HIGH PRESSURE PROCESSING AS AN ALTERNATIVE FOOD PRESERVATION TECHNOLOGY AND ITS APPLICATIONS FOR FRUITS AND VEGETABLES

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Abstract

Consumers demand for high quality, natural and fresh tasting food, free from preservatives and additives, with a clean label and an extended shelf life has increased. High pressure processing (HPP), also known as high hydrostatic pressure, is a non-thermal food preservation technique that has the potential to meet these demands. It is an opportunity to preserve food, by applying intensive pressure in the range of 300-900 MPa, without adversely affecting organoleptic, textural and nutritional qualities as thermal processing like pasteurization and sterilization may do.

In a typical high pressure batch cycle, the food prepackaged in a high-barrier flexible pouch or a plastic container is loaded into a perforated basket that goes into the pressure vessel; the pressure is then increased to the processing target pressure (come-up time); the product is held at the desired pressure for 3 to 10 minutes (pressure holding time); after which the pressure is released in usually few seconds (decompression time) and the product can be unloaded at this point. The pressure is applied uniformly in all directions simultaneously and this is known as isostatic pressure. Pressurization is usually accompanied by a moderate and uniform temperature increase called adiabatic heating. However, the food product usually rapidly returns to its initial temperature at decompression.

With the recent shift in consumer lifestyle toward healthy living and healthier food, the consumption of raw fruits and vegetables has increased in popularity. However, as per the Centers of Disease Control and Prevention, fruits and vegetables have recently been associated with multiple foodborne disease outbreaks; the effect of high pressure processing on microbial safety, quality and sensory characteristics of fruits and vegetables has therefore been widely investigated as an alternative to traditional food processing and preservation methods. HPP inactivates microorganisms and quality-deteriorating enzymes and has limited effects on covalent bonds resulting in minimal modifications of food-quality attributes such as color, flavor and nutritional values. However, depending on the fruit or vegetable, high pressure could induce chemical or biochemical reactions that can affect their quality attributes.

Table of Contents

List of Figures

Chapter 1 - Introduction

Consumers demand for high quality, natural and fresh tasting food, free from preservatives and additives, with a clean label and an extended shelf life has increased. High pressure processing (HPP), also known as high hydrostatic pressure, is a non-thermal food preservation technique that has the potential to meet these demands. It is an opportunity to preserve food, by applying intensive pressure in the range of 300-900 MPa, without adversely affecting organoleptic, textural and nutritional qualities as thermal processing like pasteurization and sterilization may do.

High pressure processing was discovered in 1899 and has been used since in chemical, ceramic, carbon allotropy, steel/alloy, composite materials, crystal, diamond and plastic processing industries. Its use in the food processing area dates back over a century to the research of Hite in 1899 for the preservation of milk and later on fruits and vegetables. It was not until early 1990s, that the first commercial high pressure processed food were available with the launch of jellies and jams by the Japanese industry in Tokyo Meidi-ya (Mertens, 1995, Thakur, 1998). In the U.S., the first successful commercial high pressure processed product application was a guacamole dip manufactured by Fresherized Foods in Texas. Currently, other high pressure processed products are on the international markets such as fruit juices, jams, jellies, rice cakes, and raw squid in Japan; fruit juices in France, Italy, United Kingdom and Portugal; and ready-to-eat meats, salsa, guacamole and in-shells oysters in the USA; apple sauce in Canada (Hugas et al., 2002).

A high pressure system consists of a pressure vessel, a pressure-transmitting fluid, a material handling system, hydraulic compressors and a heating/cooling unit. Food products can be high pressure processed in a batch system or a semi-continuous process. Liquid foods such as juices are processed in a semi-continuous system without any packaging requirements. Solid food or bulk products are processed in a batch system; in this method, the products are packaged and sealed before processing (Hogan et al., 2005; Mertens et al., 1993). In a typical batch cycle, the food is loaded into a perforated basket that goes into the pressure vessel; the pressure is then

increased to the processing target pressure (come-up time); the product is held at the desired pressure for 3 to 10 minutes (pressure holding time); after which the pressure is released in usually few seconds (decompression time) and the product can be unloaded at this point (Balasubramaniam et al., 2004, 2008).

During high pressure processing, the pressure is applied uniformly in all directions simultaneously and this is known as isostatic pressure; this is the reason why food is not crushed during treatment. This is a major advantage compared to thermal methods where the product temperature is gradually increased (Balasubramaniam et al., 2008).

High pressure processing is usually accompanied by a moderate temperature increase called adiabatic heating which depends on the composition of the food product being processed. The temperature of the water in the food increases by 3ºC per 100 MPa, whereas the temperature of the fats and oils increases about 8-9ºC per 100 MPa (Balasubramaniam et al., 2004, 2008; Hogan et al., 2005).

The effect of high pressure processing on microorganisms has been widely investigated. Microorganisms vary in their response to high pressure and indeed there can be vast high pressure sensitivity among bacterial species and even strains (Alpas et al., 1999; Benito et al., 1999). Compared to vegetative cells, endospores tend to be extremely high pressure processing resistant, requiring a combination of high pressure treatment at pressure exceeding 1000 MPa and heat treatment with a temperature of more than 80°C (Abee & Wouters, 1999; Rastogi et al., 2007; Smelt, 1998). Yeasts and molds are relatively sensitive to high pressure processing. Most vegetative yeast and molds are inactivated within a few minutes by 300-400 MPa at room temperature. However, yeast and mold ascospores may require treatment at higher pressures. Viruses show a wide range of sensitivity in response to high pressure.

The most common packaging materials used for high pressure processed food are polypropylene (PP), polyester tubes, polyethylene (PE) pouches, and nylon cast polypropylene pouches. Plastic packaging materials are the best suited for high pressure processing use because of their reversible response to compression, their flexibility and resiliency.

Packaging materials for high pressure processing must be flexible to withstand a 15% increase in volume followed by a return to original size, without losing physical integrity, sealing or barrier properties. The headspace must be minimized as much as possible (Lambert, 2000) in order to control the deformation of packaging materials and ensure efficient use of the package and space in the pressure vessel.

With the recent shift in consumer lifestyle toward healthy living and healthier food, the consumption of raw fruits and vegetables has increased in popularity. However, fruits and vegetables have recently been associated with foodborne disease outbreaks as per the centers of disease control and prevention such as cantaloupe S*almonella* outbreaks in 2011 and 2008, alfalfa sprouts S*almonella* outbreaks in 2010 and 2009, shredded romaine lettuce *E-coli 0145* outbreak in 2010, raw produce S*almonella* outbreak in 2008, tomatoes S*almonella* outbreak in 2006 and fresh spinach *Escherichia coli O157:H7* in 2006. The effect of high pressure processing on microbial safety, quality and sensory characteristics of fruits and vegetables has therefore been widely investigated as an alternative to traditional food processing and preservation methods.

Chapter 2 - High Pressure Processing Technology

Basic High Pressure Principles

Two main principles control the behavior of food under high pressure:

Le Chatelier's Principle

According to Le Chatelier principle, phenomena resulting in a decrease in volume, like phase transition, chemical reaction, or change in molecular configuration, are enhanced by pressure. On the contrary, phenomena resulting in an increased volume are slowed down by pressure (Balasubramaniam et al., 2008).

Isostatic Principle

Pressure is instantaneously and uniformly transmitted throughout the sample, independently of size and shape of the food, which is a major advantage compared to thermal processing (Smelt, 1998). A uniform pressure will be applied uniformly in all directions of the sample, thus the pressure will not damage the product which will return to its original shape when the pressure has been released. This is known as isostatic pressure (Balasubramaniam et al., 2008).
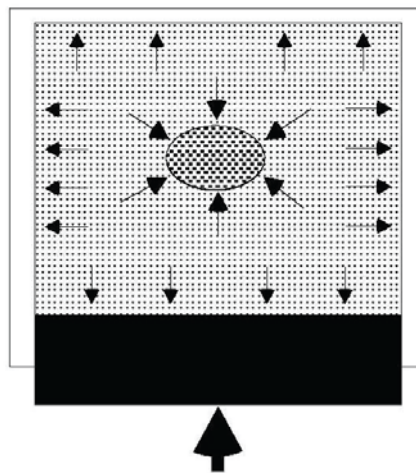
Figure 2.1: Isostatic Pressure

Critical High Pressure Processing Factors

High Pressure Processing Equipment

Although the principles of high pressure processing of food have been known since the late 1800s (Hite, 1899), it is not until relatively recently that the developments in engineering equipment design have permitted the use of high pressure processing at the industrial level.

The primary components of a high pressure processing system include:
-   A pressure vessel of cylindrical design
-   Two end closures for sealing the vessel
-   A device for restraining the end closures (yoke, threads, pin)
-   A low pressure pump
-   An intensifier which uses liquid from the low pressure pump to generate high pressure process fluid for system compression
-   A system for controlling and monitoring the pressure and temperature
-   A product-handling system, usually perforated baskets, for transferring product to and from the pressure vessel (batch system)

High pressure vessels may operate in a vertical, horizontal, or tilting mode. The pressure vessel can be built with two or more concentric cylinders. The cylinders should be made of stainless steel to avoid corrosion.

Current industrial HPP treatment of food is done using a batch or semi-continuous process; solid food can only be treated in a batch mode and need to be prepackaged, whereas liquid food can be treated in a batch (packaged food) or semi-continuous process (unpackaged food) (Hogan et al., 2005).
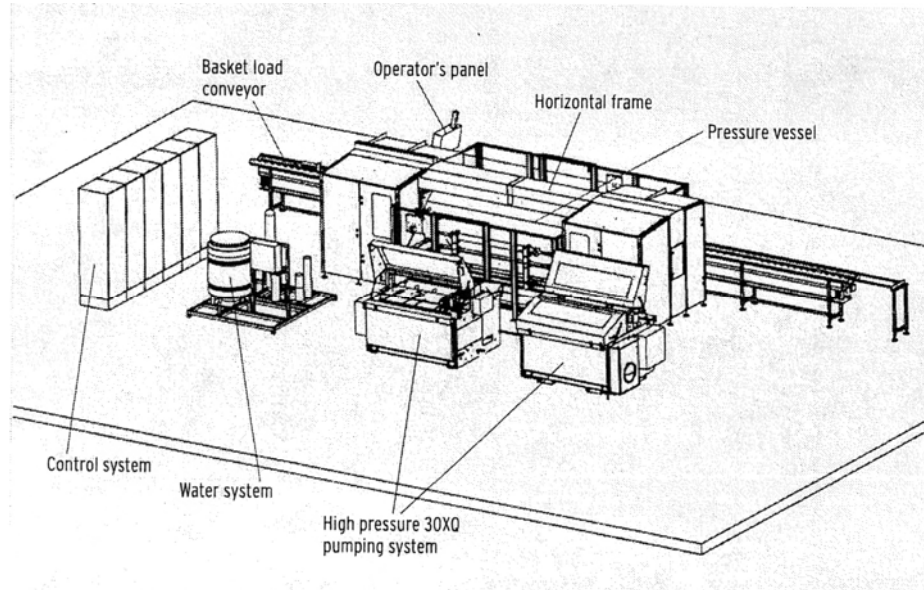
Figure 2.2: Avure Horizontal High Pressure System QFP 350L-600

Figure 2.3: Avure Horizontal High Pressure System
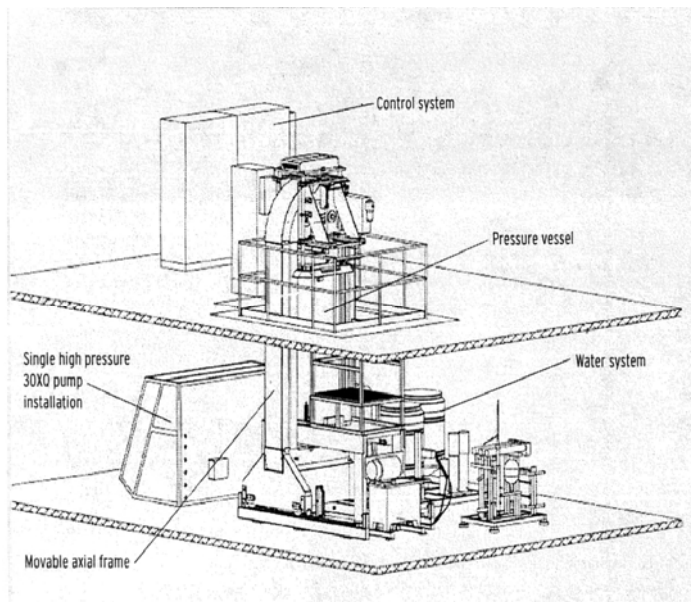
Figure 2.4: Avure Vertical High Pressure Processing System QFP 215L-600

High Pressure Processing Batch Equipment

In a typical high pressure batch process, the solid or liquid food, packaged in a high-barrier flexible pouch or a plastic container is loaded into the high pressure vessel; the vessel is then sealed and the pressure-transmitting fluid (usually water) is pumped into it, displacing any air. The pressure relief valve is then closed, and the vessel is pressurized by the use of a high-pressure pump, which injects additional pressure-transmitting fluid until the process pressure is reached. The product is held for the desired time (usually around 5 minutes) at the target pressure. The pressure is applied uniformly in all directions simultaneously. The pressure is transmitted through the package into the food itself. When the process time is completed, the pressure relief valve is opened and the vessel is decompressed by releasing the pressure-transmitting fluid. The processed product is then unloaded and stored/distributed in the conventional manner (Balasubramaniam et al., 2008, FDA, 2000).

High Pressure Processing Semi-continuous Equipment

The semi-continuous systems are used to treat unpackaged liquids. These semi-continuous high pressure equipments use two or more pressure vessels, each containing a free-floating piston that allows each vessel to be divided into two chambers. The liquid food is pumped into the first chamber; the fill valve is then closed and the pressure-transmitting fluid is pumped into the second chamber of the vessel on the opposite side of the floating piston. Pressure applied to the fluid will result in compression of the liquid food in the other chamber. After an appropriate process hold time, the product discharge valve is opened and a low-pressure pump injects pressure-transmitting fluid into the second chamber, which pushes on the piston and expels the contents of the product chamber through the discharge valve. The treated liquid is discharged from the pressure vessel to a sterile hold tank through a sterile discharge port. The treated liquid food can then be filled aseptically into pre-sterilized containers (Balasubramaniam et al., 2008, Farkas and Hoover, 2000, US Food and Drug Administration, 2000).
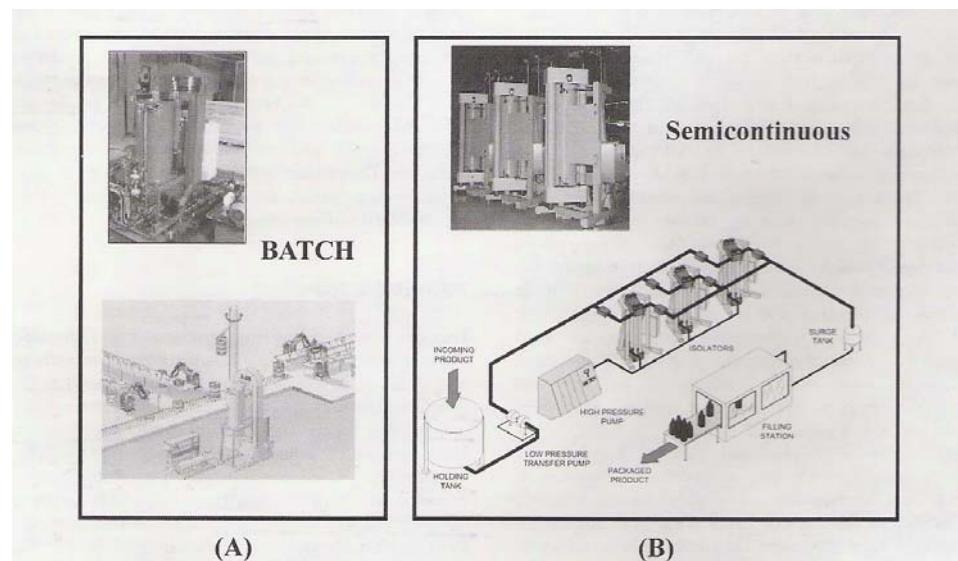
Figure 2.5: Batch (A) and Semi-continuous (B) High Pressure Food Processors (Balasubramanium, 2003).

High Pressure Processing Time (Balasubramaniam et al., 2004, 2008)

Come-up time

The come-up time is the time required for the pressure of the treated sample to increase from the atmospheric pressure Ps (0.1MPa) to the processing pressure P1 (Figure 2.6). This time depends on the:

- sample rate of compression
- transmitting fluid rate of compression
- power of the high pressure pump
- target process pressure

Most high pressure equipments use one to three minutes pressure-come-up time to reach the process pressure.

Pressure holding time

The pressure holding time is the time between the end of the come-up time and the beginning of decompression. High pressure holding time is usually around three to ten minutes. For an economically effective commercial high pressure process, the industry should target reduced pressure holding times to maximize productivity. Other options that may help reduce the processing time could be pulsed pressure processing (multiple compression-decompression cycles), combination of high pressure with temperature treatment or other processing technologies, or increasing the processing pressure.

Decompression time

The decompression time is the time required to bring the sample from process pressure back to atmospheric pressure (0.1 MPa). Most high pressure equipments need just few seconds to depressurize.
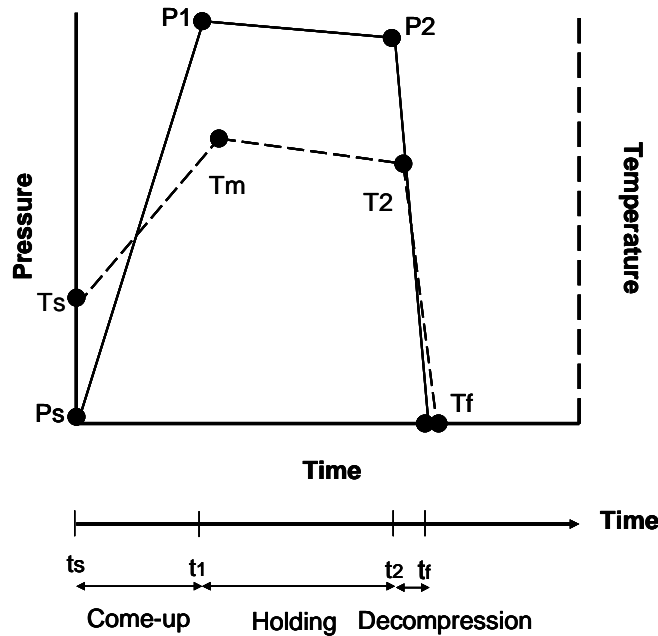
Figure 2.6: Pressure-Time-Temperature Curves for a Batch HPP Treatment (Balasubramaniam et al., 2004).

High Pressure Product and Process Temperatures

Initial Temperature

The initial temperatures of the product, pressure-transmitting fluid and the process vessel have major influence on the microbial inactivation and thus need to be documented. An increase or decrease in the initial food temperature above or below room temperature enhances the microbial inactivation rate (Kalchayanand et al., 1998). Products are usually chilled for food pasteurization applications; however, for sterilization of low-acid foods, products are often preheated. It is important to allow sufficient equilibrium time of the product, pressure transmitting fluid and vessel to ± 0.5% of the target initial temperature before high pressure processing (Balasubramaniam et al., 2004).

Adiabatic Heating (Balasubramaniam et al., 2004, 2008; Hogan et al., 2005)

Pressurization is usually accompanied by a moderate and uniform temperature increase called adiabatic heating. The heat of compression of food materials depends on the final pressure, the initial temperature and the food composition. Food materials have specific heat of compression values; during compression, the water temperature increases about 3ºC/100 MPa at room temperature, and the fats and oils temperature increases about 8-9ºC/100 MPa. The temperature of the food that contains a significant amount of water will then increase by approximately 3ºC per 100 MPa; whereas the temperature rise of the food that contains a significant amount of fat like oil, butter or cream will be around 8-9ºC per 100 MPa.

In an insulated system, the food product rapidly returns to its initial temperature at decompression. However, heat can be lost to or gained from the walls of the pressure vessel during decompression and holding time. It is thus important to control and monitor the temperature of the walls of the pressure vessel and hold it to the desired temperature in order to truly achieve isothermal conditions. In general, at decompression, the product returns to a temperature (Tf) slightly lower than its initial temperature (Ts) due to heat exchange with the surroundings (Figure 2.6).

pH, Water Activity (Aw) and Food Composition

Many food components provide microorganisms a protection effect against the high pressure treatment. Processing time and pressure will then depend on the composition of the food and its environment.

Pressurization of food causes an increase in ionization which leads to a decrease in pH (Hoover et al., 1989; Heremans, 1995; Patterson et al., 2005). Heremans (1995) reported a decrease of 0.2 units in apple juice pH for an increase of pressure by 100 MPa.

The reduced pH works in synergy with pressure in eliminating microorganisms and inhibits the recovery or outgrowth of sub-lethally injured cells. Hoover et al. (1989) noted that

high pressure pasteurization reduces the pH range for microorganism's growth by inhibiting the membrane ATPase, vital enzyme in the acid-base physiology of cells. Patterson et al. (2005) and Alpas et al. (2000) indicated that pH shifts, due to high pressure, depend on the chemical nature of the surrounding environment. A low pH buffer enhances pathogens sensitivity to high pressure. pH shifts are reversible after the treatment pressure is released.

A low water activity inhibits pressure inactivation of microorganisms and protects cells against high pressure pasteurization. Microorganism cells injured by HPP are more sensitive to water activity (Smelt, 1998).

Carbohydrates, proteins, lipids, and other food components can provide microorganisms a protective effect (Simpson and Gilmour, 1997; Patterson et al., 1999 and Cheftel, 1995). This may be due to the fact that high pressure pasteurization only affects non-covalent bonds like ionic, hydrophobic and hydrogen bonds and does not denature primary protein structures.

Simpson and Gilmour (1997) indicated that phosphate-buffer-saline (PBS) modified with bovine serum albumin (protein), glucose (carbohydrate), and olive oil (lipid) protected *Listeria monocytogenes* against pressure inactivation when compared to phosphate-buffer-saline (PBS) alone.

Cheftel (1995) reported that salt and sucrose protected pathogens from high pressure pasteurization inactivation effect.

Patterson et al. (1999) reported that ionic solutes such as NaCl and $CaCl_2$ granted a better protection of *Bacillus coagulans* compared to nonionic solutes such as sucrose and glycerol.

Hauben et al. (1998) indicated that Ca^{2+} provided a dose-dependent baroprotection to *Escherichia coli*.

Pressure-transmitting Fluids

Pressure-transmitting fluids transmit pressure uniformly and instantaneously to the sample. The most common transmitting fluids are water, food-grade glycol–water solutions, silicone oil, sodium benzoate solutions, ethanol solutions, and castor oil (Balasubramaniam et al., 2004; Hogan et al., 2005).

Air pockets-containing products

Air pockets-containing products such as strawberries and lettuce can be damaged by high pressure processing due to the collapse of air voids under pressure and the infiltration of liquid from the surrounding structure into the collapsed air pockets. The air displacement due to high pressure can cause irreversible physical shrinkage and shape distortion of the air pockets-containing food (Balasubramaniam et al., 2004; Hogan et al., 2005).

High Pressure Process Packaging

High pressure processing of food is done either on bulk or prepackaged products so it is crucial to investigate the effect of high pressure processing on packaging materials in order to select the correct packaging.

Packaging materials for high pressure processing must be flexible to withstand a 15% increase in volume followed by a return to original size, without losing physical integrity, sealing or barrier properties. Metal cans, glass bottles and paperboard-based packages cannot be used in high pressure processing because of their irreversible response to high pressure treatment, and their tendency to deform or fracture (Lambert, 2000; Caner et al., 2004).

Temperature and adiabatic heating during high pressure processing may also affects packaging materials and need to be considered when selecting the correct packaging.

The headspace must be minimized as much as possible (Lambert, 2000) in order to control the deformation of packaging materials and ensure efficient use of the package and space in the pressure vessel.

The most common packaging materials used for high pressure processed food are polypropylene (PP), polyester tubes, polyethylene (PE) pouches, and nylon cast polypropylene pouches. Plastic packaging materials are the best suited for high pressure processing because of their reversible response to compression, their flexibility and resiliency.
However, plastic-based packaging materials may have a multilayer structure including metal foil, such as aluminum, a thin paper layer or an inorganic coating, such as aluminum trioxide (Al_2O_3) and silicon dioxide (SiO_2).
A metal layer will negatively affect the barrier properties of the packaging material, whereas inorganic coatings will positively affect its mechanical and barrier properties (Caner et al., 2004).

For an efficient high pressure treatment, it is crucial for the quality and safety of the food, that the process does not affect the integrity of the food package, its mechanical properties, delamination and sealing integrity, and its barrier properties (permeation (oxygen, water, carbon dioxide), sorption (aroma loss) and migration (additives, odors)).

Barrier Properties

Permeation

Masuda et al. (1992) studied the water vapor permeability (at 40°C and 90% RH) and oxygen vapor permeability (at 23°C and 90% RH) of different plastic films (PP/EVOH/PP, OPP/PVOH/PE, KOP/CPP, PET/Al/CPP) after high pressure treatment at 400 MPa and 600 MPa for 10 minutes and did not report significant changes in water and oxygen permeability properties.

Ochiai et al. (1992) investigated the effect of high pressure (400 MPa, 10 minutes) on the barrier properties (oxygen and water permeability) and total migration of laminated plastic packages filled with water and sealed. The food simulant used was (4% acetic acid, 20% ethyl alcohol, n-heptane). The authors reported no significant changes in barrier properties and total migration of all high pressure treated packaging materials.

Nachamansion (1995) showed that high pressure treatment at 400 MPa at 25°C for 30 minutes did not affect the barrier properties and structural characteristics of polymer based packaging material.

Pastorelli (1997) examined the effect of high pressure processing (400 MPa, 60°C, 30 minutes) on the barrier properties (oxygen and water permeability) of LLDPE/EVA/EVOH/EVA/LLDPE and PET/AL/PP multilayer packaging materials. The oxygen permeability of LLDPE/EVA/EVOH/EVA/LLDPE increased by 0.11 cc/m^2/day, while water permeability increased by 0.1 g/ m^2/day compared to untreated control samples. The oxygen permeability of PET/AL/PP increased by 0.27 cc/m^2/day, while water permeability increased by 0.01 g/ m^2/day compared to untreated control samples. The authors concluded that the increases in barrier properties of both films were within acceptable limits and high pressure processing did not cause significant changes in barrier properties of studied packaging materials.

Lambert et al. (2000) studied the effect of HPP (200, 350 and 500 MPa, 30 minutes, ambient temperature) on the permeability and package integrity of multilayer plastic structures (PA/PE, PET/PVDC/PE, PA/PP/PE, PA/MDPE, PA/LLDPE) in contact with different simulants (water, 3% acetic acid, 15% ethyl alcohol and olive oil). Simulants are substances that exhibit behavior similar to that of the packaged food with respect to solutes (aqueous, acidic, alcoholic or fatty). The authors reported no significant changes in the water and oxygen vapor permeability (Oxygen permeability increase: PA/LLDPE (12%), PET/PVDC/PE (12%), PA/PP/PE (12%), PA/MDPE (25%), PA/PE (16%); water permeability were higher but within acceptable limits). The authors showed that a 12% change in oxygen and water vapor barrier properties of packaging materials after high pressure treatment is an allowable deviation.

Lambert et al. (2000) also studied the effect of HPP (800 MPa, 2 minutes, 25°C) on PE coated with SiOx and PP and reported no significant oxygen barrier property change. HPP treatment of 400 MPa for 30 minutes at 60°C on LLDPE/EVA/EVOH/EVA/LLDPE and PET/Al/PP did not show a significant water-vapor barrier property change.

Caner et al. (2000, 2004) investigated the effect of high pressure (600 and 800 MPa for 5, 10, and 20 minutes at 45°C) on pouches made from PET/SiOx/LDPE, PET/Al₂O₃/LDPE, ET/PVDC/Nylon/HDPE/PE, PE/Nylon/EVOH/PE, PE/Nylon/PE, Metallized-PET/EVA/LLDPE, PP/nylon/PP and PET/PVDC/EVA. The pouched were filled with distilled water, heat-sealed and then treated with high pressure. The authors reported no significant changes in the permeability of oxygen, carbon dioxide, and water vapor of PET/SiOx/LDPE, PET/Al₂O₃/LDPE, ET/PVDC/Nylon/HDPE/PE, PE/Nylon/EVOH/PE, PE/Nylon/PE, PP/nylon/PP and PET/PVDC/EVA; whereas a significant increase (up to 150%) was observed in the permeability of oxygen, carbon dioxide, and water vapor of Metallized- PET/EVA/LLDPE; the water vapor transmission being the most severely affected by HPP.

Schauwecker et al. (2002) investigated the effect of high pressure processing (400, 600 and 827 MPa, 30, 50 and 75°C, 10 minutes) on the migration of a pressure transmitting- fluid, the 1,2-propanediol (PG) through pouches made from Polyester/nylon/aluminum/Polypropylene and Nylon/EVOH//PE and filled with 95% ethanol as food stimulant and sealed. Water was used as the food simulant at temperatures of 30, 75, 85, 90 and 95°C and at pressures of 200, 400, 690 and 827 MPa, in order to investigate any structural changes of the films during high pressure processing. The authors reported no detectable PG migration into the Polyester/nylon/aluminum/Polypropylene pouches after pressure. However, PG migration into the EVOH pouches significantly decreased when treated with high pressure at 30, 50 and 75°C compared to control samples treated with atmospheric pressure; it was noted that PG migration was significantly higher than the amounts detected at 30°C.

Rubio et al. (2005) investigated the effect of high pressure treatment (400 and 800 MPa, at 40°C and 75°C, for 5 and 10 minutes) on the oxygen barrier and morphological properties of EVOH

based packaging materials. The authors indicated that high pressure treatment barely affects packaging materials, especially when compared to the detrimental changes induced by retorting.

Halim et al. (2009) examined the effect of high pressure processing (800 MPa, 70°C, 10 minutes) on the barrier properties (oxygen transmission rate and water vapor transmission rate) of Nylon 6 (N6), Nylon 6/Ethylene Vinyl Alcohol (N6/EVOH) and Nylon 6/Nanocomposites (N6/nano) films. The films were coextruded with low-density polyethylene (PE) as the heat-sealing layer. The oxygen transmission rate of N6 and N6/Nano increased after HPP by 16.9% and 39.7% respectively, while it decreased by 53.9% in the N6/EVOH. The HPP treatment increased the water vapor transmission rate of N6, N6/EVOH and N6/nano by 21%, 48.9% and 21.2% respectively. The authors also examined the thermal characteristics and morphologies of the samples using differential scanning calorimetry (DSC) and X-ray diffraction (XRD) and reported that the enthalpy and percent crystallinity increased by 2.3% to 6.5% in the N6/nano when compared with the N6 material after high pressure treatment.

Galotto et al. (2010) studied the effect of high pressure processing (400 MPa, 30 minutes, 20 or 60°C) on the barrier properties (oxygen transmission rate and water vapor transmission rate) and total migration of four different packaging materials, polyethylene/ethylene-vinyl-alcohol/polyethylene (PE/EVOH/PE), metallized polyester/polyethylene, polyester/polyethylene (PET/PE), and polypropylene-SiOx (PPSiOx), filled with food simulants (distilled water and olive oil) and sealed. When water was used as food stimulant, the authors observed a low total migration after high pressure treatment, lower than in the untreated control samples. In the packages where oil was used as food stimulant, the total migration after high pressure treatment was higher compared to the control due to the damage that occurred to the structures after pressurization. The gas permeability of all films increased after high pressure processing, compared to the control, due to the structures damages caused by pressure treatment. The PET/PE film presented minimum changes in properties after high pressure processing.

Migration

The quality and safety of the packaged food can be compromised by migration to the food of functional additives, such as catalysts, antioxidants, heat stabilizers, plasticizers or colorants which may be added to the flexible plastic packaging. This transfer of materials may deteriorate the quality and safety of the food, increase the risk of chemical hazards, cause formation of off-flavor and consequently have an adverse effect on acceptability of the food.

Caner and Harte (2005) investigated the effect of high pressure processing (800 MPa, 60°C, 10 minutes) and storage for 20 days at 40 and 60°C on the migration of a typical antioxidant, Irganox 1076, from polypropylene flexible pouches containing 10 or 95% ethanol aqueous food simulating liquids. It was reported that no significant Irganox 1076 migrated from the polypropylene flexible structures into the food simulating liquids immediately after high pressure treatment. However, significant increase in Irganox 1076 migration into the food simulating liquids was noted during storage time; migration into foods is likely, especially if there is a long contact period. The authors showed that migration level into 95% ethanol was significantly higher than the one into 10% ethanol; the contact phase properties such as fat, alcohol content and acid content can influence migration behavior. Also, the migration of Irganox 1076 into food simulating liquids increases with high pressure processing temperature increase.

Sorption

Masuda et al. (1992) reported that a high pressure treatment at 400 MPa for 10 minutes decreased the sorption of D-limonene by low-density polyethylene (LDPE) and ethylene vinyl acetate (EVA) films.

Kuebel et al. (1996) investigated the effect of high pressure processing (0.1 – 450 MPa) on the sorption of aroma compounds, p-cymene and acetophenone, by flexible polymeric films (LDPE/HDPE/LDPE, PET/LDPE, and HDPE). The authors filled the internal pouches (70 x 12 mm) with a solution of H_2O:Ethanol:Cymene or H_2O:Ethanol:Acetophenone; pouches were then

sealed and placed into external pouches that were filled with a solution of ethanol-water and then sealed, high pressure processed and compared to control atmospheric pressured samples. The sorption rates were measured after high pressure treatment. The authors reported that the sorption of aroma compounds was lower in films exposed to higher pressure (450 MPa) compared to unpressured samples. It was suggested that the decrease in the sorption of the aroma compounds after high pressure treatment was due to the transition of the films to the glassy state at higher pressures. It was also showed that the distribution of aroma compounds to films was a function of their polarity.

Goetz and Weisser (2002) studied the high pressure processing effect (50 MPa, 23°C) on the sorption of a volatile aroma compound, *p*-cymene (0.25 vol. %), by LDPE/HDPE/LDPE (12/12/12 μm) polymer and reported a decrease in the permeation rate of *p*-cymene to LDPE/HDPE/LDPE after high pressure treatment and indicated that the extent of sorption was dependent on the processing pressure and time; the permeation rate decreased with increasing pressure.

Caner et al. (2004) evaluated the sorption behavior of D-limonene by different packaging films, a monolayer polypropylene (PP), a multilayer polyethylene/nylon/ethylene vinyl alcohol/polyethylene film (PE/nylon/EVOH/PE), and a metalized polyethylene terephthalate/ethylene–vinyl acetate/linear low-density polyethylene (metalized PET/EVA/LLDPE) after high pressure treatment at 800 MPa and 60°C for 10 minutes. The films were filled with simulants liquids (10% ethanol or 3% acetic acid solutions) and D-limonene, heat-sealed and then high pressure processed. Untreated controls were prepared at 1 atm, 60°C and 40°C. The quantity of absorbed D-limonene was analyzed in both the films and the simulants liquids. The authors reported no significant absorption of D-limonene in both the PP and PE/nylon/EVOH/PE films after high pressure treatment; whereas the metalized PET/EVA/LLDPE showed lower D-limonene sorption compared to control samples. The results also showed that sorption behavior of all three films was significantly affected by changes in temperature.

Mechanical Properties

Mechanical strength

Masuda et al. (1992) measured the tensile strength of PP/EVOH/PP, OPP/PVOH/PE, KOP/CPP, PET/Al/CPP films after high pressure treatment at 400 MPa and 600 MPa for 10 minutes at 20°C and 40°C and reported that the tensile strength of the studied packaging materials was not affected by high pressure processing.

Ochiai et al. (1992) investigated the effect of high pressure (400 MPa, 10 minutes) on the mechanical properties of laminated PP/PVDC/PP packages filled with water and sealed and reported no change in tensile strength of the films.

Mertens, B. (1993) evaluated the effect of high pressure treatment at 400 MPa and 60°C for 30 minutes on the mechanical properties of flexible packaging structures LLDPE/EVA, EVOH/EVA/LLDPE and PET/Al Foil/PP and reported no changes in the tensile strength, elongation and heat-seal strength and integrity of the films after high pressure processing.

Pastorelli (1997) examined the effect of high pressure processing (400 MPa, 60°C, 30 minutes) on the mechanical properties of LLDPE/EVA/EVOH/EVA/LLDPE and PET/AL/PP multilayer packaging materials and reported no significant tensile strength changes.

Lambert et al. (2000) studied the effect of high pressure processing (200, 350 and 500 MPa, 30 minutes, ambient temperature) on flexible structures, PA/PE, PET/PVDC/PE, PA/PE surlyn and PA/PP/PE and reported an increase of less then 25% (which is considered an allowable deviation) in the tensile strength of the films that became more rigid and less flexible.

Goetz and Weisser (2002) showed that a high pressure treatment at 500 MPa for 5 minutes of PA/PE packaging films flushed with CO_2 did cause the loss of transparency of the pouches and the appearance of cracks and folds in the films; whereas films flushed with O_2 were not affected by the high pressure processing.

Caner et al. (2004) investigated the effect of high pressure (600 and 800 MPa for 5, 10, and 20 minutes at 45°C) on the mechanical properties of pouches made from PET/SiOx/LDPE, PET/Al$_2$O$_3$/LDPE, ET/PVDC/Nylon/HDPE/PE, PE/Nylon/EVOH/PE, PE/Nylon/PE, Metallized-PET/EVA/LLDPE, PP/nylon/PP and PET/PVDC/EVA. The pouched were filled with distilled water, heat-sealed and then treated with high pressure. The authors reported no significant mechanical properties changes of the studied films. However, structural damages were observed in metalized-PET/EVA/LLDPE when the authors checked the C mode scanning acoustic microscopy (C SAM) and scanning electron microscopy (SEM) micrographs.

Caner et al. (2004) also studied the effect of high pressure processing (400 MPa, 30 minutes, 60°C) on LLDPE/EVA, EVOH/EVA/LLDPE and PET/Al/PP films and reported that the changes observed in tensile strength and elongation were not significant.

Galotto et al. (2009) investigated the effect of high pressure processing at 500 MPa for 15 minutes at 50°C on the physical properties (tensile strength, percent elongation and modulus of elasticity) of a biopolymer, polylactic acid coated with silicon oxide (PLASiOx/PLA), and a synthetic polymer, polyethyleneterephthalate coated with aluminum oxide (PET-AlOx). The pouches were filled with food stimulant (olive oil and distilled water), sealed and then high pressure treated (high pressure transmitting fluid used was glycol-water 25:75). The authors reported significant changes in mechanical properties of both PLASiOx/PLA and PET-AlOx. An induced crystallization was also noticed in both films. Many pinholes and cracks were formed during high pressure processing in PET-AlOx film and this may be the reason why a large reduction in the percent elongation was noted in this film; most of the properties changed more in the presence of oil as food stimulant. However, the PLASiOx/PLA film showed larger changes when in contact with water, that acted as a plasticizer.

Delamination

Lambert et al. (2000) reported that, after high pressure treatment, packaging films materials prepared by cast co-extrusion are susceptible to delamination, whereas the one prepared by tubular extrusion are more robust, have higher barrier properties and overall

integrity. A high pressure treatment at 200, 350, and 500 MPa for 30 minutes did not cause delamination in PA/PE, PET/PVDC/PE, PA/PE surlyn and PA/PP/PE films, whereas it did show delamination in PA/PE films.

Goetz and Weisser (2002) showed that a high pressure treatment at 500 MPa for 5 minutes did cause delamination of PA/PE packaging films.

The type of glue used, the presence of air pockets in packaging materials and the compressibility of laminated materials, can cause delamination when treated by high pressure (Lambert et al. (2000); Goetz and Weisser (2002)).

Schauwecker et al. (2002) investigated the effect of high pressure processing (400, 600 and 827 MPa, 30, 50 and 75°C, 10 minutes) on the delamination of pouches made from Polyester/nylon/aluminum/Polypropylene and Nylon/EVOH//PE and filled with 95% ethanol as food stimulant and sealed. Water was used as the food simulant at temperatures of 30, 75, 85, 90 and 95°C and at pressures of 200, 400, 690 and 827 MPa, in order to investigate any structural changes of the films during high pressure processing. The authors reported that, after a pressure treatment at pressures higher than 200 MPa, at 90°C for 10 minutes, visible signs of delamination between the polypropylene (PP) and aluminum (Al) layers were observed. However, there were no high pressure processing-induced molecular changes to the treated pouches as the differential scanning calorimetric analyses and Fourier transform infrared (FTIR) spectra of high-pressure treated pouches were similar as their control samples.

Sealing

Masuda et al. (1992) studied the heat-seal performance of PP/EVOH/PP, OPP/PVOH/PE, KOP/CPP, PET/Al/CPP films after high pressure treatment at 400 MPa and 600 MPa for 10 minutes at 20°C and 40°C and reported that the heat-seal performance of the studied packaging materials was not affected by high pressure processing.

Lambert et al. (2000) studied the effect of high pressure treatment at 200, 350, and 500 MPa, 20°C for 30 minutes of PA/PE, PET/PVDC/PE, PA/PE surlyn and PA/PP/PE films; the results showed that the heat-seal strength of the multilayered packaging films was not modified by high pressure processing.

Dobias et al. (2004) evaluated the effect of high pressure processing at 600 MPa for 60 minutes on mechanical properties (tensile and seal strengths) of single and multilayered packaging material films filled with food simulants (95% ethanol, isooctane, water or olive oil) and reported significant heat sealability losses.

Pathogens under Pressure

Effectiveness of high pressure processing on inactivation of vegetative cells

Recent years have seen significant research on the inactivation of microorganisms in foods by high hydrostatic pressure treatment. There can be vast high pressure sensitivity among bacterial species and even strains (Alpas et al., 1999; Benito et al., 1999; Pagan & Mackey, 2000; Patterson, 2005).

Generally, bacterial inactivation by high pressure processing is caused by damages on the cell membrane including alteration in membrane permeability, inactivation of intracellular enzymes and rupture of the cell wall (Hoover et al., 1989; Hoover, D.G., 1993; Casadei et al., 2002; Smelt et al., 1994).

Normally, gram-positive bacteria are more resistant to environmental stresses such as heat and pressure than gram-negative bacteria, and cocci are more resistant than rod or spirochete-shaped bacteria (Alpas et al., Patterson, 2005; 1999; Smelt, 1998). This is due to the complexity and abundance of protein, phospholipids, and lipopolysaccharide in the gram negative outer wall (Patterson, 2005; Pilavtepe-Celik et al., 2008; Ritz, et al., 2000).

Changes that are induced in the cell morphology of the microorganisms are reversible at low pressures, but irreversible at higher pressures where microbial death occurs due to permeabilization of the cell membrane.

The growth phase of microorganisms also plays a role in determining their pressure sensitivity. Cells in the lag or stationary phase of growth are generally more pressure resistant than those in the log or exponential phase; this is probably due to the fact that the cellular membrane is more robust and stress induced genes, that synthesize proteins that protect against stress conditions, can be turned on more readily in the stationary phase cells (Alpas et al., 1999; Bowman et al., 2008; McClements et al., 2001; Manas & Mackey, 2004). Therefore, stationary phase cells should be used in high pressure microbial inactivation research experiments, so that the process is evaluated against the most resistant cells.

High-pressure treatments are generally effective in inactivating most vegetative pathogenic and spoilage microorganisms at pressures between 200 and 600 MPa and temperatures at or below ambient temperature. The rate of inactivation is influenced by the peak pressure (Patterson, 2005). Although, in order to accelerate the inactivation process, higher pressures are commercially preferred, unless protein denaturation needs to be avoided.

Alpas et al. (1999) investigated the pressure resistance of six *Salmonella* strains at 345 MPa for 5 to 15 minutes at 25 and 50°C. The lower temperature value yielded a reduction of 5.5-8.3 log cycles while the higher temperature level resulted in more than 8 log-cycles within 5 minutes.

Patterson and others (1995) investigated the pressure resistance of several vegetative microorganisms. At 275 MPa for 15 min, *Yersinia enterocolitica* was reduced by 5-log cycles in phosphate-buffer-saline (PBS). For 5-log reduction, *Salmonella Typhimurium* required 350 MPa, *L. monocytogenes* required 375 MPa, *Salmonella Enteritidis* 450 MPa, *E. coli* O157:H7 required 700 MPa, and *S. aureus* 700 MPa.

Alpas et al. (1999) reported an 8 log-cycles reduction of a cocktail of six *E. coli O157:H7* strains at 345 MPa for 5 minutes at 50°C.

Ritz et al. (2001) investigated the damage done to *Listeria monocytogenes* cells treated by high pressure for 10 minutes at 400 MPa in pH 5.6 citrate buffer. No cell growth occurred after 48 hours on plate count agar.

Chen and Hoover (2003) obtained 8 log-cycles inactivation of *Listeria monocytogenes Scott A* in UHT milk with 500 MPa at 50°C for 5 minutes.

Effectiveness of high pressure processing on inactivation of spores

Bacterial spores are not by themselves a hazard; it is the germination of the organism that results in toxification or spoilage of the food.
Compared to vegetative cells, endospores tend to be extremely high pressure resistant, requiring treatment at pressure exceeding 1000 MPa and temperature of more than 80°C (Abee & Wouters, 1999; Rastogi et al., 2007; Smelt, 1998).

Effectiveness of the combination of high pressure processing and heat on spores

High pressure processing was proved to inactivate bacterial spores more effectively when used in combination with heat (Okazaki, 1996; Ahn et al., 2007, Reddi et al., 1999, Heinz and Knorr (2002)).

Okazaki et al. (1996) have studied the effect of high pressure and heat on the inactivation of four different strains of *Bacillus*. Each strain revealed different survival behavior; The survival curves of *B. subtilis* and *B. stearothermophilus* spores became convex at 400 MPa, 65°C and 120°C for 50 minutes, while survival curves of *B. coagulans* and *C. sporogenes* spores were linear in a temperature range of 50-110°C and a pressure range of 0.1- 400 MPa.

Ahn et al. (2007) studied the combined pressure/thermal inactivation kinetics of spores from three strains of anaerobic (*Clostridium sporogenes, C. tyrobutylicum,* and

Thermoanaerobacterium thermosaccharolyticum), and six strains of aerobic (*Bacillus amyloliquefaciens and B. sphaericus*) bacteria at 700 MPa and 121°C for 1 min. Inactivation up to 7–8 log was shown for some of the spores tested.

Reddi et al. (1999) reported a 5-log reduction of *Clostridium botulinum* type E spore's strains Alaska and Beluga at 50°C and 55°C respectively at 827 MPa for a processing time of 5 min.

Roberts and Hoover (1996) reported that spores of *Bacillus coagulans* were more sensitive to pressure both at lower pH and at higher treatment temperatures. At 400 MPa and 45°C, a 1.5 log reduction was observed when pH was lowered from 7.0 to 4.0. They also reported a 4 log reduction of spores of *Bacillus coagulans* when the temperature was increased from 25°C to 70°C during pressurization at 400 MPa.

Effectiveness of high pressure pulsed application on spores

Studies have shown that low pressures can induce germination of bacterial spores. (Smelt, 1998; Black et al., 2005; Wuytack et al., 2000). Spores germinated at lower pressures were in turn more sensitive to subsequent pressure treatments. This led research to prove that spores could be killed by applying pressure in two stages. The first treatment, at low pressure will germinate the spores, while the second treatment, at higher pressures, will kill the germinated spores.

Wuytack et al. (1998) reported that germination of *Bacillus subtilis* spores could be achieved using both low and high pressure treatments. However, spores germinated at lower pressures were then more sensitive to subsequent pressure treatments.

Paidhungat et al. (2002) studied the mechanisms of induction of germination of *Bacillus subtilis* spores by high pressure; they concluded that a pressure of 100 MPa activates the germinant receptors which induce spore germination and a pressure of 550 MPa induce the release of dipicolinic acid from the spore core, which leads to the later steps in spore germination.

Setlow et al. (2001) confirmed that the process of germination could happen in two stages. Stage I consist of the activation of the germinant receptors and release of dipicolinic acid and Stage II consist of the cortex hydrolysis and the associated swelling and water uptake by the spore core.

Hayakawa et al. (1994b) investigated the oscillatory compared with continuous high pressure sterilization on *Bacillus stearothermophilus* spores. Six cycles of pulsed pressure for 5 minutes at 400 MPa and 70°C decreased the spore count from 10^6 to 10^2/ml, and at 600 MPa, complete sterilization was achieved. Oscillatory pressurization was more effective for spore sterilization.

Butz and others (1990) reported that pre-exposure of bacterial spores at low pressures (60 - 100 MPa) enhanced the inactivation of spores at high pressure with temperatures of 25°C to 40°C.

Effectiveness of high pressure processing on inactivation of yeasts and molds

Yeasts and molds are relatively sensitive to high pressure processing. Most vegetative yeast and molds are inactivated within a few minutes by 300-400 MPa at 25°C. However, heat-resistant molds such as *Byssochlamys*, *Neosartorya* and *Talaromyces* are generally considered to be extremely resistant to high pressure. These fungi produce resistant structures know as ascospores. Ascospores are, like bacterial endospores, structured to withstand unfavorable environmental conditions and are resistant to heat, pressure, and desiccation.

Butz et al. (1996) reported that *Byssochlamys nivea* was the most resistant of the fungi studied; depending on strain, a treatment of 700 MPa at 70 ∘C for 15 min resulted in a 0.4- to 1.0-log reduction, and after 60 min at 70 ∘C and 700 MPa, a 3.2- to 4.0-log reduction was observed.

Maggi et al. (1994) studied the inactivation of *Byssochlamys, Neosartorya*, and *alaromyces* ascospores in apricot nectar and water and reported that a 900 MPa treatment for 20 minutes at 20°C inactivates *Talaromyces flavus* ascospores, and reduces ascospores of *N. fischeri* by 2 log

but did not affect *B. fulva* and *B. nivea* ascospores. A complete inactivation of ascospores was achieved for all species in 1 to 4 min at 800 MPa (50°C) or 1 to 2 min at 700 MPa (60°C).

Voldrich et al. (2004) studied the resistance of vegetative cells and ascospores of heat resistant mould *Talaromyces avellaneus* to the high pressure treatment in apple juice; they reported D_{600MPa} of 32 min at 17°C, but at 60°C D_{600MPa} was reduced to 10 min.

Palou and others (1998) investigated the efficacy of continuous versus oscillatory pressure treatments on inactivation of *B. nivea* ascospores suspended in apple and cranberry juice concentrates adjusted by dilution to water activities of 0.98 and 0.94 at 21 and 60°C. Continuous pressure treatment at 689 MPa and 60°C did not led to an inactivation of *B. nivea* ascospores after 25 minutes. With a water activity of 0.98, oscillatory pressure treatment inactivated *B. nivea* ascospores after three or five cycles of pressurization at 60°C. With a water activity of 0.94, *B. nivea* ascospores were reduced by 1 log after five pressure cycles. At 21°C, no effect on *B. nivea* ascospores viability was reported with continuous or oscillatory treatments. Palou and others (1998) also proposed that the efficacy of oscillatory high pressure treatment was due to spore lysis promoted by increased wall permeability at high temperatures and pressures as observed by scanning electron microscopy.

Aleman and others (1994 and 1996) reported that pulsed pressure treatments were more effective than static applications in the inactivation of *Saccharomyces cerevisiae* in pineapple juice. A pulsed pressure treatment at 0–270 MPa at 0.1, 1 and 2 cycles/s with 100 seconds total on-pressure time resulted in 3.3, 3.5, and 3.3 decimal reductions, respectively; the static pressure treatment resulted in 2.5 decimal reductions.

Effectiveness of high pressure processing on inactivation of viruses

Pressure inactivation of viruses has not been extensively researched in comparison to foodborne pathogenic bacteria.

In response to high hydrostatic pressure, viruses show a wide range of sensitivity. Studies have shown that virus inactivation by pressure is due to the denaturation of capsid proteins essential for host cell attachment to initiate infection (Khadre & Yousef, 2002; Kingsley et al., 2002; Buckow et al., 2008)

Noroviruses or "Norwalk-like viruses" from the Caliciviridae family are the most common cause of outbreaks and sporadic cases of acute gastroenteritis. Two surrogate viruses from the family of Caliciviridae, feline calicivirus (FCV) and murine norovirus (MNV) are normally used in pressure inactivation studies.

Buckow et al. (2008) reported an inactivation of feline calicivirus by more than 7 logs PFU per ml in cell culture medium or mineral water at 75°C for 2 min at ambient pressure and at 450 MPa and 15°C for 1 min.

Kingsley et al. (2002) and Murchie et al. (2007) reported a total inactivation of feline calicivirus (FCV) and only a reduction of 1.8 PFU/ml of MNV with pressure around 300 MPa for 5 minutes.

The inactivation rate of feline calicivirus (FCV) by high pressure processing at temperatures close to ambient is acceptable, however temperatures above 50°C and below 0°C are more effective (Chen et al., 2005; Buckow et al., 2008).

Hepatitis A virus (HAV) is the main foodborne virus of the Picornaviridae group. Generally, treatments with pressure higher than 400 MPa are the most effective but reduction rates significantly depend on the processing time and temperature. Temperatures higher than 30°C are the most effective; HAV is more resistant at temperatures below 0°C. Oscillatory high pressure processing does not increase the inactivation rate of HAV (Kingsley et al., 2006).

Kingsley et al. (2002) investigated the impact of high pressure processing on the inactivation of hepatitis A virus (HAV), poliovirus and a Norwalk virus surrogate and reported a complete inactivation of HAV after 5 minutes at 450 MPa, while poliovirus was unaffected by a treatment

at 600 MPa for 5 minutes and feline calcivirus was completely inactivated after 5 minutes at 275 MPa.

Rotaviruses from the family of *Reoviridae* are involved in acute food and waterborne gastroenteritis.
Khadre et al. (2002) studied the susceptibility of human rotavirus to high pressure treatment at 300 MPa and 25°C for 2 minutes; a reduction of 8 log-cycles was obtained but times longer than 2 minutes did not additionally decrease rotavirus, a small fraction of the virus population remained resistant to pressure treatments at 800 MPa for 10 minutes.

High Pressure Processing Cost

The many advantages of using high pressure processing in food production have been known for over a century. However, the technology and equipment required to efficiently and reliability generate the extreme pressures (up to 600 MPa / 87,000 psi) used in high pressure processing have only recently become commercially viable.

Avure Technologies, NC Hyperbaric, and Uhde are major suppliers of commercial scale pressure equipment. Both horizontal and vertical pressure vessel configurations are available. Commercial batch vessels have internal volumes ranging from 30 liters (7.92 gallons) to more than 600 liters (158.5 gallons). Commercial-scale, high pressure processing systems cost approximately $500,000 to $2.5 million, depending on equipment capacity (processing pressure vessels volumes, complete cycle time and higher horsepower intensifiers) and extent of automation (package and vessel loading and pc-based control system monitoring operator, time, lot, pressure, temperature, de-aeration of the vessel prior to pressure and faults during cycles) (Balasubramaniam et al., 2008).

HPP treatment costs are currently ranging from 4–10 cents/lb, including operating cost and depreciation (Sàiz et al., 2008). Pressure-processed products currently cost 3 to 10 cents per pound more to produce than thermally processed products (Ramaswamy et al., 2004). As

demand for high pressure processing equipment grows, innovation is expected to further reduce capital and operating costs.
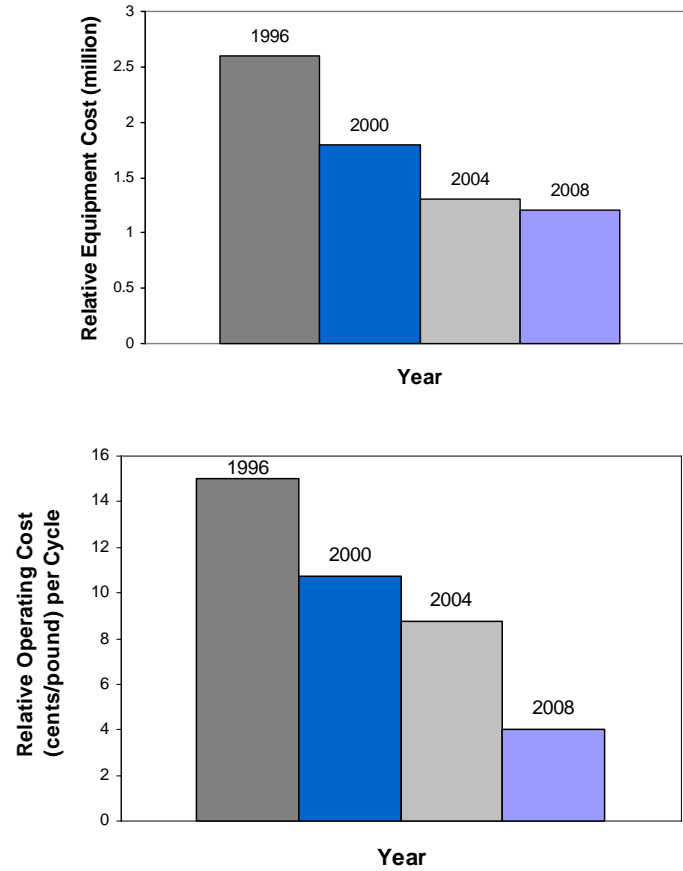
Figure 2.7: High Pressure Process: Equipment and Operating Costs (Balasubramaniam et al. (2008) through Hewson, 2008).

Chapter 3 - High Pressure Processing Application on Fruits and Vegetables

The use of high hydrostatic pressure on fruits and vegetables processing is of great interest because of its ability to inactivate microorganisms and quality-deteriorating enzymes and its limited effects on covalent bonds resulting in minimal modifications of food-quality attributes such as color, flavor and nutritional values. Application of high pressure preserves the freshness and extends the shelf life of fruits and vegetables. However, depending on the fruit and vegetable, high pressure could induce chemical or biochemical reactions that can affect their quality and nutritional attributes (Oey et al., 2008; Sila et al., 2008).

Effect of High Pressure Processing on Fruits

Apple and Apple Juice

Weemaes et al. (1998) reported a pressure inactivation of polyphenoloxidase (PPO), enzyme responsible for fruit browning and flavor loss, in apples treated at 600 MPa and at room temperature (25°C).

Novotna et al. (1999) compared the aroma of apple juice treated by high pressure or pasteurized at 80°C for 20 min and reported that high pressure treated samples were better than pasteurized samples.

Riahi and Ramaswamy (2003) evaluated the high pressure inactivation of pectin methylesterase (PME), the enzyme which normally tends to lower the viscosity of fruits products and adversely affect their texture, in apple juice and reported that almost a full decimal reduction in the activity of commercial PME was achieved by high pressure treatment at 400 MPa for 25 min.

Donsì et al. (2010) evaluated the effectiveness of a pulsed high pressure treatment on Annurca apple juice at pressure levels of 150 to 300 MPa, temperature levels of 25 to 50°C, and pulse number of 1 to 10. They reported a reduction of the initial microbial load from 20 to 7 CFU/mL approximately. Also, there were no difference with the brightness and a* values of the juice immediately after the treatment, however a significant increase of the brightness and a* values was detected during storage under refrigerated conditions (4°C) for 21 days.

Landl et al. (2010) compared, at an industrial scale, the effect of high pressure treatments (400 and 600 MPa/5 min/20°C) and a mild conventional pasteurization at (75°C/10 min) on total vitamin C, ascorbic acid and total phenolic content of an acidified apple purée product that was stored for 3 weeks at refrigerated temperatures (5 °C ± 1 °C). They reported that treatment at 400 MPa did not affect the total vitamin C, ascorbic acid and total phenolic contents, although a first-order reaction kinetic loss of total vitamin C was described at 9.3 to 10.3 days of storage for all three treatments. Treatment at 600 MPa affected the total phenolic content. The mild pasteurization treatment did not affect the total vitamin C but slightly reduced the total phenolic contents.

Avocado Puree and Guacamole

Lopez et al. (1998) studied the polyphenoloxidase activity, color changes and microbial inactivation during storage at 5, 15, or 25°C of avocado puree treated with high hydrostatic pressure at 345, 517, or 689 MPa, for 10, 20, or 30 min at initial pH of 3.9, 4.1, or 4.3. Polyphenoloxidase (PPO) activity was significantly less ($p \leq 0.05$) with increasing pressure and decreasing initial pH. The avocado puree with a residual PPO activity of < 45% maintained an acceptable color for at least 60 days when stored at 5°C. Standard plate and yeast and mold counts were <10 cfu g^{-1} during 100 days of storage at 5, 15, or 25°C.

Weemaes et al. (1998) reported that inactivation of avocado polyphenoloxidase (PPO) at ambient temperatures (25°C) and pH 6-7 was possible at pressure of 800 MPa.

Palou et al. (2000) have analyzed the effects of continuous and oscillatory high pressure treatments on guacamole. Significantly less (*P*<0.05) residual polyphenoloxidase (PPO) and lipoxygenase (LOX) activities were obtained by increasing the process time and number of pressurization–decompression cycles. A 15 minutes continuous treatment or oscillatory high pressure was sufficient to inactivate LOX. After four high pressure cycles at 689 MPa with a holding time of 5 minutes each, the lowest residual PPO activity value (15%) was obtained. Standard plate and yeast and mold counts of high pressure-processed guacamole were <10 cfu/g. Sensory acceptability and color of high pressure guacamole were not significantly different (P>0.05) from those of a guacamole control. Browning during storage was mainly due to changes in the hue attributed to a decrease in the green color. A shelf-life of 20 days was achieved at < 15°C compared to control samples which spoiled within 5 days at 5°C.

Jacobo-Velázquez and Hernández-Brenes (2010) also reported a decrease in polyphenoloxidase (PPO) and lipoxygenase (LOX) in avocado paste when treated with high pressure at 600 MPa for 3 minutes and stored for 45 days at 4°C. However, a reactivation of both enzymes was observed at 10 to 15 days of storage as well as a cell disruption and a gradual migration of intracellular components such as organic acids. Lactic acid bacteria counts were very low during storage. pH was consistently declining during the first 20 days of storage.

Berries (Strawberry, Raspberry and Blackberry)

Lambert et al. (1999) evaluated the effect of high pressure (200, 500 and 800 MPa, 20°C, 20 min) on the aromatic volatile profile (furaneol (2,5-dimethyl-4-hydroxy-furan-3-one) and nerolidol (3,7,11-trimethyl 1,6,10-dodecatrien-3-ol)) of strawberry purée and indicated that pressure treatments of 200 and 500 MPa at 20°C for 20 minutes did not affect the aroma profile of the strawberry purée; whereas a pressure of 800 MPa significantly changed the aroma profile by inducing the synthesis of new compounds such as 3,4-dimethoxy-2-methyl-furan and γ-lactone.

Zabetakis et al. (2000) studied the effect of high pressure (200-800 MPa) on flavor compounds (acids (butanoic acid, 2-methyl-butanoic acid and hexanoic acid), ketone (2,4,6-heptatrione) and furanones (5-hexyl-dihydro-3H-furan-2-one, 2,5-dimethyl-4-hydroxy-2H-furan-3-one and 2,5-dimethyl-4-hydroxy-2H-furan-3-one-glucoside) of strawberries (*Fragaria × ananassa*, cv. Elsanta) after storage for 24h at 4, 20 and 30°C and indicated that the highest flavor stability was obtained with lower pressures treatments and storage temperatures of 4 and 30°C.

Suthanthangjai et al. (2005) evaluated the anthocyanin content and color stability (cyanidin-3-glucoside and cyanidin-3-sophoroside) of high pressure processed (200-800 MPa, 18-22°C, 15 minutes) raspberry purée (*Rubus idaeus*) stored at 4°C, 20°C and 30°C for up to 9 days and reported that the highest stability of the anthocyanins was observed when raspberries were processed at 200 and 800 MPa and stored at 4°C.

Shiferaw Terefe et al. (2009) indicated that high pressure treatment at 600 MPa for 10 minutes combined with temperature of 60°C induced up to 58% inactivation of peroxidase in strawberries and did not have significant effect on the total polyphenol and total anthocyanin content of strawberries. Although, after storage at refrigerated temperature for 3 months an average of $22 \pm 13\%$ loss of total polyphenol content and $27 \pm 10\%$ loss of total anthocyanin contents were observed.

Patras et al. (2009) evaluated the immediate effect of high pressure processing (400-600 MPa/15 min/10–30°C) and thermal treatment (70°C/2 min) on total antioxidant activity and color of strawberry and blackberry purées and did not report significant changes in ascorbic acid and anthocyanins contents or color in pressure treated purées, whereas conventional thermal treatment caused degradation of ascorbic acid by 21%, reduction of anthocyanins levels and color change compared to unprocessed samples. Antioxidant activities were significantly higher after high pressure processing compared to thermally processed samples. Patras et al. concluded that high pressure is an efficient quality preservation method of strawberry and blackberry purées.

Grape Juice

Moio et al. (1994) evaluated the effect of high hydrostatic pressure on red and white grape musts and reported that pressure of 500 MPa for 3 minutes sterilized the white grape must; however red grape must were not fully sterilized by a pressure of 800 MPa for 5 minutes because of the higher pressure stability of the natural microflora present in red grape. Little changes in physicochemical properties were reported.

Castellari et al. (1997) demonstrated that pressures between 300 and 600 MPa had limited inactivation of grape polyphenoloxidase (PPO). The use of a combination of high pressure and mild thermal treatments (40°C – 50°C) was necessary to completely inactivate grape PPO.

Weemaes et al. (1998) reported that inactivation of grape polyphenoloxidase (PPO) at ambient temperatures (25°C) and pH 6-7 was possible at pressure of 700 MPa.

Rastogi et al. (1999) reported that polyphenoloxidase (PPO) and peroxidase (POD) in red grape juice can be inactivated by a combined treatment of pressure and temperature. At 60°C, the lowest polyphenoloxidase (41.86%) and peroxidase (55.75%) activities were achieved with pressure of 100 MPa and 600 MPa respectively.

Daoudi et al. (2002) demonstrated that based on L*, a* and b* values, no visual color differences are noted immediately after high pressure treatments of white grape juice at 400 MPa/2°C, 500 MPa/2°C or 400 MPa/40°C/10 minutes.

Grapefruit

Naringin is the bitter component in grapefruit juice; Naringinase is the enzyme that hydrolyzes naringin to naringenin, which is tasteless. Ferreira et al. (2008) reported that high hydrostatic pressure treatment (160 MPa, 37°C, 20 minutes), combined with the use of naringinase can increase the reduction of naringin to naringenin and achieve a debittering of 75%

of grapefruit juice. In model solution and at atmospheric pressure (0.1 MPa), the naringin reduction was only 35%.

Guava

Gow and Hsin (1996) indicated that high pressure treatment (600 MPa, 25°C, 15 minutes) of guava puree inactivated microorganisms to less than 10 CFU mL^{-1}. The reported shelf-life of the guava puree stored at 4°C was 40 days with no change in color, pectin, cloud and ascorbic acid content.

Gow and Hsin (1999) showed that the high pressure treatment (25 °C, 600 MPa, 15 min) effectively sterilized guava juice but partially inactivated enzymes in the juice and this is the reason behind the gradual changes of the volatile components (increase in methanol, ethanol, and 2-ethylfuran with decreases in other components) during storage periods beyond 30 days; the volatile distribution of 600 MPa treated guava juice was similar to that of freshly extracted juice when stored at 4 °C for 30 days.

Lychee

Phunchaisri and Apichartsrang (2005) showed that a combination of high pressure and heat treatment (600 MPa, at 60°C for 20 min) is needed for an extensive inactivation of peroxidase (50%) and polyphenol oxidase (90%) in fresh lychee; inactivation rates were less for lychee processed in syrup. A pressure treatment of 200 MPa and 20-60°C increased POD activity. Compared to thermal processing, pressure treatment caused less loss of visual quality in fresh and syrup processed lychee.

Mango

Boynton et al. (2002) pointed out that high pressure treatment of sliced mangos at 300 MPa and 600 MPa for 1 minute slightly reduced fresh mango flavor and increased off-flavor and sweetness. After storage for 9 weeks at 3°C, high pressure treatments at 300 MPa and 600 MPa reduced microbial levels by 2 and 3 logs CFU/mL respectively.

Ahmad et al. (2005) reported that color parameters of mango pulps remained constant after high pressure treatment (100–400 MPa for 15 or 30 min at 20 °C) indicating that no significant variation in color was observed. It has also been demonstrated that the consistency index of fresh pulp increased with pressure level from 100 to 200 MPa while a steady decrease was observed for canned mango pulp. The flow behavior index of fresh pulp decreased with high pressure treatment, while the canned pulp flow behavior index increased.

Guerrero-Beltran et al. (2006) evaluated the effect of high hydrostatic pressure on mango puree (pH 3.5) containing ascorbic acid at 500 ppm and stored at 3°C for one month. They reported that pressure treatment at 207, 345, 483 and 552 MPa decreased the residual polyphenoloxidase (PPO) activity to 35.8 ± 6, 21.5 ± 13.2, 46.8 ± 53.2 and $61.8 \pm 5.8\%$ respectively. Total plate counts and yeasts were inactivated (<10 cfu/g) at pressure treatments of 483 or 552 MPa. The authors also proved that setting the pH to 3.5 and the addition of ascorbic acid reduced the rate of browning during storage.

Melon and Watermelon Juice

Wolbang et al. (2008) demonstrated that high pressure processing did not affect the total titratable acidity and total soluble solids of fresh cut melon, but it significantly increased the β-carotene levels and decreased the ferric iron reducing capacity and the vitamin C content.

Zhang et al. (2011) compared thermal, ultraviolet-c, and high pressure treatments on quality parameters of watermelon juice. They reported that high pressure treatment holds all-*trans*-

lycopene, and *cis*-lycopene of watermelon juice; High pressure was the best way to keep quality (color, browning degree and dynamic viscosity) of watermelon juice compared to thermal and ultraviolet-c treatments.

Ultraviolet-c treatment was the fastest and the most effective in inactivating the watermelon juice pectin methylesterase (PME) compared to other treatments.

Orange Juice

Cloud loss is considered to be a major quality defect in orange juice; Goodner et al. (1999) evaluated the effect of high pressure processing (700 MPa, 1 minute) on cloud preservation and shelf life of freshly squeezed orange juice and reported a 90 days shelf life under refrigeration conditions considering cloud preservation and microbiologically stable product.

Polydera et al. (2003) compared the effect of high pressure (500 MPa, 35°C, 5 minutes) and conventional thermal processing (80°C, 30 seconds) on the shelf life of reconstituted orange juice stored at 0–15°C and concluded that high pressurized juice had longer shelf life compared to the thermally processed one as it had lower ascorbic acid degradation rate. The high pressure processed samples had also higher viscosity values and better sensory characteristics.

Bull et al. (2004) studied the quality and shelf life of high pressure processed (600 MPa, 20°C, 1 minute) Valencia and Navel orange juices stored at 4°C for 12 weeks. The microbial load, yeast and other fungi were reduced to a non detectable level immediately after high pressure treatment and after storage at 4°C for up to four weeks; the microbial load was less than 2 $\log_{10}$ cfu/ml after storage for up to 12 weeks. A 7-log reduction of *Salmonella* was achievable. High pressure processing did not have any effect on °Brix, viscosity, titratable acid content, alcohol insoluble acids, browning index, color, ascorbic acid and β-carotene concentrations. Pectinmethylesterase (PME) activity was not completely deactivated in Valencia juice (pH 4.3) but was significantly reduced in Navel juice (pH 3.7).

Noma et al. (2004) compared the effect of a slow decompression (SD, 2 min) versus a rapid decompression (RD, 30 sec) on the inactivation of *E. coli 0157:H7* during storage of orange juice at 4°C after a high pressure treatment and reported that RD showed a higher inactivation effect of *E. coli 0157:H7* than SD, whereas untreated samples did not show any inactivation after 5 days of storage.

Butz et al. (2004) reported that high pressure processing at 600 MPa and 25°C for 5 minutes showed a good retention of folates; excess ascorbate strongly protected folates against pressure.

Polydera et al. (2005) demonstrated that high pressure at 600 MPa at 40°C for 4 minutes of fresh novel orange juice preserves 49% of ascorbic acid content after storage at 15°C, and 112% after storage at 0°C compared to thermally pasteurized samples. High pressure treated samples had better sensory characteristics, flavor and apparent viscosity values compared to thermally processed samples. The color change was linearly correlated to the ascorbic acid loss. Polydera et al. also reported a decrease in total antioxidant activity mainly due to ascorbic acid loss. Compared to conventional pasteurization, high pressure processing led to a better retention of the antioxidant activity of orange juice.

Baxter et al. (2005) reported that odor and flavor (volatile components) of high pressure processed navel orange juice was acceptable to trained sensory panel and consumer acceptance panel after storage at 10°C for up to 12 weeks.

Bayındırlı et al. (2006) demonstrated that an inoculum of *Staphylococcus aureus* 485, *Escherichia coli O157:H7* 933 and *Salmonella enteritidis* FDA in orange juice was completely inactivated at 350 MPa and 40°C in 5 min. A residual pectinesterase activity of $7 \pm 1.6\%$ was observed after a high pressure treatment at 450 MPa and 50°C for 30 min; whereas a residual pectinesterase activity of $12 \pm 0.2\%$ was reported after high pressure processing at 450 MPa and 40°C for 60 min. The enzyme inactivation is irreversible and is not reactivated upon storage.

Plaza et al. (2011) explored the effect of high pressure combined with heat processing (400 MPa/40 °C/1 min) on the carotenoid and flavanone content of freshly squeezed orange juice

during refrigerated storage for 40 days at 4°C and compare it to pulsed electric fields (PEF) (35 kV cm−1/750 μs) and low pasteurization (LPT) (70 °C/30 s). They reported an immediate increase of total carotenoid (45.19%), flavanone (15.46%) and on vitamin A value (30.89%) contents in high pressure processed orange juice compared to untreated samples. Whereas, storage at 4°C decreased the flavanone content by 50% during the first 20 days and the carotenoid content by 11% during the last 20 days. Compared to pulse electric fields and low pasteurization, high pressure treated orange juice had the higher content of carotenoids and flavanones after storage at 4°C for 40 days.

Torres et al. (2011) studied the stability of anthocyanins and ascorbic acid of high pressure processed (400-600 MPa, 15 min) blood orange juice during storage at 4°C for 10 days and reported a 99% anthocyanins content retention and a 94.5% ascorbic acid content retention immediately after high pressure treatment. During storage at 4°C for 10 days, retention rates for anthocyanins and ascorbic acid were 93.4 and 85.0% respectively, at a pressure treatment of 600 MPa for 15 min. The degradation kinetics of processed samples followed first order kinetics during storage.

Passion Fruit

Laboissière et al. (2007) demonstrated that high pressure treatment (300 MPa, 5 minutes, 25°C) of yellow passion fruit pulp improved sensory quality of yellow passion fruit juice compared to commercial juices.

Peach

Sumitani et al. (1994) showed that high pressure treatment (400 MPa, 20°C, 10 minutes) partially inactivated β-glucosidase in white peach; therefore benzaldehyde content in high pressure treated white peach increased during storage.

Dogan and Erkmen (2004) studied the high pressure kinetics of *Listeria monocytogenes* inactivation in broth, milk, peach and orange juices. The authors reported D values for aerobic bacteria and *Listeria monocytogenes* of 2.13 and 1.52 min, respectively in peach juice; the z value of *Listeria monocytogenes* in peach juice was 506 and the k values for *Listeria monocytogenes* in peach juice ranged from 0.3733 to 1.5151 min^{-1}.

Kingsly et al. (2009) indicated that high pressure treatment at 300 MPa and 25°C combined with citric acid (1-1.2%) inactivates peach polyphenoloxidase (PPO). High pressure processing increased permeability of cells and therefore enhanced the drying rate and reduced drying time. The authors demonstrated that high pressure processing of peach slices in acidic medium is an alternative for hot water blanching as pretreatment of peach fruits.

Persimmon Puree

Ancos et al. (2000) showed that persimmon puree treated with high pressure (50, 300 and 400 MPa, 15 min, 25°C) had higher levels of extractable carotenoids, which was related with the increase of vitamin A.

Pineapple

Rastogi et al. (2000) reported that diffusion coefficients (for water absorption and solute diffusion) of high pressure pretreated and osmotically dehydrated pineapples cubes (100, 300, and 500 MPa for 10 min at 5, 25, and 35°C) were lower compared with ordinary osmotically dehydrated samples; the diffusion coefficients decreased with increase in treatment pressure. The decrease in diffusion coefficients is the result of the permeabilization of cell membranes, the release of cellular components, and the structural changes of cell materials.

Buzrul et al. (2008) compared continuous (single pulse) and pulse pressure treatment (350 MPa, 20°C for 60s×5 pulses) on inactivation of *Escherichia coli* and *Listeria innocua* in pineapple

juice and reported that pulse treatment significantly increased the inactivation ($p < 0.05$) of both bacteria. Microbial inactivation level further increased after storage of pulsed pressured pineapple juice at 4, 20 and 37°C for 3 weeks and no injury recovery bacteria were detected.

Kingsly et al. (2009) studied the effect of high pressure (50, 100, 300, 500 and 700 MPa at 25°C for 10 minutes) on texture and drying (70°C) behavior of pineapple slices and reported that hardness, springiness and chewiness of pineapple slices were reduced by high pressure, whereas cohesiveness was not significantly affected. Higher pressure reduced drying time during dehydration at 70°C. The study showed that high-pressure blanching of pineapple can be an alternative for hot water blanching, before dehydration.

Tomatoes

Tangwongchai et al. (2000) evaluated the effect of high pressure (200−600 MPa, 20 minutes, 20°C) on the texture of cherry tomatoes and on softening enzymes pectinmethylesterase and polygalacturonase and reported that pressure up to 400 MPa resulted in an increased texture damage, while pressure between 400 MPa and 600 MPa caused less apparent damage compared to untreated samples. No significant inactivation of pectinmethylesterase in cherry tomatoes was reported even after treatment at 600 MPa, while polygalacturonase was completely inactivated after treatment at 500 MPa.

Polygalacturonase (PG) is responsible for decreasing the viscosity of tomato-based products. Fachin et al. (2003) explored the inactivation kinetics of PG in high pressure-heat treated (200-500 MPa/5-50°C) tomato juice and reported that a combination of high pressure and heat treatment inactivates PG without applying high temperatures and that polygalacturonase inactivation follows a first order kinetic model.

Plaza et al. (2003) reported a 4-log reduction of total microbial counts and an inactivation of polyphenoloxidase, peroxidase and pectinmethylesterase in tomato purée treated at 400 MPa. In

the presence of NaCl (0.8%) the viscosity of the tomato purée increased with increasing pressure up to 400 MPa.

Rodrigo et al. (2006) explored thermal and high pressure inactivation of polygalacturonase (PG) and pectinmethylesterase (PME) from four different tomato varieties and reported that PG is inactivated by pressure of 300-500 MPa at room temperature or by a 5 minutes heat treatment of 65°C or 90°C (depending on PG isoform); whereas PME activity is reduced by 50% after a high pressure treatment of 850 MPa for 15 minutes at 25°C and thermally inactivated after treatment at 70°C for 5 minutes.

Sánchez-Moreno et al. (2006) studied tomato purée subjected to high-pressure (HP) (400 MPa/25 °C/15 min), low pasteurization (LPT) (70°C/30 s), high pasteurization (HPT) (90 °C/1 min), freezing (F) (−38°C/15 min), and HPT plus F (HPT + F) and reported that CIELab uniform color parameters (lightness L*, green-red tonality a*, and blue-yellow tonality b*) and individual, total carotenoids, and provitamin A carotenoids were significantly higher in high pressure processed tomato purée compared to other treatments; whereas ascorbic acid and total vitamin C were lower in high pressure and thermal treatments compared to untreated and frozen tomato purées.

Verlent et al. (2006) studied the rheological properties of tomato purée and indicated that a pressure treatment of less than 300 MPa caused significant losses in rheological properties; a combined high pressure/heat treatment (60°C, 500 MPa) improved the rheological properties of tomato purée but caused formation of a tomato gel structure; a pressure treatment of 500 MPa and temperatures higher than 60°C did not have any effect on the rheological properties and gel formation of the tomato purée.

Qiu et al. (2006) evaluated the effect of high pressure (100-600 MPa, 12 minutes, 20 ± 1°C) on lycopene stability of tomato purée stored at 4 ± 1°C or 24 ± 1°C and the highest lycopene stability reported was in samples pressurized at 500 MPa and stored at 4 ± 1°C.

Oxidative enzymes, such as lipoxygenase (LOX) and hydroperoxide lyases (HPL) catalyze oxidation of unsaturated fats, producing peroxides and volatile aldehydes. Although, the volatile aldehydes play a major role in forming the aroma of many fruits and defending the plant against pest and pathogen attack, the oxidative rancidity causes off odors and can lead to a bleaching of the deep-red tomato color.

Rodrigo et al. (2007) studied the thermal (25 - 90°C) and high pressure (100 – 650 MPa) stability of tomato lipoxygenase (LOX) and hydroperoxide lyase (HPL) in tomato juice and reported that a thermal treatment of 60°C for 12 minutes was needed to completely inactivate LOX and a heat treatment of 40°C for 12 minutes is needed to reduce 50% of HPL activity; whereas a pressure treatment of 650 MPa for 12 minutes was able to inactivate 80% of hydroperoxide lyase.

Rodrigo et al. (2007) also studied the combined effect of high pressure and thermal processing (300–700 MPa, 65°C, 60 minutes) on tomato puree color and did not report any color degradation.

Hsu et al. (2008) evaluated the microbial inactivation and physicochemical properties of pressurized tomato juice (300 – 500 MPa/25 °C/10 min) during refrigerated storage at 4°C for 28 days. The authors demonstrated that a pressure treatment of 500 MPa for 10 minutes at 25°C would be an alternative for thermal processing of tomato juice as it significantly inactivates microorganisms and pectolytic enzymes, improves color and extractable carotenoids and lycopene contents and retains vitamin C even after storage at 4°C for 28 days.

Patras et al. (2009) reported that, compared to untreated and thermally processed samples (70 °C/2 minutes), high pressure (400–600 MPa/15 minutes/20 °C) significantly retained ($p < 0.05$) antioxidant activity in tomato purée; more than 90% of ascorbic acid was retained after a high pressure treatment of 600 MPa. High pressure and thermal treatments did not affect the phenolic content in tomato puree but significantly affected color parameters.

Effect of High Pressure Processing on Vegetables

Alfalfa Seeds

Alfalfa sprouts have been implicated in multiple *Salmonella* and *Escherichia coli O157:H7* outbreaks in 2009 and 2010 and therefore high pressure processing has been investigated as a treatment method of alfalfa seeds used for sprouting as they appear to be the primary source of pathogens.

Ariefdjohan et al. (2004) inoculated alfalfa seeds with *Escherichia coli O157:H7* and *Listeria monocytogenes* and then treated the seeds with high pressure at 275 to 575 MPa for 2 minutes or at 475 MPa for 2 to 8 minutes (40°C). Ariefdjohan et al. reported reductions of 1.4 logs and 2.0 logs of *Escherichia coli O157:H7* at 575 MPa (2 minutes) and 475 MPa (8 minutes) respectively; whereas *Listeria monocytogenes* counts were only reduced by 0.8-log and 1.1-log at the same pressures and time. The authors concluded that high pressure did not completely eliminate *E. coli O157:H7* and *L. monocytogenes* in alfalfa seeds.

The high pressure treatment did reduce the germination rates of the alfalfa seeds to 34% compared to 95% for the control untreated samples.

Neetoo et al. (2008) inoculated alfalfa seeds with a cocktail of five different strains of *E. coli O157:H7* and treated them, in a dry or wet state, with high pressure of 500 and 600 MPa for 2 minutes at 20°C. The results showed that *E. coli O157:H7* counts of the immersed seeds were reduced by 3.5 logs and 5.7 logs at 500 and 600 MPa respectively compared to < 0.7 log for dry seeds at both pressure levels. Neetoo et al. recommended a treatment of 650 MPa for 15 minutes for a complete elimination of a population of *E. coli O157:H7* of > 5 logs. The high pressure processing did not affect the germination rates as these were the same for high pressure processed alfalfa seeds and untreated seeds.

Later on, in 2009, Neetoo et al. evaluated the effectiveness of a combined treatment of high pressure (600 MPa for 2 minutes) and mild heat (4, 20, 25, 30, 35, 40, 45, and 50°C.) in decontaminating alfalfa seeds from *E. coli O157:H7* and reported that the optimal treatment for a

5 log reduction of *E. coli O157:H7* with no adverse effect on seed viability was at 600 MPa for 2 minutes at 40°C.

In another study, Neetoo et al. (2009) reported that a 5 cycles oscillatory pressure treatment at 600 MPa and 20°C with a holding time of 2 minutes per cycle did not eliminate *E. coli O157:H7* from inoculated alfalfa seeds. The study also showed that the pressure inactivation of *E. coli O157:H7* is enhanced by soaking the alfalfa seeds before the pressure treatment.

Neetoo et al. (2010) also investigated the effect of high hydrostatic pressure on *Salmonella* contaminated alfalfa seeds and reported that a pressure of 600 MPa for 25 minutes at 4°C and 20°C could not completely eliminate *Salmonella*. However, a pressure treatment at 600 MPa for 25 minutes at 40, 45 and 50°C did completely inactivate *Salmonella*. The authors showed that a treatment at 500 MPa for 2 minutes at 45°C of pre-soaked alfalfa seeds is successful in eliminating both *Salmonella* and *E. coli O157:H7* without affecting seed viability.

In 2011, Neetoo and Chen looked at the combined effect of high hydrostatic pressure and heat treatment on alfalfa seeds inoculated with *Salmonella* and *E. coli O157:H7*. In order to get a 5 logs reduction in *Salmonella* and *E. coli 0157: H7* populations, alfalfa seeds had to be heat treated at 65°C for 10 days or 70°C for 24 hours; whereas a heat treatment at 55, 60, 65 and 70°C for 96, 24, 12 and 6 hours respectively, followed by high pressure processing at 600 MPa for 2 minutes at 35°C were able to achieve the 5 logs reduction in *Salmonella* and *E. coli 0157: H7* in alfalfa seeds. Sprouting yield was reduced when seeds were heat treated at 65°C for 10 days or high pressure treated at 600 MPa for 2 minutes at 35°C after heat treatments at 60 and 65°C for 24 and 12 hours respectively; in both cases, germination percentage of alfalfa seeds was not significantly affected.

Penas et al. (2008) researched the optimized combinations of time, pressure and temperature to reduce total aerobic mesophilic bacteria, total and faecal coliforms and yeast and molds populations without affecting the germination capacity of alfalfa seeds and reported 40°C and 100 MPa as optimal treatment conditions.

Later on, in 2009, Penas et al. studied the combined effect of pressure, temperature and antimicrobial compounds, hypochlorite and carvacrol, on the reduction of microbial loads and germination capacity of alfalfa seeds. Microbial loads were reduced with increasing pressure and antimicrobial compounds concentrations. A treatment of 200 MPa and hypochlorite

concentration of 18,000 ppm achieved reductions between 4.5 and 5 log CFU/g without significantly affecting seed viability; whereas a treatment of 250 MPa and carvacrol concentration of 1500 ppm achieved microbial safety of alfalfa seeds but reduced germination percentage to unacceptable levels.

Bamboo Shoots

Miao et al. (2011) studied the texture changes of bamboo shoots after high pressure treatment for 10 minutes at room temperature (25°C) and storage for 7 days at 4°C. High pressure processing delayed the enzyme activities of phenylalanine ammonia lyase (PAL) and peroxidase (POD), retarded the accumulation of lignin and cellulose and reduced the firmness of the water bamboo shoots, extending thus their shelf life.

Broccoli

Van Loey et al. (1998) showed that, in broccoli juice, chlorophylls a and b exhibit extreme pressure stability at room temperature but a combined pressure-heat treatment with temperature higher than 50°C significantly reduces chlorophyll content.

Butz et al. (2002) studied the effect of high pressure treatment (600 MPa) on the content of health promoting substances (e.g. vitamins, antioxidants, antimutagens), water retention, glucose retardation, changes in extractability and in-vitro bioavailability of carrots, tomatoes and broccoli. Butz et al. found that in general high pressure did not induce loss of beneficial substances but altered some physico-chemical properties such as higher glucose retardation index and water retention or reduced extractability; high pressure did not significantly impacted chlorophylls a and b in broccoli.

Houska et al. (2006) evaluated high pressure processing (500 MPa for 10 minutes) of apple-broccoli juice and noted that pressure inactivates more than 5 logs of the microbial population

and preserves the content of sulforaphane, a compound that exhibits anticancer, antidiabetic and antimicrobial properties to broccoli. Houska et al. also reported that the vitamin C content is independent of the pressure level but depends on the holding time; sensory quality of the treated juice was similar to the frozen juice for up to 70 days of storage.

Eylen D. et al. (2007, 2009) evaluated the effect of a combined treatment of high pressure and heat on myrosinase, glucosinolates and isothiocyanates. The Brassicaceae family is reach in glucosinolates, which can be hydrolyzed by myrosinase to produce isothiocyanates sulforaphane and phenylethyl isothiocyanate which have an anticarcinogenic activity. Eylen D. et al. reported that at pressures up to 200 MPa and temperatures of 50°C and above, pressure retarded thermal inactivation of myrosinase and that at pressures between 600 and 800 MPa and temperatures between 30-60°C, isothiocyanates were found to be relatively thermolabile and pressure stable. In a later research study, Eylen D. et al. showed that pressure treatment limits the loss of glucosinolates and consequently the health benefits of their degradation products. At 100-500 MPa and 20°C, there was no glucosinolates degradation reported after 15 minutes but after 35 minutes at 200-300 MPa and 20°C, a reduction of 20% of glucosinolates content was noted; however treatment at 100-500 MPa and 40°C showed a significant degradation of glucosinolates after 15 minutes.

Verlinde et al. (2008) reported that high pressure treatment of broccoli (0.1–600 MPa, 25–45°C, 30 min) significantly induced folates losses (48–78%), whereas broccoli folates were stable after heat treatment at temperatures up to 90°C.

Cabbage

Wennberg and Nyman (2004) indicated that treatment of white cabbage (*Brassica oleracea* var. *capitata*) with high pressure at 500 MPa reduced the proportion of soluble fiber without significantly affecting the total dietary fiber (TDF) content.

Li et al. (2010) evaluated the effect of high pressure processing (200-600 MPa; 10-30 minutes) on microbial loads of sour Chinese cabbage after storage at 4, 27 and 37°C for 90 days and reported a reduction of total aerobic bacteria (TAB) counts by 2.7–4.0 $\log_{10}$ CFU/g at 400 MPa and 4.2–4.5 $\log_{10}$ CFU/g at 600 MPa; lactic acid bacteria (LAB) were reduced by 2.4 – 4.3 $\log_{10}$ CFU/g at 400 MPa and completely inactivated at 600 MPa; yeasts counts were reduced by 1.5–2.0 $\log_{10}$ CFU/g at 400 and 600 MPa. Li et al. reported a shelf life of 15 days when pressurized (400 MPa) sour Chinese cabbages are stored at 27 and 37°C and a shelf life of 60 days when stored at 4°C.

Carrots

Stute et al. (1996) studied high pressure processing of vegetables at ambient temperature and results showed destruction of carrot, potato and green beans cell membranes and loss of soluble pectin causing softening of vegetables.

Sila et al. (2004, 2005) demonstrated that subjecting carrots (*Daucus carota*) to high pressure pretreatment (400 MPa, 60°C for 15 minutes) before thermal processing results in less texture loss. High pressure pretreatment combined with calcium infusion significantly improved textural properties of thermally processed carrots.

Araya et al. (2007) showed that all high pressure treatments of carrots studied (100, 200 and 300 MPa at 20°C) resulted in a significant loss of hardness (5, 25 and 50% respectively). Increase in pressure levels did not induce greater texture losses. Cell deformation, shape factors, elongation and turgidity loss were also observed.

Nguyen et al. (2007) indicated that pressure-assisted thermal processed carrots (500-700 MPa, 95 to105°C) had better quality attributes such as color and carotene content compared to thermal processed ones.

Rastogi et al. (2008a) demonstrated that carrot's pretreatment with, pressure (100–400 MPa),

temperature (50–70 °C), calcium chloride (0.5–1.5% w/v), and their combinations, after pressure-assisted thermal processing (PATP) and thermal processing, increased product hardness; pressure (200 MPa), heat (60 °C) and calcium chloride (1.0%) pretreatments increased pressure-assisted thermal processed carrots hardness by 1.2, 2.0 and 2.4 times respectively, and thermally processed carrots hardness by 2.7, 3.6 and 2.4 times respectively. Combined pretreatments increased the hardness of PATP carrots by 9.16 times and thermally processed carrots by 13.22 times.

Zhou et al. (2009) evaluated the effect of high pressure carbon dioxide on the quality of carrot juice and reported an increase in L-value and a-value, whereas b-value was similar to the control. The study also showed a decrease in pH, browning degree and a significant increase in cloud, titratable acidity and juice viscosity.

Green Beans

Stute et al. (1996) studied high pressure processing of vegetables at ambient temperature and results showed destruction of green beans cell membranes and loss of soluble pectin causing softening of the green beans.

Krebbers et al. (2002) indicated high pressure processing (500 MPa, 25°C) and two-pulse pressure treatment increased shelf life of green beans as treated products did not show outgrowth of microorganisms after 1 month storage at 6°C or 20°C and significantly retained firmness and ascorbic acid content. Two-pulse pressure treatment of green beans induced more than 99% inactivation of peroxidase, whereas high pressure did not have a significant effect on peroxidase as 76% of its initial activity remained after high pressure processing.

Mushroom

Matser et al. (2000) demonstrated that polyphenoloxidase, enzyme responsible for browning, is very pressure-resistant in mushrooms and a pressure of 950 MPa is needed to inactivate it.

Navy Beans

Ramaswamy et al. (2005) demonstrated that treatment of navy beans with moderate pressure (33 MPa), achieved high initial moisture uptake (0.59 to 1.02 kg/kg dry mass) and reduced loss of soluble materials over a soaking time of less than one hour.

Onion

Butz et al. (1994) demonstrated that treatment of fresh onions at 350 MPa, 25°C and 40°C for 30 minutes significantly reduced the microbial load of fresh onions but did damage onions membrane without inactivating undesirable enzymes which lead to changes in the odor and flavor of fresh onions. Pressures above 100 MPa damaged the cell structure, releasing polyphenoloxidase (PPO) and inducing then browning of the onions. Browning rate increased with increasing pressure.

Roldán et al. (2009) studied the combined effect of high pressure (100-400 MPa) and thermal processing (5°C) on flavonol content (Quercetin and quercetin glucosides) and antioxidant activity of onions (*Allium cepa* L. var. *cepa*, 'Grano de Oro'). Roldán et al. demonstrated that the combination of high pressure and thermal process resulted in a better extraction of flavonols and increased antioxidant activity. Treatment at 400 MPa and 5°C, increased quercetin glucosides extraction by 33% compared to untreated controls and maintained initial antioxidant activity.

Gonzalez et al. (2010) evaluated the effect of high pressure processing (50, 100, 200, 300, or 600 MPa; 5 minutes holding time) and thermal processing (40, 50, 60, 70, or 90 °C; 30 minutes) on onions membrane integrity and texture and indicated that a membrane destabilization is observed at pressure of 200 MPa and above and temperature of 50°C. Membrane rupture was observed at 300 MPa and above and 60°C and above.

Pepper

Castro et al. (2008) compared the effect of pressure treatment (100 and 200 MPa; 10 and 20 minutes) to thermal blanching (70°C, 80°C and 98°C; 1 and 2.5 min) on sweet green and red bell peppers and indicated that high pressure processing resulted in a lower reduction of soluble protein and ascorbic acid contents and better firmness. Compared to untreated red peppers, pressure treatment showed an increase in ascorbic acid content by 15-20%. However, high pressure treatment resulted in comparable polyphenol oxidase activity, higher pectin methylesterase and peroxidase activities than thermal blanching. Microbial loads were similar in pressurized and thermally treated green peppers, whereas pressurized red peppers showed higher microbial loads than blanched ones.

Potato

Eshtiaghi and Knorr (1993) compared the effect of high hydrostatic pressure treatment (400 MPa, 15 minutes, 5–50°C) to hot water blanching (100°C, 30–180 seconds) and indicated that microbial loads reduction and potatoes softness were comparable for both treatments. The authors also showed that a combination of high pressure with citric acid solution (0.5%) treatments resulted in a complete inactivation of polyphenoloxidase at 20°C. A 20% reduction in leaching of potassium was observed after high pressure treatment of potatoes. High pressure processing resulted in retention of 90% of ascorbic acid at 5°C and 35% at 50°C.

Stute et al. (1996) studied high pressure processing of vegetables at ambient temperature and results showed destruction of carrot, potato and green beans cell membranes and loss of soluble pectin causing softening of vegetables.

Luscher et al. (2005) studied the effect of pressure-shift freezing at pressures up to 400 MPa on quality attributes of potatoes tissues such as texture, color and visual appearance and showed considerable improvements compared to conventional freezing.

Benet et al. (2006, 2007) investigated the effect of high pressure low temperature processing on quality attributes of potatoes and reported that after freezing and thawing processes, polyphenoloxidase (PPO) activity of pressurized potatoes was not increased and color, drip loss, texture and microstructure of pressurized potatoes were better than in atmospheric control samples. Benet et al. also showed a reduction in processing time of pressure-induced thawing at pressure levels of 290 MPa.

Chapter 4 - Conclusion

High pressure processing has a significant potential as a novel technology in the food industry as it permits manufacturing of value-added food products with extended shelf life by inactivating microorganisms and enzymes at low temperatures without changing organoleptic and nutritional properties of foods and without the use of additives and preservatives.

The use of high pressure processing has expanded from U.S. and Japan to reach the international market, which reflects the important growth of the technology. Hormel Foods, Kraft Foods, Perdue, Foster Farms, Wellshire Farms (ready-to-eat deli meats), Maple Leaf Foods (ready meals), Avomex (guacamole, salsa, avocado pieces, juices, ready meals), Pressure Fresh, Australia (fruit, vegetable and herb products), Leahy Orchards (applesauce), Winsoms of Walla Walla (chopped onions), Motivatit Seafoods, Nisbet Oyster, Joey Oysters (oysters), Zwanenberg, The Netherland (mousse and spreads), Infantis, Greece/Germany (deli meats), Fressure Foods, New Zealand (avocados and guacamole), DGG Marketing, Australia/Singapore (red & white grape juice) are examples of companies that already have successfully utilized the technology for a variety of products.

HPP treatment costs are currently ranging from 4–10 cents/lb, including operating cost and depreciation (Sàiz et al., 2008). Pressure-processed products currently cost 3 to 10 cents per pound more to produce than thermally processed products (Ramaswamy et al., 2004). As demand for high pressure processing equipment grows, innovation is expected to further reduce capital and operating costs.

Although high pressure processing can not be used across the board on all food products, it does have its niche applications.

In this report we have covered the application of high pressure processing on fruits and vegetables but this technology has been also used in other type of foods such as meats, seafood and dairy.

High pressure processing has been very effective in the seafood industry as a shucking process of shellfish (oysters, mussels, clams, scallops, crabs and lobsters). The hign pressure processing technology denatures the adductor muscle, which will enable easy opening of the shellfish shell and this offers economical advantages such as reducing the labor cost, eliminating the risk of physical injuries, improving meat recovery, yields and product quality (Murchie et al., 2005; Torres & Velazquez, 2005). High pressure processing also increases microbiological safety and shelf life, eliminates *Vibrio spp.* in raw shellfish and *Listeria spp.* in other seafood products.

High pressure processing has been very successful in the treatment of ready-to-eat deli meats (beef, pork, turkey, chicken), sliced and diced cooked meat and dry cured meat products, by eliminating *Listeria*, *E. coli*, *Salmonella*, *Vibrio*, yeasts and molds and is already being used as a treatment method in many deli-meats industries. Besides satisfying the USDA alternative 1 rule for *Listeria monocytogenes* control in ready-to-eat meats, high pressure processing eliminates the use of preservatives, improves sensory properties, and extends shelf-life. The U.S Department of Agriculture/Food Safety and Inspection Service has already approved the use of high pressure processing as an acceptable method for eliminating *Listeria monocytogenes* in processed meat products (Hayman et al., 2004; USDA, Food Safety Inspection Service, 2006). High pressure processing can influence muscle's protein conformation and induce protein denaturation, aggregation, or gelation. It may also tenderize or toughen the meat depending on the meat protein system, the pressure, the temperature and the duration of the pressure treatment. Myoglobin, lipid oxidation, meat color, juiciness and chewiness can also be affected by high pressure processing (Cheftel and Culioli, 1997; Balny et al., 1993). Combination of high pressure processing, antimicrobial and refrigerated storage could be very effective to obtain value-added ready-to-eat products (Marcos et al., 2008a).

High pressure processing, combination of pressure and temperature and periodic oscillations of pressure are effective methods to reduce microorganisms and extend shelf-life of milk (Hite et al., 1899; Vachon et al., 2002; Mussa and Ramaswamy, 1997). High pressure processing is also related to the increase in curd firming rate, the reduction in rennet coagulation

time, the increase in cheese yield, and the acceleration of cheese ripening (Zobrist et al., 2005; Lopez et al., 1996; Molina et al., 2000, O'Reilly et al., 2001).

The combination of high pressure processing with non-thermal technologies such as gamma irradiation, alternating current, ultrasound, carbon dioxide or anti-microbial agent such as lacticin, nisin, or lactoperoxidase has been shown to work synergistically to enhance microbial lethality (Haas, 1989; Park et al., 2002; Crawford et al., 1996; Shimada, 1992; Knorr, 1995).

Consumers are usually conservative and skeptical towards new technologies and changes overall, independently on the advantages that the novel techniques are offering. However, according to a study done in June 2000 by the TRD Frameworks research company based in Seattle, on consumers acceptance of high pressure processing, 500 U.S. primary shoppers rated the technology between 6-7 on a 1-7 scale, showing the great potential of high pressure processing.

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