjected to pressures of 270 (K1) and 340 MPa (K2) for 15 min at room temperature (Table 8).

Step-pulsed and 100 s static pressure experiments were conducted at the R&D facilities of Flow International Corporation (Kent, WA). In step-pulsed experiments (R3 and R5), the come-up, come-down and off-pressure times were 0.5, 0.2 and 0.02 s, respectively. In the 100 s static pressure treatment (R2), the come-up and come-down times were 0.45 and 0.2 s, respectively (Table 8). Pineapple samples were exposed to step-pulsed pressures in a modified cylinder of an UHP direct-drive triplex pump with water at room temperature as the hydrostatic fluid. An oscilloscope and pressure transducers were used to record the pressure-time profile for each treatment with an estimated ±1% precision at 270 MPa. Digital oscilloscope pressure readings were calibrated against a precision bourdon tube test gauge. Static pressure treatments were 100 s at 270 MPa (R2) while pulsed treatments were 0-270 MPa for 100 s, 300 s and

600 s. Two cycle frequencies were used, 0.1 (R3) and 2 (R5) pulse/s (Fig. 4). To obtain 100 s, 300 s and 600 s step-pulsed pressure treatments, the total number of pulses for R3 was 10, 30 and 60 and for R5 was 200, 600 and 1200, respectively.

TABLE 8

Ultra high pressure processing of pineapple chunks to evaluate the effectiveness of static and step-pulsed UHP treatments

Run	UHP treatment time	Process conditions
K1	9000 s	static 270 MPa come-up time = 100 s come-down time = 20 s
K2	9000 s	static 340 MPa come-up time = 140 s come-down time = 25 s
R2	100 s	static 270 MPa come-up time = 0.45 s come-down time = 0.2 s
R3	100 s, 300 s, 600 s 10, 30 and 60 pulses at 0.1 pulses/s	0-270 MPa step-pulses come-up time = 0.5 s come-down time = 0.2 s off-pressure time = 0.02 s
R5	100 s, 300 s, 600 s 200, 600 and 1200 pulses at 2 pulses/s	0-270 MPa step-pulses come-up time = 0.5 s come-down time = 0.2 s off-pressure time = 0.02 s

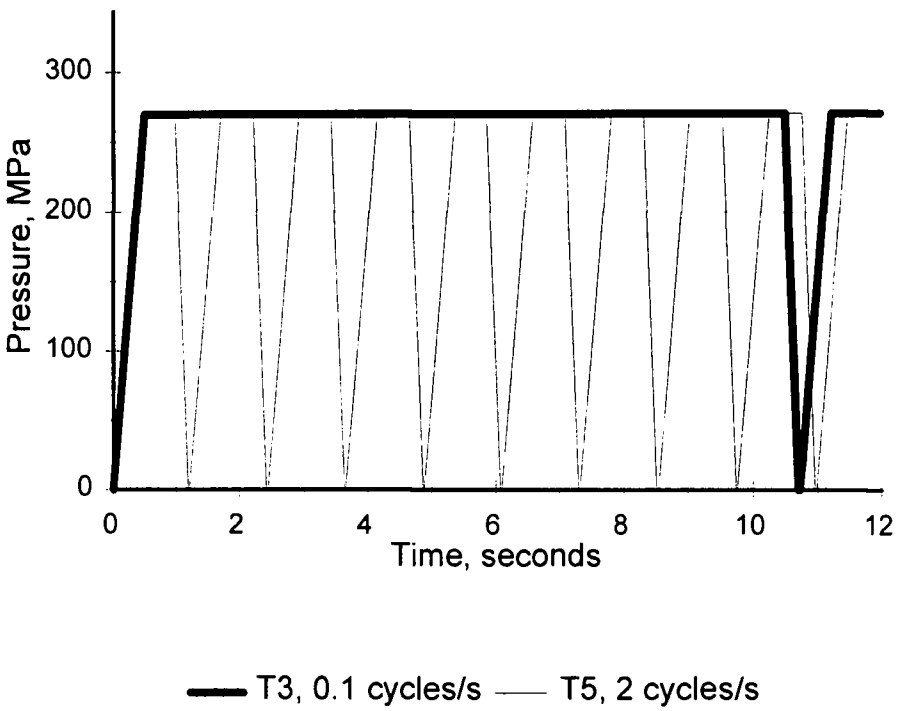

FIGURE 4

Step-pulsed pressure treatments (T3 and T5)

Microbiological assays

Controls and pressure treated samples were stomached using 0.1% peptone water and plated immediately after treatment to determine microbial counts. Appropriate dilutions were then made with aliquotes plated using the pour plate technique. Plate count agar (PCA; Difco Laboratories, Inc., Detroit, MI) was used to estimate bacterial counts. Yeast extract peptone dextrose agar medium containing 1% yeast extract, 2% peptone, 1% dextrose and 1.5% agar. (YEPD; Difco) incubated 48 hours at 32°C were used to estimate yeast counts. Samples were tested immediately after treatment, and after 5, 10, 15. 30, and 60 days of refrigerated storage (4°C). Average counts were reported only when CFU/plate ≥25 after a 1/10 fruit sample dilution, i.e., ≥250 CFU/g sample. Lower microbial counts were reported as (-) to indicate 0 colony counts and (+) to indicate 1-24 CFU/plate.

Statistical analysis

Multifactor ANOVA using Statgraphics Plus (Windows V.2, North Hollywood, CA) was used to evaluate the relative effect of each treatment on microbial inactivation. A randomized block design was used to evaluate the effect of two levels of static pressure on bacteria and yeast for up to 60 days of refrigerated storage. Each pressure treatment was composed of three runs/treatment (N1-N3). Samples were stored refrigerated at 4°C for up to 60 days and randomly removed from storage for microbial counts.

Samples with a total treatment time of 100 s for R2, R3, and R5 generated in 2 runs/treatment/sampling-day (runs N1 and N2) were stored refrigerated for 30 days and randomly removed for microbial testing. Single runs of treatments R3 and R5 of 300 s and 600 s duration were tested in duplicates at 0, 5, 10, 15 and 30 days after refrigerated storage.

RESULTS AND DISCUSSION

Static pressure treatment

Bacteria and *S. cerevisiae* counts treated at 270 and 340 MPa for 15 min are shown in Table 9. The reproducibility of the experiment was confirmed by analysis of the triplicate runs ($p >> 0.05$). Both pressure treatments reduced yeast counts by 5-log cycles and total bacteria and yeast counts remained <25CFU/plate after a 1/10 sample dilution during 60 days of storage. Statistical analysis (ANOVA) showed an interaction effect between pressure and storage ($p < 0.001$). In both treatments, counts decreased during storage time for up to 30 days and increased slightly at day 60. The exception was a slight increase in counts at day 5 for 270 MPa treated samples.

Step-pulsed pressure treatments

Step-pulsed pressure treatments (0-270 MPa) for 100 s at 0.1 cycles/s (10 pulses R3) and 2 cycles/s (200 pulses R5) were more effective than a 100 s static treatment at 270 MPa (R2). Again, the reproducibility of the experiment was

confirmed by analysis ($p >> 0.05$). After 30 days of refrigerated storage only R2 samples showed $>10^3$ CFU/g in bacteria and yeast counts (Table 10). Both 300 and 600 s pulsed 0-270 MPa treatments (Table 11) were as effective as 15 min static 270 and 340 MPa treatments (Table 9). R3 and R5 (0-270 MPa) for 300 s yielded 0-4 CFU/plate after a 1/10 sample dilution during 30 days of storage and recovery of pressure-injured cells was not observed. Static 15 min pressure treatments at 270 MPa and 340 MPa resulted in ≤ 10 CFU/plate after a 1/10 sample dilution during the same 30 days of storage for duplicate plates.

TABLE 9

Effects of pressure and storage time at 4 °C on the microbial stability of fresh cut pineapple pieces subjected to static pressure treatments for 15 minutes at ambient temperature

a. 270 MPa with 100 s and 20 s pressure come-up and come-down time, respectively

Storage Time (days)	PCA (bacterial counts)[a]				YEPD (yeast counts)			
	Untreated	N1	N2	N3	Untreated	N1	N2	N3
		A A B B	A A B B	A A B B		A A B B	A A B B	A A B B
0	$1.2x10^4$	+ + + +	+ + + +	+ + + +	$1.3x10^4$	+ + + +	+ + + +	+ + + +
5	$4.1x10^4$	+ + + +	+ + + +	+ - + +	$2.5x10^4$	+ + + +	+ + + +	+ + + +
10	$5.2x10^4$	+ + + +	+ + + +	+ + - +	$6.5x10^4$	+ - - +	- - + +	+ + + +
15	$4.2x10^4$	+ + - +	- - - -	+ - - -	$3.5x10^4$	+ + - +	- - - +	- + + +
30	$2.9x10^4$	- - - +	- - - -	- - - -	$3.3x10^4$	- - - -	- + - -	- - - +
60	$2.5x10^6$	+ + + +	- + + +	- + + +	$2.4x10^6$	+ + + +	+ + + +	- + - +

TABLE 9 - continued

b. 340 MPa for 15 min with 140 s and 25 s pressure come-up and come-down time, respectively

Storage Time (days)	PCA (bacterial counts)				YEPD (yeast counts)			
	Untreated	N1	N2	N3	Untreated	N1	N2	N3
		A A B B	A A B B	A A B B		A A B B	A A B B	A A B B
0	$1.2x10^4$	+ + + +	+ + + +	+ + + +	$1.3x10^4$	+ + + +	+ + + +	+ + + +
5	$4.1x10^4$	- - - +	- + - +	+ + - -	$2.5x10^4$	- - - +	- + - -	- + - +
10	$5.2x10^4$	- + + -	+ - - -	+ + + -	$6.5x10^4$	- + - -	- - + +	- + + +
15	$4.2x10^4$	- - - +	- - - +	- - - -	$3.5x10^4$	- - - -	- - - -	- - - -
30	$2.9x10^4$	- + - +	- - - -	- - - -	$3.3x10^4$	- + - +	- - - -	- - - -
60	$2.5x10^6$	- + - +	- - - -	- - - -	$2.4x10^6$	+ + - +	- - - +	- - - +

[a]Three replicate runs (N1, N2, and N3) and two samples (A, B) per run were tested by duplicate plating and reported as an average only when CFU/plate≥25 after a 1/10 fruit sample dilution, i.e. ≥250 CFU/g sample. Lower microbial counts were reported as AABB with (-) = 0 and (+) = 1-24 CFU/plate.

TABLE 10

Effects of pressure and refrigerated storage time on the microbial stability of fresh cut pineapple pieces subjected to static 270 MPa and step-pulsed 0-270 MPa treatments at ambient temperature

Storage Time (days)	PCA[a] (bacterial counts)						
	Untreated	T1[b]		T3[b]		T5[b]	
		N1	N2	N1	N2	N1	N2
0	$1.3x10^4$	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
5	$1.7x10^4$	+ + + +	+ + + +	+ + + -	+ + + +	+ + - -	- - - -
10	$1.9x10^4$	+ + + +	+ + + +	- - + -	- + + -	- - - -	- - - +
15	$5.4x10^4$	- - + -	+ - + -	- - + -	- - - -	- - - -	- - - -
30	$4.5x10^6$	$3.6x10^3$	$4.6x10^3$	+ + + +	+ + + -	+ + - -	- - - +

TABLE 10 - continued

Storage Time (days)	YEPD (yeast counts)						
	Untreated	R2		R3		R5	
		N1	N2	N1	N2	N1	N2
0	$8.2x10^3$	+ + + +	+ + + +	+ + + +	- - + -	+ + + +	- - + +
5	$1.9x10^4$	- - - -	+ + - -	+ - - -	- - - -	- + - -	- - - -
10	$2.7x10^4$	- + - -	- - - -	- - - -	- - + +	- - - -	- - - -
15	$2.2x10^5$	+ - + -	+ - - -	- - - -	- - + -	- - - -	+ - + -
30	$6.2x10^6$	$4.8x10^3$	$5.5x10^3$	+ + + +	+ - - +	+ + + -	+ - - +

[a]Two replicate runs (N1 and N2) and two samples (A, B) per run were tested by duplicate plating. See Table 9 footnote for further details.
[b]R2 = 100 s static, R3 = 10 pulses at 0.1 cycles/s, R5 = 200 pulses at 2 cycles/s (see Fig. 4).

TABLE 11

Step-pulsed pressure treatments at 0-270 MPa and samples stored at 4°C

Storage time (days)	PCA[a] (bacterial counts)				
	Untreated	300 s treatment		600 s treatment	
		R3[b]	R5	R3	R5
0	$1.3x10^4$	+ - - -	+ - + -	+ + + +	- - - -
5	$1.7x10^4$	- - - +	- - - -	- - - -	- - - -
10	$1.9x10^4$	- - + -	- - - -	- - - -	- - + -
15	$5.4x10^4$	- + + -	+ + - -	- - - -	- - + +
30	$4.5x10^6$	- + - -	+ + - -	- - - -	- - + +

	YEPD (yeast counts)				
	Untreated	300 s treatment		600 s treatment	
		R3	R5	R3	R5
0	$8.2x10^3$	+ - - -	- - + -	+ - + -	+ + - -
5	$1.9x10^4$	- - - -	- - - -	- - - -	- - - -
10	$2.7x10^4$	+ + + -	+ - - -	- - - -	- - - -
15	$2.2x10^5$	- - - -	- - - +	- - - -	- - - -
30	$6.2x10^6$	- - - -	+ - - -	- - - -	+ + - -

[a]Two samples (A, B) per run were tested by duplicate plating. See Table 9 footnote for further details.
[b]R3 = 30 and 60 pulses at 0.1 cycles/s, R5 = 600 and 1200 pulses at 2 cycles/s (see Fig. 4).

CONCLUSIONS

This research and previous work (Alemán et al., 1996) demonstrate the effectiveness of step-pulsed pressure treatments on reducing microbial populations when compared to static pressure treatments. Storage tests showed that pulsed pressure at 0-270 MPa at 0.1 and 2 pulses/s for 100s were comparable to 15 min static pressure treatments at 270 MPa. The recovery of pressure-injured cells in fresh pineapple held at 4°C was not observed. This suggests that fresh pineapple might have a microstatic effect on pressure-injured cells.

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VI. CONCLUSIONS

Ultra High Pressure food processing is currently the focus of major research efforts around the world. This technology has the potential to offer consumers minimally processed foods of high quality, extended shelf life and free of additives. Conventional food preservation methods do not meet these requirements due to alterations of food texture, flavor and nutritional value.

UHP treatment at 340 MPa for 15 min produced a commercially acceptable reduction of native microflora on fresh cut pineapple. Longer process times (40 and 60 min) at this pressure did not result in further significant count reductions. This research demonstrated for the first time the effectiveness of step-pulsed pressure treatments on reducing microbial populations when compared to static pressure treatments. Storage tests showed that pulsed pressure at 0-270 MPa at 0.1 and 2 pulses/s for 100s were comparable to 15 min static pressure treatments at 270 MPa. The recovery of pressure-injured cells in fresh pineapple held at 4°C was not observed. This suggests that fresh pineapple might have a microstatic effect on pressure-injured cells. The characteristics of the step-pressure pulses need to be further optimized and it would be advantageous to conduct in-depth studies to determine the kinetics of the UHP effect on microbial cells. An understanding of this high pressure effect on microbial survival and other properties of interest to the food industry will lead to new products and applications.

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