MSC THESIS

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Effect of HHP Treatment on Foodborne Pathogenic Bacteria in Fruit Purees

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Effect of HHP Treatment on Foodborne Pathogenic Bacteria in Fruit Purees

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1. Introduction

Healthy and high-quality cuisine is becoming increasingly popular. Consumers are more concerned about what they eat and the processes that food has been subjected to. This is why smoothies and fruit purees, which are nutritionally useful due to their high concentration of bioactive components and antioxidant activity, are getting more popular (Barba et al., 2012). Smoothies and fruit purees can be prepared quickly and easily from various combinations of fruits, vegetables, milk and milk products, or water. They are available on the market as well as being prepared at home. Consumers are increasingly interested in food that retains practically all of its organoleptic qualities after processing. As a result, there is an urgent need for the development of new food processing processes to replace the old ones in the food business. A high hydrostatic pressure processing method is one such form of food processing.

High hydrostatic pressure is a fantastic new approach for processing that substitutes old procedures while ensuring minimal changes in sensory, nutritional, and textural properties. The little increase in temperature and the short processing time, which can range from a few seconds to 30 minutes, generate minimal changes in processed food. In addition to assuring minimum loss of nutritional value and sensory qualities, high hydrostatic pressure improves the shelf life and ensures the product's microbiological quality. The use of high hydrostatic pressure records an increase in the production of fruit purees and smoothies, where it is unnecessary to add preservatives to maintain durability (Heinz and Buckow, 2009).

This paper analyzed the effects of high hydrostatic pressure on the pathogenic bacteria in smoothie and strawberry puree.

2. Goal of the Thesis

High Hydrostatic Pressure (HHP) treatment is a food preservation technique that uses high pressure to inactivate bacteria and enzymes in food. Both *Salmonella enterica* and *Listeria monocytogenes* are dangerous bacteria that can cause severe sickness. *Salmonella enterica* is a Gram-negative bacterium with a low fatality rate that causes foodborne sickness (CDC, 2021), whereas *Listeria monocytogenes* cause listeriosis, which has a high mortality rate (Kirk et al., 2014).

The primary goals of this work were:

- To determine whether using the HHP treatment on *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in food samples will reduce or eliminate these pathogenic bacteria, which can cause foodborne illness,
- To improve food safety, by reducing the pathogen load in food samples,
- To assess the effectiveness of this treatment,
- To determine the extent of injured cells caused by the HHP treatment, because injured cells might compromise food safety as under optimal conditions injured cells can regenerate and grow again and injured cells that are not able to grow on selective media can escape detection and result in false positive samples during microbiological investigations,
- To optimize HHP treatment parameters, by studying the effects of different HHP treatment parameters, such as pressure level and treatment time, on *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in food samples.

3. Literature Review

3.1. High Pressure Processing

High hydrostatic pressure (HHP) is a food processing method that has gained popularity in recent years due to its ability to maintain food quality, nutritional content, and safety (Figure 1). HHP is the process of submitting food products to high hydrostatic pressure, typically between 100 and 800 MPa, to inactivate or eliminate microbes, enzymes, and other food spoilage causes while keeping nutritional and sensory characteristics. The temperature rises slightly throughout processing, and the time ranges from a few seconds to 30 minutes (Rastogi, 2013). High hydrostatic pressure, which is utilized as an alternative to heat treatments, has various advantages.

Figure 1. High pressure technology

One of the primary advantages of HHP is its ability to enhance food shelf life without the use of chemical preservatives. HHP does not alter food's taste, texture, or nutritional content because it does not use heat. Furthermore, HHP can be used on a variety of meals, such as meats, fruits, vegetables, and dairy products, making it a versatile technique for food manufacturers. Another advantage of HHP is that it can increase food safety by reducing hazardous germs including *E. coli*, *Salmonella*, and *Listeria* species. HHP can inactivate

bacteria cell membranes by applying high pressure to foods, rendering them unable to replicate or cause harm. This makes HHP an effective alternative to traditional thermal processing methods, such as pasteurization, which can damage food quality and nutritional value (Thakur and Nelson, 1998).

Some of the other benefits of HHP include:

- lower energy consumption,
- it affects food regardless of its shape, size and composition,
- shorter processing time,
- low concentration of waste products.

However, there are several drawbacks to using HHP technology. One of the most significant constraints is the cost of the equipment necessary to execute HHP. High-pressure chambers can be costly to buy and maintain, and the technology requires specialized knowledge and training to use. Furthermore, because some foods may be sensitive to the high pressures required, HHP may not be acceptable for all types of meals.

In general, HHP is good for most foods, as long as they contain enough water and have no air space. The main foods treated using HP today are meat products, fruit, and vegetable products, aquatic products, and beverages (Table 1) (Huang et al., 2016).

Table 1. Examples of food treated with the HHP treatment

Product	*Process parameters*	*Purpose of treatment*
Juices and beverages	400 – 600 MPa; 1-5 minutes	Microbial safety and extension of shelf life
Meat products	400 – 600 MPa; 1-5 minutes	Microbial safety
Fruit and vegetable preparations (dips, salsa, baby food, etc.)	500 – 600 MPa; 5-10 minutes	Shelf life extension and inactivation of enzymes
Seafood and shellfish	250 – 350 MPa; 30-90 seconds	Removal of shell, increasing yield, inactivation of *Vibrio*
Ready-to-eat meals	400 – 600 MPa; 1-5 minutes	Microbial safety and extension of shelf life
Dairy products	400 – 600 MPa; 1-5 minutes	Microbial safety and extension of shelf life

3.2. Historical development of high hydrostatic pressure

Bert Hite and colleagues at West Virginia University discovered the effect of high hydrostatic pressure on food at the end of the nineteenth century. Hite then employed high hydrostatic pressure (up to 600 MPa) to maintain the quality of milk, and later vegetables and fruits, as well (Hite, 1899; Hite et al., 1914). Following that, interest in high hydrostatic pressure as an alternative to existing thermal food processing technologies is developing. In Japan, a big revolution occurred in 1992, when a product treated with high hydrostatic pressure was released onto the market (Knorr, 1993).

The groundbreaking goods comprised jellies, jams, and fruit sauces that were made without the use of high temperatures (Thakur and Nelson, 1998). As a result of extensive research conducted over the years, new pressure-treated items, such as guacamole in the United States and sliced cooked ham in Spain, were put into the market in the 1990s. Over the last two decades, there has been continuous development in the application of HP technology in the treatment of various foods. As a result, several manufacturers of HHP equipment that produce industrial-scale machines have emerged, including Hiperbaric (Burgos, Spain), Avure (Middletown, OH, USA), Uhde (Hagen, Germany, merged with Multivac in 2011), and several other smaller companies. More than ten businesses manufacture HHP equipment in total (Balasubramaniam et al., 2015).

3.3. Principle of operation of high hydrostatic pressure

The high hydrostatic pressure impacts the food uniformly from all sides and does not harm it unless the meal is hollow or has an empty area. There is a significant reduction in the number of microbes and protein denaturation during food processing, whereas high hydrostatic pressure has no effect on the bonds between molecules (Elamin et al., 2015). The application of high hydrostatic pressure can increase the product's durability, prevent chemical reactions that cause vitamin loss and the formation of disagreeable flavors, and inactivate harmful bacteria (Rastogi, 2013). The principles that explain the behavior of foods under the influence of high hydrostatic pressure are Le Chatelier's principle, isostatic rule, electrostriction, and compression of energy and heat (Elamin et al., 2015).

When a chemical system is in equilibrium and is affected by some change, such as the effect of increased pressure on a closed system, it tends to diminish this change by accompanying reactions, according to Le Chatelier's principle. That is, processes that result in a loss in volume are accelerated, whereas reactions that result in an increase in volume are inhibited. Similarly, according to Le Chatelier's principle, any change accompanied by a drop in volume, such as a chemical reaction, phase transition, or change in molecular configuration, will result in an increase in pressure (Pauling, 1964). Non-covalent bonds, such as ionic, hydrogen, and hydrophobic, are very susceptible to the effect of high hydrostatic pressure due to this phenomenon, known as Le Chatelier's principle. High hydrostatic pressure, on the other hand, has a minor influence on the covalent bonding of food constituents. As a result, food components with a high molecular mass are subject to changes in functional properties and conformation, whereas food components with a low molecular mass and a low proportion of secondary, tertiary, and quaternary structures are insensitive to the action of high hydrostatic pressure. That is, the components responsible for organoleptic characteristics and nutritional value, such as vitamins and bioactive substances, are unaffected by high hydrostatic pressure (Balci and Wilbey, 1999).

According to the isostatic principle, when food products are compressed by uniform pressure from all directions and then returned to their original stage and the pressure is released, food products are compressed independently of product size and geometry, because pressure transmission to the core is not mass/time-dependent, which is why HHP is also known as an isostatic processing technique (Martinez-Monteagudo and Balasubramaniam, 2016).

Pressure, according to electrostriction, increases ionization because water molecules arrange more compactly around electric charges. The chemical composition of the buffer and the biological reaction is controlled, resulting in more or less significant negative and reversible pH shifts (Balci and Wilbey, 1999). Due to the principle of microscopic ordering, an increase in pressure causes an increase in the degree of ordering of a molecule in a constant-temperature system (Yordanov and Angelova, 2010).

During processing, compression work is maintained by raising the temperature of the product through adiabatic heating. The intensity of the temperature increase depends on the composition of the sample being treated. In the case of water and foods that contain a higher

proportion of water, it is 3 °C for every 100 MPa, if the sample contains a higher proportion of fat, the temperature change can be up to three times higher (8–9 °C for every 100 MPa) (Karlović, 2015). Compression can be reflected in a change in pH value, if there is a decrease in pH, microorganisms become more sensitive to the action of high hydrostatic pressure, which increases the efficiency of the process along with increased temperature. The food, that is, the sample cools down to its initial temperature during decompression, if no heat was generated or lost from the wall of the pressure capillary during the pressure retention phase (Lovrić, 2000).

Foodstuffs, or samples subjected to high hydrostatic pressure, must be packed in flexible packaging that has the elasticity to convey pressure to the product while also ensuring a high sealing ability. In that instance, the packaging used to process samples must be able to adapt to a 15% volume reduction and return to its previous form and size (Marangoni et al., 2019). For the application of this technology, polymers, and copolymers are most often used. That is vials of different volumes made of poly(vinyl–alcohol)–PVAL and ethylene/vinyl–alcohol copolymer (E/VAL) (Norton et al., 2008).

3.4. High hydrostatic pressure treatment equipment

The high-pressure treatment system consists of a high-pressure tank and system for creating pressure, a temperature control system, and a material handling system. The high-pressure tank is the most important component of high-pressure processing equipment. When pressurizing the product, liquids in the tank are used to transfer pressure uniformly and instantaneously to the product. The most commonly used fluids are aqueous glycol solutions, silicone oil, sodium benzoate solutions, ethanol solutions, inert gases, and castor oil. Food products should be packed in flexible packaging. The packages are inserted into the high-pressure chamber. The container is closed and filled with a medium (liquid) for pressure transmission. High pressure is usually achieved with water as the hydraulic fluid for ease of operation and compatibility with food materials. The basic and fundamental application of high pressure in food is primarily product compression with water surrounding the treated food product. Since the effect of high pressure on liquids causes a small change in volume, there is no danger in operation as with processes in which compressed gases are used. When

the desired pressure is reached, the pump or piston is stopped, the valves are closed and the achieved pressure can be maintained without further input of energy (Singh et al., 2019).

3.5. Effect of high hydrostatic pressure on microorganisms and nutritional quality

High hydrostatic pressure affects microbial inactivation differently depending on the type of microorganism (yeasts, molds, bacteria), the form in which they are found (vegetative cells, spores, Gram-positive or Gram-negative), the genus, species, strain, and growth phase (adaptation phase, exponential phase, stationary phase, dying phase). That is, various microorganisms are more sensitive to stress, resulting in increased hydrostatic pressure (Mañas and Pagán, 2005; Daryaei et al., 2016). They are mostly bacteria that are heat and high hydrostatic pressure resistant. Gram-positive bacteria, for example, are more resistant than Gram-negative bacteria, spores are more resistant than vegetative cells, and cells in the exponential growth phase are more sensitive than those in the stationary phase (Lovrić, 2000). Yeasts and molds, unlike bacteria, are more sensitive to stress generated by high hydrostatic pressure (Daryaei et al., 2016). Microorganisms are inactivated due to damage in many areas of the cell caused by changes in proteins, ribosomes, cell membrane permeability, protein synthesis, and enzyme activity (McKay et al., 2011). Thus, with a pressure of 50 MPa, protein synthesis in microorganisms can be inhibited and the number of ribosomes can be reduced, with a pressure of 100 MPa, protein denaturation can occur, with the application of a pressure of 200 MPa, damage occurs to the cell membrane and the internal cellular structure is destroyed, while with the application of pressures of 300 MPa and more leads to irreversible protein denaturation, cell membrane rupture and inactivation of vegetative bacteria (bacterial death) (Abe, 2007). In general, processing at pressures of 200 to 700 MPa inactivates vegetative bacteria, yeasts, and molds, while bacterial spores, which are resistant to high hydrostatic pressure, stay viable even at pressures of 1200 MPa. As a result, the inactivation of some microorganisms (vegetative bacteria, bacterial spores) is accomplished through a combination of process-specific parameters such as treatment time, treatment temperature, and adiabatic heating (McKay et al., 2011).

HHP has the following effects on *Salmonella enterica* serovar Hartford and *Listeria*

monocytogenes:

- Inactivation, which means that HHP therapy can kill these bacteria by damaging their biological components, such as the cell membrane and DNA.
- Reduction of pathogen load: HHP treatment can considerably reduce the amount of viable *Salmonella* and *Listeria monocytogenes* cells in food, lowering the risk of foodborne disease.
- Preservation of food quality: because HHP treatment does not use high heat or chemical preservatives, it can help maintain the nutritional value, flavor, and texture of food products.
- Potential for synergistic effects: HHP treatment can act in tandem with other food processing procedures, such as heat and chemical treatments, to lower the risk of *Salmonella* and *Listeria* contamination even further.

Compounds influence nutritional value, which is described as micronutrients (minerals and vitamins) and macronutrients (carbohydrates, fats, and proteins), which are separated based on the amount we require from our diet (Gibney et al., 2009). Bioactive substances, in addition to micro and macronutrients, are extremely important. Carotenoids, phytosterols, polyphenols, fatty acids, and peptides are phytochemicals that regulate metabolic processes and improve human health (Correia et al., 2012).

Fruits and vegetables are extremely rich in polyphenols, such as phenolic acids, flavonols, flavones, proanthocyanidins, flavanones, anthocyanins, and catechin monomers, which reduce the risk of various degenerative diseases, premature death, cardiovascular diseases and reduce body inflammation and oxidative stress (Sánchez-Moreno et al., 2009). High hydrostatic treatment pressure has a different effect on minerals, vitamins, and bioactive compounds in food, but due to the minimal effect of high hydrostatic pressure on covalent bonds, they are safer than other food components. However, a direct consequence of the effect of high hydrostatic pressure on bioactive compounds is a change in the functional properties of these food ingredients, which includes changes in antioxidant activity, carcinogenic activity, and bioavailability (Mahadevan and Karwe, 2016).

3.2.1. Pathogenic bacteria

Pathogenic bacteria are microorganisms that have the ability to infect people and cause illness and disease. These bacteria can be present in a variety of settings, including food, water, and soil, and can be transmitted via a number of routes, including direct touch, ingestion, or inhalation. Pathogenic bacteria can take many forms, including spherical, rod-shaped, and spiral. They are classified according to their staining properties, which might be Gram-positive or Gram-negative. Pathogenic bacteria can also create virulence factors like toxins and adhesins that allow them to infiltrate and colonize host tissues. Pathogenic bacteria can also elude detection by the immune system by generating enzymes that destroy host antibodies or by altering the expression of their own surface antigens (Doron and Gorbach, 2008).

Some pathogenic bacteria can form biofilms, making them more resistant to antibiotics and host defenses. Pathogenic bacteria's processes for causing disease can differ based on the species and location of infection. Some bacteria, such as *Staphylococcus aureus*, can produce toxins that cause food poisoning, whereas others, such as *Streptococcus pneumoniae*, can colonize the respiratory tract and cause pneumonia or meningitis. Pathogenic bacteria can enter the bloodstream and produce sepsis, a potentially fatal illness characterized by organ failure and death. Controlling the spread of dangerous microorganisms is critical for disease outbreak prevention. Proper food handling and preparation, appropriate personal hygiene practices, and effective sanitation measures in healthcare settings are all strategies for preventing harmful germs. Antibiotics can also be used to treat bacterial infections; however, misuse can lead to the development of antibiotic-resistant bacteria, making treatment more difficult (Todar, 2012).

3.2.1.1. Pathogenic bacteria in food

Foodborne pathogenic bacteria are a major public health concern, causing a variety of foodborne illnesses. Foodborne infections can cause moderate symptoms like nausea and diarrhea, as well as more serious complications like sepsis and death. Pathogenic bacteria of several sorts can contaminate food and cause sickness in people (Bintsis, 2017). Some of the most common are as follows:

1) *Salmonella* species: found in raw and undercooked eggs, poultry, meat, unpasteurized milk and dairy products, and raw fruits and vegetables;
2) *Campylobacter*: found in raw and undercooked poultry and unpasteurized milk;
3) *Escherichia coli* (*E. coli*): found in undercooked beef and other meats, unpasteurized milk, and raw fruits and vegetables;
4) *Listeria monocytogenes*: found in ready-to-eat meats, soft cheeses, unpasteurized milk, and raw fruits and vegetables;
5) *Clostridium botulinum*: found in improperly canned or preserved foods (Bintsis, 2017).

Pathogenic bacteria can infect food at any moment during manufacture, processing, storage, and preparation. The following are some prevalent sources of contamination:

1) Animal feces that: can contaminate water, soil, and crops;
2) Poor sanitation which that: can lead to contamination of food preparation surfaces and equipment;
3) Improper food storage which: can lead to the growth of bacteria in foods;
4) Contaminated water that: can be used to irrigate crops or used in food preparation (Alegbeleye et al., 2018)

Preventing contamination of food with pathogenic bacteria requires a multi-faceted approach. Some of the measures taken to prevent contamination include:

1) Food safety education: educating consumers on safe food handling practices can reduce the risk of foodborne illness.
2) Food safety regulations: governments implement regulations that mandate food safety standards for food producers and processors.
3) Proper food storage and handling: food should be stored at the correct temperature and handled using proper hygiene practices.
4) Use of antibiotics: antibiotics should be used judiciously in food-producing animals to prevent the development of antibiotic resistance.

In addition to classic pathogenic bacteria management strategies, research has concentrated on the creation of novel ways, such as the use of bacteriophages, which are viruses that infect and kill bacteria. Other techniques include the use of probiotics, which are good bacteria that can help prevent pathogenic bacteria from colonizing, and the creation of vaccinations that can boost the immune system to protect against bacterial infections (Uddin et al., 2021). Pathogenic bacteria are a major public health concern because they can cause a wide range of illnesses and disorders. Pathogenic bacteria's features, illness causes, and control techniques are all major areas of research in microbiology and public health. Ongoing research into the development of novel approaches for controlling pathogenic bacteria will be essential to preventing the spread of bacterial infections and improving public health outcomes (Doron and Gorbach, 2008).

3.2.2. *Salmonella* spp.

Salmonella is a genus of Gram-negative rod-shaped bacteria that cause foodborne diseases in people and animals (Figure 2). The bacteria can be found in soil, water, and animal excrement, and they can contaminate food products during production, processing, or storage. *Salmonella* is a facultative anaerobic bacteria that does not generate spores. Because of the presence of peritrichous flagella, the bacteria are motile and can grow at temperatures ranging from 5°C to 45°C, with 37°C being the ideal growth temperature. It can also survive in a wide variety of pH levels, making it suitable for survival in the acidic environment of the stomach. *Salmonella enterica* and *Salmonella bongori* are the two species of *Salmonella.* *Salmonella enterica* is further divided into six subspecies, which are responsible for most human infections. *Salmonella* causes disease by invading the intestinal epithelial cells of the host and producing enterotoxins and cytotoxins. These bacteria's enterotoxins cause diarrhea by increasing the secretion of water and electrolytes into the intestinal lumen. The cytotoxins produced can cause inflammation and tissue damage to the intestinal mucosa (Giannella, 1996). Infections can range in severity from minor self-limiting gastroenteritis to serious systemic infections like sepsis and meningitis. The severity of the disease is determined by the virulence of the *Salmonella* strain and the host's immunological state. Young children, the elderly, and immunocompromised individuals are at higher risk of developing severe infections. *Salmonella* is a significant public health concern globally, with millions of cases of foodborne illness and thousands of deaths reported each year. According to the World

Health Organization (WHO), *Salmonella* is responsible for an estimated 93.8 million cases of foodborne illness and 155,000 deaths each year (Eng et al., 2015).

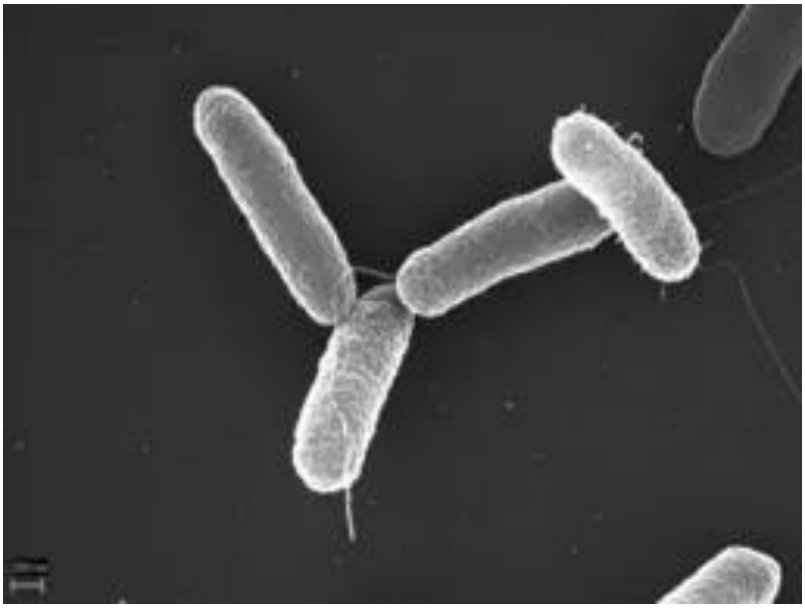

Figure 2. *Salmonella* bacteria (Internet 1).

Salmonella is a type of bacteria that can infect food. It is found in a variety of foods, including fruits and vegetables, meat, and dairy products. *Salmonella* can contaminate fruits through a variety of means, including contaminated soil or water, contaminated surfaces, or contact with diseased animals. Fruit is a widely consumed food by people all over the world. Fruits are well-known for their nutritional content as well as their health advantages. When fruits become contaminated with this bacteria, however, they can create major health problems for consumers.

Preventing *Salmonella* contamination in fruit is crucial for public health. Here are some preventative measures that can be taken:

1) Practice good hygiene: wash hands thoroughly with soap and warm water before and after handling fruits;
2) Purchase fruits from reputable sources: buy fruits from reputable stores or farmers' markets that follow good hygiene practices;
3) Clean and store fruits properly: clean fruits thoroughly before eating or cooking them. Store fruits in a clean and dry place;

4) Avoid cross-contamination: keep fruits away from raw meat, poultry, and eggs. Use separate cutting boards and utensils for fruits and other foods (CDC, 2023).

If *Salmonella* contamination is suspected in fruits, several control measures can be taken to prevent further contamination:

1) Recall contaminated fruits: if contaminated fruits have already been distributed to stores, they should be recalled immediately.
2) Investigate the source of contamination: the source of contamination should be investigated to prevent future outbreaks.
3) Sanitize equipment and surfaces: all equipment and surfaces that came into contact with contaminated fruits should be thoroughly cleaned and sanitized.
4) Implement food safety measures: food safety measures should be implemented to prevent future contamination, such as good hygiene practices, regular testing of water and soil, and proper cleaning and sanitation of equipment and facilities.

3.2.2.1. *Salmonella* spp. and HHP treatment

Gouvea, et al., (2020), conducted a research where they investigated the effects of high-pressure processing (HPP) on microbial inactivation in açaí juices with variable pH and soluble solids content (SSC). Açaí juice with pH 4.3 and 2.9°Brix was infected with cocktails of 5 strains of *E. coli* O157:H7, *Listeria monocytogenes*, or *Salmonella* spp. and processed at 5°C at different pressures (300, 400, and 600 MPa) and dwelling durations (1 and 3 min). At 400 MPa for 3 minutes, the lethality was greater than 6-log CFU/mL. To investigate the effect of pH and SSC on the inactivation of *Salmonella* spp. by HPP, the pH of açaí juice samples was altered to a range of 4.0 to 5.5, and SSC was modified to a range of 2.9 to 14.9°Brix. With rising pH and SSC, HPP's ability to deliver a 5-log reduction in the population of *Salmonella* spp. was diminished. The juices with pH 4.0 and 2.9°Brix showed >6-log reductions immediately after HPP, while the juice with 8.9°Brix showed a 5-log reduction. The juices (pH 4.0-14.9°Brix and pH 4.5-2.9°Brix) also showed a >6-log reduction in *Salmonella* spp. concentration after one week of refrigerated storage (7 °C). These findings indicated that a less intense process (below commonly recommended commercial conditions - 600 MPa/3 min) could be used for açaí juice, ensuring required

safety, as well as additional microbial inactivation verified during refrigerated storage.

3.2.3. *Listeria monocytogenes*

Listeria monocytogenes is a bacterium found in the environment that causes listeriosis, a serious infection that can lead to severe illness and death, particularly in vulnerable populations such as pregnant women, newborns, the elderly, and immunocompromised individuals. *Listeria monocytogenes* is a Gram-positive, rod-shaped bacterium that is facultatively anaerobic, which means it can thrive with or without oxygen (Figure 3). It is motile and can form biofilms, making it resistant to washing and disinfection. It may also live and develop at low temperatures, making it especially dangerous in refrigerated and frozen foods. One of the potential sources of *Listeria monocytogenes* contamination is fresh produce, including fruits (Rogalla and Bomar, 2023).

Listeriosis outbreaks have been linked to several foods, particularly when they are not properly kept, cooked, or handled. It can infect fruits at any point in the manufacturing process, from the farm to the customer. Once the bacterium is present on the fruit, it can multiply and spread to other fruits during storage and transportation, potentially leading to listeriosis outbreaks. While Listeria monocytogenes contamination of fruit is uncommon in comparison to other food products, outbreaks have occurred in recent years, emphasizing the importance of understanding risk factors and implementing effective prevention strategies. (Zhu et al., 2017).

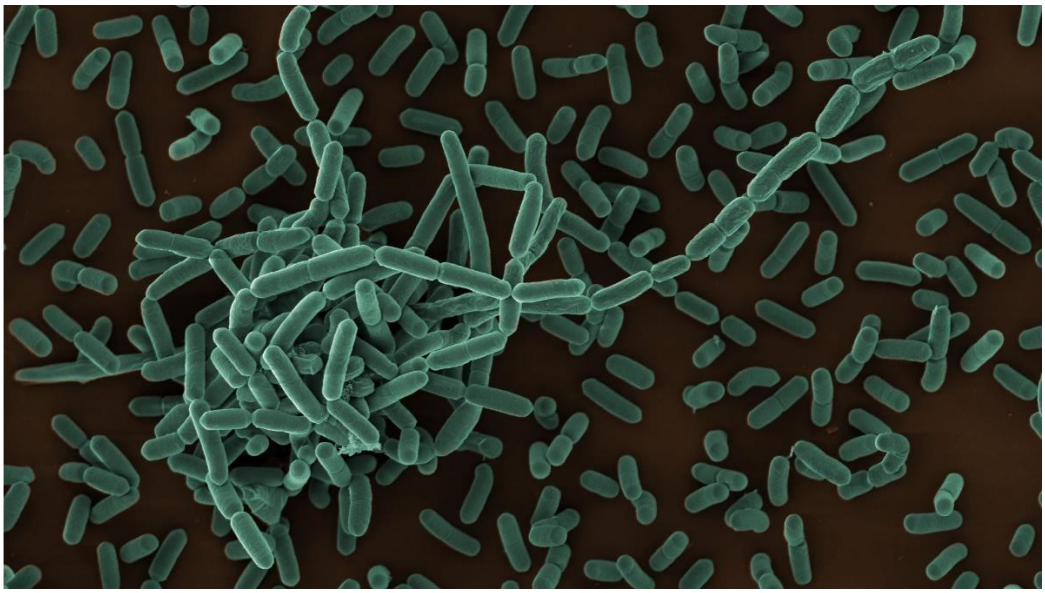

Figure 3. *Listeria monocytogenes* bacteria (Internet 2).

Several factors contribute to the risk of *Listeria monocytogenes* contamination in fruit. These include:

1) Contaminated water sources used for irrigation
2) Contaminated soil or manure used for fertilization
3) Poor hygiene and sanitation practices during harvesting and processing
4) Inadequate temperature control during storage and transportation

Prevention of *Listeria monocytogenes* contamination in fruit involves a combination of measures at different stages of production, from the field to the consumer. Some of the strategies for preventing the spread of *Listeria monocytogenes* in fruit include:

1) Use of clean water sources for irrigation
2) Proper handling of fertilizers and manure to avoid contamination
3) Good hygiene and sanitation practices during harvesting and processing, including handwashing and the use of disinfectants
4) Use of refrigeration and temperature control during storage and transportation
5) Regular monitoring and testing for Listeria monocytogenes contamination in fruit and in the production environment

Listeria monocytogenes contamination in fruit is a serious food safety issue that can lead to outbreaks of listeriosis. Understanding the elements that contribute to contamination risk and adopting effective preventative techniques at all stages of production are critical for assuring fresh produce safety. Growers, processors, and consumers must be aware of the risks and take the appropriate precautions to prevent the spread of this bacteria in fruits and other fresh produce. *Listeria monocytogenes* can cause everything from mild flu-like symptoms to severe invasive infections like meningitis and sepsis. Once inside the body, *Listeria monocytogenes* can invade and replicate within host cells, leading to tissue damage and inflammation (Shamloo et al., 2019).

3.2.3.1. *Listeria monocytogenes* and HHP treatment

Alpas and Bozoglu (2003), carried out a study where the goal was to compare high-pressure resistance of *Listeria monocytogenes* strains at 25°C and 50°C at 350 MPa, as well as to use high pressure (250 MPa and 350 MPa) at 30°C and 40°C to inactivate the relatively most pressure resistant strain inoculated in pasteurized apple, apricot, cherry, and orange juices. *L. monocytogenes* was discovered to be the relatively most pressure-resistant strain, and raising the pressure from 250 MPa to 350 MPa at 30°C resulted in an additional three to four-log cycle drop in viability, with viable cells remaining after 5 minutes. When 350 MPa was applied at 40°C for 5 minutes, the cell population of all fruit juices was reduced by more than eight log cycles.

4. Materials and Methods

4.1. Sample Collection

For this research strawberry puree, smoothie, and distilled water were used as food matrices. The strawberry puree was prepared from frozen strawberries that were purchased at a Lidl supermarket in Budapest, Hungary. The strawberries were then homogenized with a blender in the laboratory of the Department of Food Microbiology, Hygiene, and Safety of the Hungarian University of Agriculture and Life Sciences. The smoothie was made from the frozen fruit mixture that includes strawberries (37,2%), banana (24,1%), avocado (13,3%), and almond milk (25,4%). All the ingredients of the smoothie were also purchased in the Lidl store. Distilled water was prepared in the lab and autoclaved for 20 minutes at 120 °C.

4.2. Bacterial Strains

Salmonella enterica serotype Hartford and *Listeria monocytogenes* CCM4699 were obtained from the laboratory of the Department of Food Microbiology, Hygiene, and Safety of the Hungarian University of Agriculture and Life Sciences. The bacterial strains were kept in the department's freezer, and the strains were recultivated on TSA media before the experiments. Before using the strains, they were subcultured on TSA agar and incubated at 37 °C for one day, so we could have fresh and active strains, in order to minimize errors during the experiments.

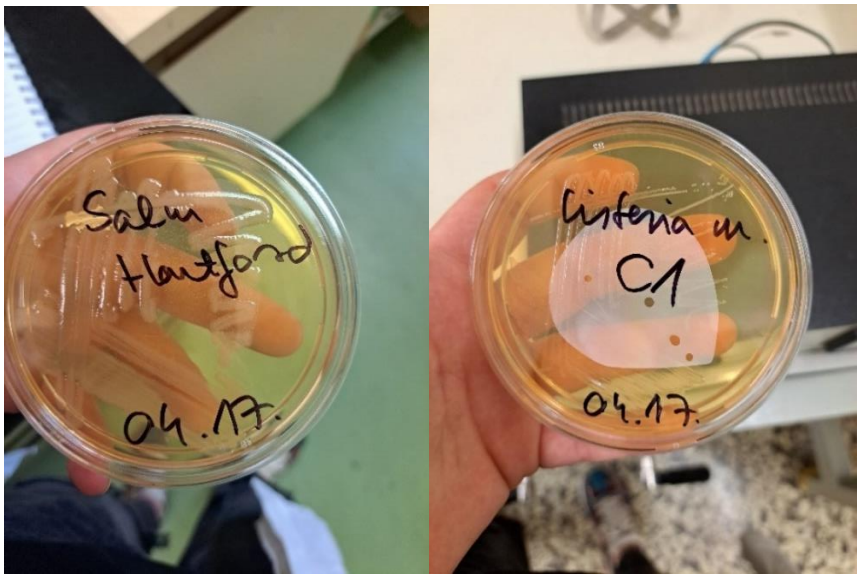

Figure 4. *Salmonella enterica* serotype Hartford *Listeria monocytogenes* CCM4699

4.3. Culture Media

PALCAM Agar is a differential and highly selective medium used to isolate and detect Listeria ssp., particularly *L. monocytogenes* from food and clinical specimens. Palcam agar was prepared in the laboratory according to the manufacturer's instructions, which state that 68.8 grams of the agar powder need to be dissolved in one liter of distilled water. The agar was autoclaved at 120 ℃ for a duration of 20 minutes. The media was then cooled down to 50 ℃, and the dissolved contents of two vials of Palcam Listeria Selective Supplement were added. For each vial, 5 ml of distilled and sterilized water was added, and the contents of the vials were dissolved before being added to the agar.

Xylose Lysine Deoxycholate (XLD) Agar is a selective medium for the isolation of *Salmonella* and *Shigella* spp from clinical specimens and food samples. XLD Agar is both a selective and differential medium. XLD agar was prepared following the instructions provided by the manufacturer, which state that 56.7 grams of the agar powder have to be dissolved in one liter of distilled water (Figure 5). The medium must not be autoclaved, rather it has to be heated slightly in the microwave until we can't see the agar particles on the walls of the bottle. Before pouring the plates agar was kept in the water bath at 50 °C.

Tryptone Soy Agar (TSA) agar is a culture medium used in microbiology for aerobic and anaerobic low-demand bacteria. It is a versatile, non-selective medium that provides sufficient nutrients to allow the growth of a wide variety of microorganisms. TSA agar was prepared by following the manufacturer's instructions. In order to prepare 500 mL of TSA, 15 grams of tryptic soy broth (TSB) powder and 7.5 grams of agar were measured and dissolved in 500 mL of distilled water. The agar was autoclaved at 120 ℃ for a duration of 20 minutes. Before pouring the plates, the agar was kept in the water bath at 50 °C.

Figure 5. Prepared culture media

4.4. Diluent solution

The diluent solution, which is used for serial dilutions, was prepared by adding 8.5 grams of sodium chloride (NaCl) and one gram of peptone to one liter of distilled water. 9 mL of the resulting solution was then distributed into tubes and autoclaved at 120 °C for 20 minutes.

4.5. HHP Treatment

The treatment was carried out in three different matrices: sterile distilled water, strawberry puree, and smoothie. The matrices were inoculated with a mixture (1:1) of fresh cultures of *Salmonella* Hartford and *Listeria monocytogenes* to reach 10^6 CFU/ml initial count of the pathogens in the matrices directly before the high hydrostatic pressure treatment. The cell count was adjusted with the help of a Densitometer.

In the first set of experiments, strawberry puree, smoothie, and distilled water were used. 5 mL of each of the matrices was transferred into small flexible bags. The bags were later labeled and inoculated with the bacteria. During the first experiment, 24 samples were prepared and treated with HPP treatments using different parameters. Also, 3 control samples for each matrices were prepared without HPP treatment. The control samples were used to ensure that any observed effects are not due to factors other than the HHP treatment.

Three replications were performed for each HHP treatment to ensure data accuracy and reproducibility.

After the inoculation, the bags were sealed and put under the HHP treatment. The pressures used during the first set of experiments were: 150, 200, 250, 300, 450, and 600 MPa and the time of the treatment was 5 minutes. In the second set of experiments, distilled water, smoothie, and strawberry puree were treated by using the HPP treatment at 200 MPa for different times (0, 5, 10, and 15 minutes). After the treatment ten-fold serial dilutions were prepared from the samples. The appropriate dilutions of strawberry or smoothie samples were inoculated onto the XLD or Palcam with and without using the thin agar layer (TAL) method to enumerate the number of non-injured and injured *Listeria monocytogenes* and *Salmonella* Hartford that resulted from HHP treatment. In the TAL method injured cells can resuscitate and grow on the top layer of TSA agar while selective agents of the bottom agar (XLD or Palcam agar) diffuse from the selective agar to the TSA layer. Then colonies form typical reactions with the selective components of the XLD agar, which enable the differentiation of pathogen colonies from background microflora (Kang and Fung, 2000).

Using the TAL method to enumerate *Listeria monocytogenes*, samples were inoculated on Palcam agar and parallel on Palcam agar which was overlaid with TSA agar. In the case of Salmonella, samples were inoculated on XLD agar and parallel on XLD agar overlaid with TSA agar. Inoculation was done by spread plating and by the “drop” method. In the case of the “drop” method, 10 μL of the desired dilution was pipetted on the sterile agar surfaces allowing multiple dilution members to be inoculated on a single plate.

In the case of the distilled water, samples were inoculated parallel onto the XLD and TSA agar to enumerate Salmonella Hartford, and onto Palcam, and TSA agar to enumerate Listeria monocytogenes. In this case, TSA plates were equivalent to the TSA overlaid plates used for strawberry puree and smoothie samples. Inoculation was done by spread plating and by the “drop” method, as described earlier. After each experiment, the plates were incubated at 37°C.

5. Results and Discussion

5.1. Survival of *Salmonella* Hartford and *Listeria monocytogenes* after HHP treatment in distilled water

Since, in the case of the TAL method, the injured cells can resuscitate and grow on the top layer of TSA agar while selective agents of the bottom agar (XLD or Palcam) diffuse from the selective agar to the TSA layer during incubation, that allow differentiation of the pathogen from the background microbiota, TAL plates show the total number (non-injured and injured) of cells.

Figures 6, 7, and 8 represent the survival of total count, non-injured, and injured cells of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water, after the HPP treatment which was done at different pressures (150, 200, 250, 300, 450, and 600 MPa). Results show that the highest survival rate for the total cell count and the injured cells (difference between the total cell count and non-injured cells) was at 150 MPa for both strains, (total cell count: *Salmonella* 6.1 ± 0.137 log CFU/mL, *Listeria* 7.3 ± 0.067 log CFU/mL; injured cells: *Salmonella* 5.9 ± 0.174 log CFU/mL, *Listeria* 7.2 ± 0.067 log CFU/mL). In the case of non-injured cells, the two strains showed the highest survival rate at different pressures (*Salmonella* at 150 MPa: 5.5 ± 0.031 log CFU/mL, *Listeria* at 200 MPa: 6.5 ± 0.034 log CFU/mL). The largest reduction for salmonella was detected at the same pressure treatment (250 MPa) for all types of counted cells. For *Listeria* the samples treated with 450 MPa had the lowest survival rate for both total cell count and injured cells, and for the non-injured cells, it was detected at 300 MPa. The largest reduction values were obtained when the cell count for both pathogens was reduced below the detection limit for both strains.

Based on these results it is clear that high hydrostatic treatment resulted in a high proportion of injured cells, especially in *Listeria monocytogenes*. Therefore when applying this technology it is important to keep in mind that if they are placed in favorable conditions after pressure treatment these injured cells can regenerate which could have an impact on food safety. As a result, the technology should be combined with, for example, refrigeration, low pH, the addition of natural antimicrobial compounds, or other environmental factors.

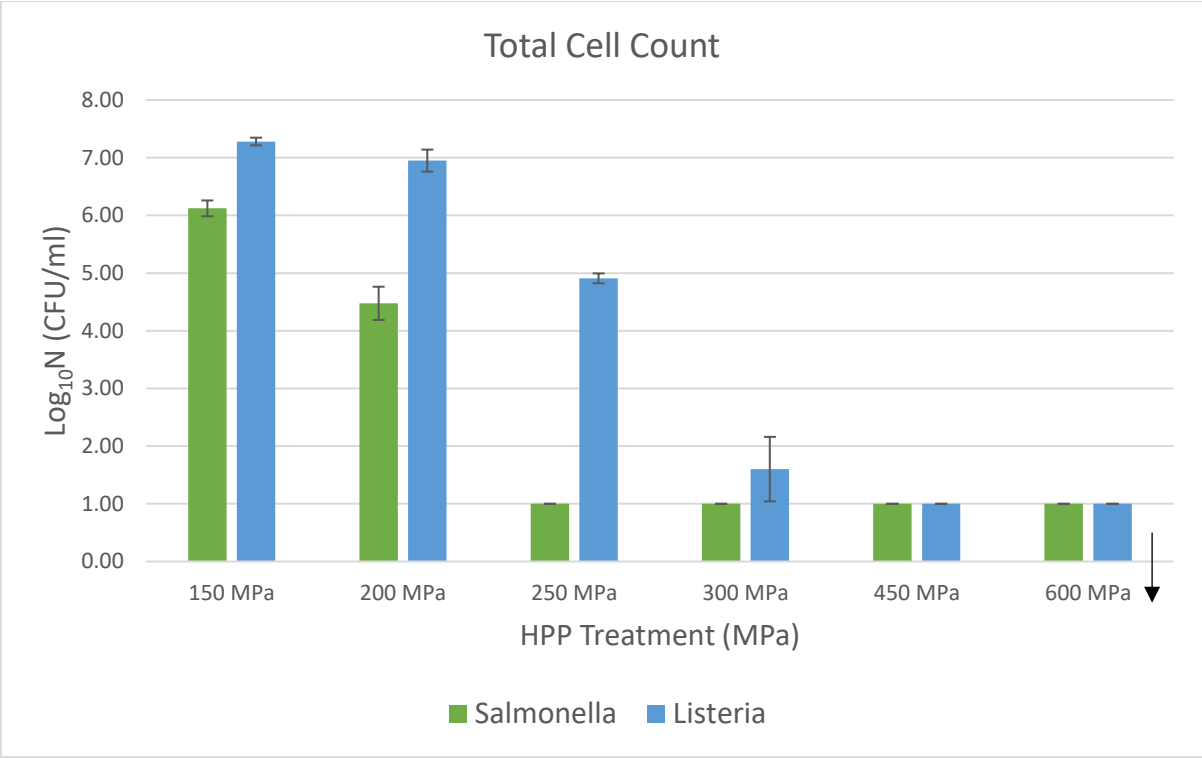

Figure 6. Total count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water after the HPP treatment (arrows indicate a cell count below the detection limit)

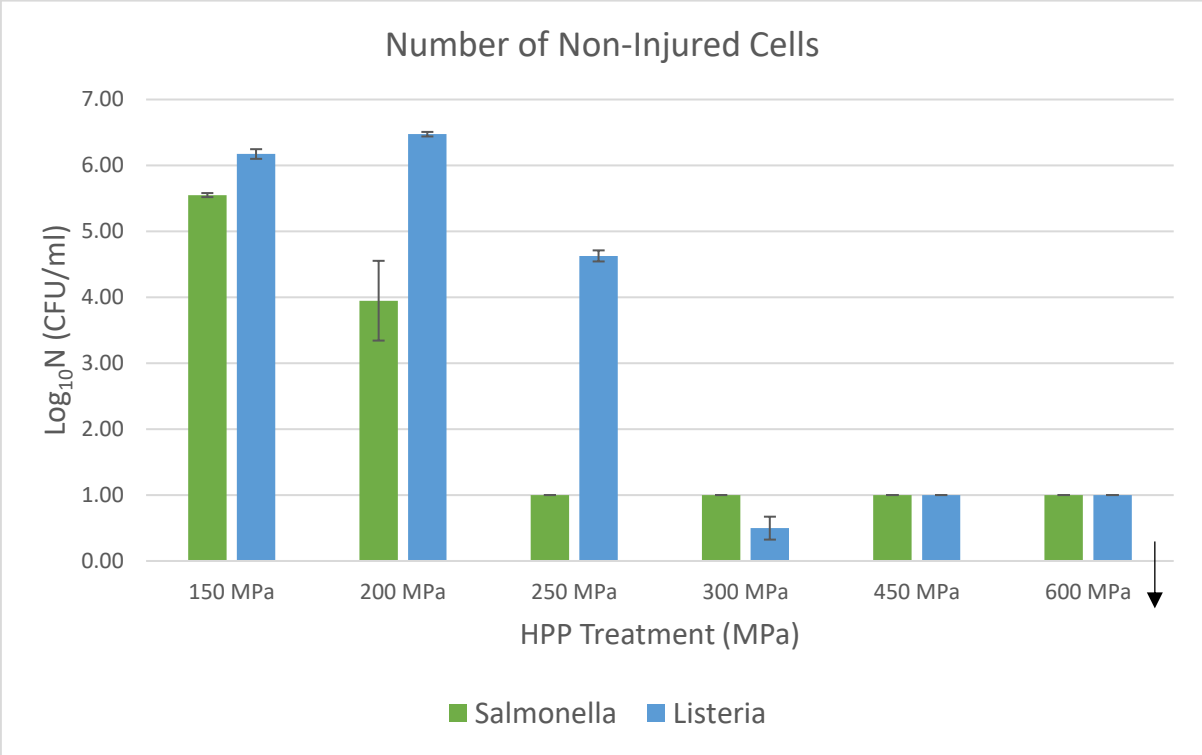

Figure 7. Non-injured cell count of *Salmonella enterica* serovar Hartford and *Listeria*

monocytogenes in distilled water after the HPP treatment (arrows indicate a cell count below the detection limit)

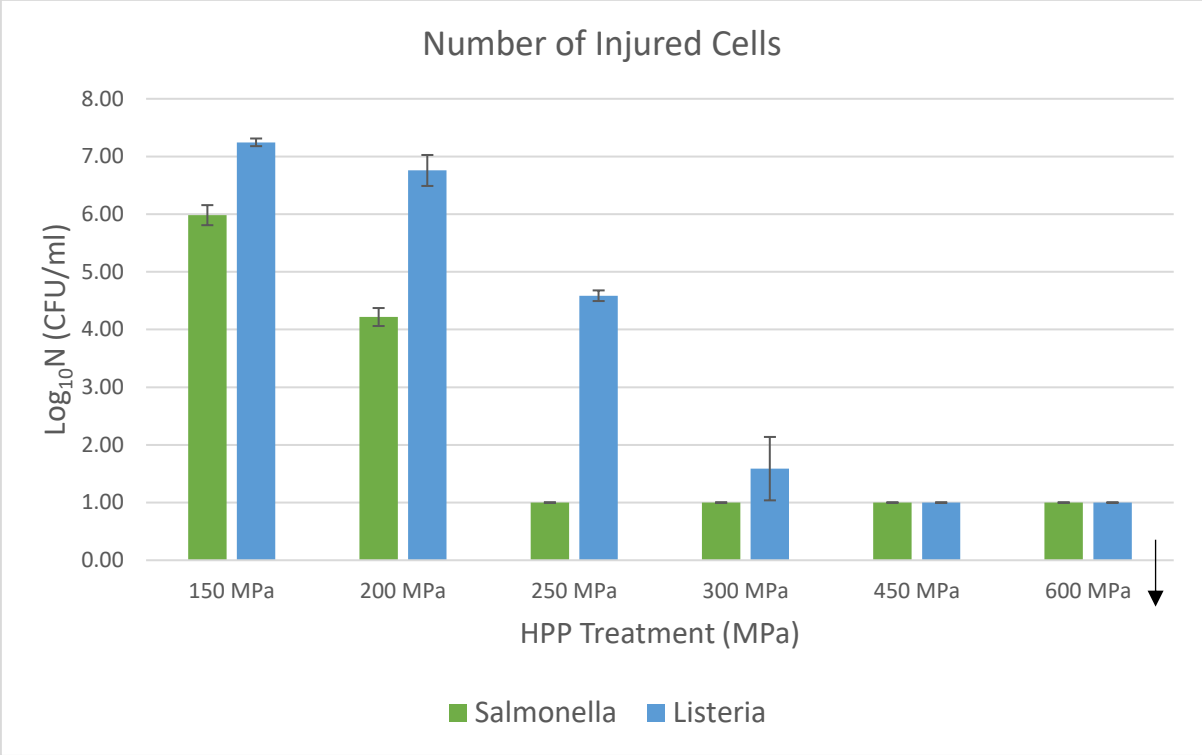

Figure 8. Injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water after the HPP treatment (arrows indicate a cell count below the detection limit)

5.2. Survival of *Salmonella* Hartford and *Listeria monocytogenes* after HHP treatment in strawberry puree

The survival of total count, non-injured, and injured cells of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after HPP treatment at different pressures (150, 200, 250, and 300 MPa) is depicted in Figures 9–11. The maximum survival rate for total cell count and injured cells (difference between total cell count and non-injured cells) for *Salmonella* occurred at 150 MPa (total cell count: 3.6 ± 0.498 log CFU/mL, injured cells: 2.5 ± 1.481 log CFU/mL). *Salmonella* had the best survival rate in non-injured cells at 150 MPa as well (3.5 ± 0.089 log CFU/mL). For all categories of counted cells, the greatest reduction in *Salmonella* was observed at the same pressure treatment (200 MPa). There was no evidence of growth in the instance of *Listeria*. For both strains, the largest

reduction values were obtained when the cell count for both pathogens was reduced below the detection limit.

Compared to the results of distilled water the low survival rate in strawberry puree can be explained by the low pH value of this food matrix. This low pH value in combination with HHP treatment resulted in significant microbial destruction even at low-pressure treatment values.

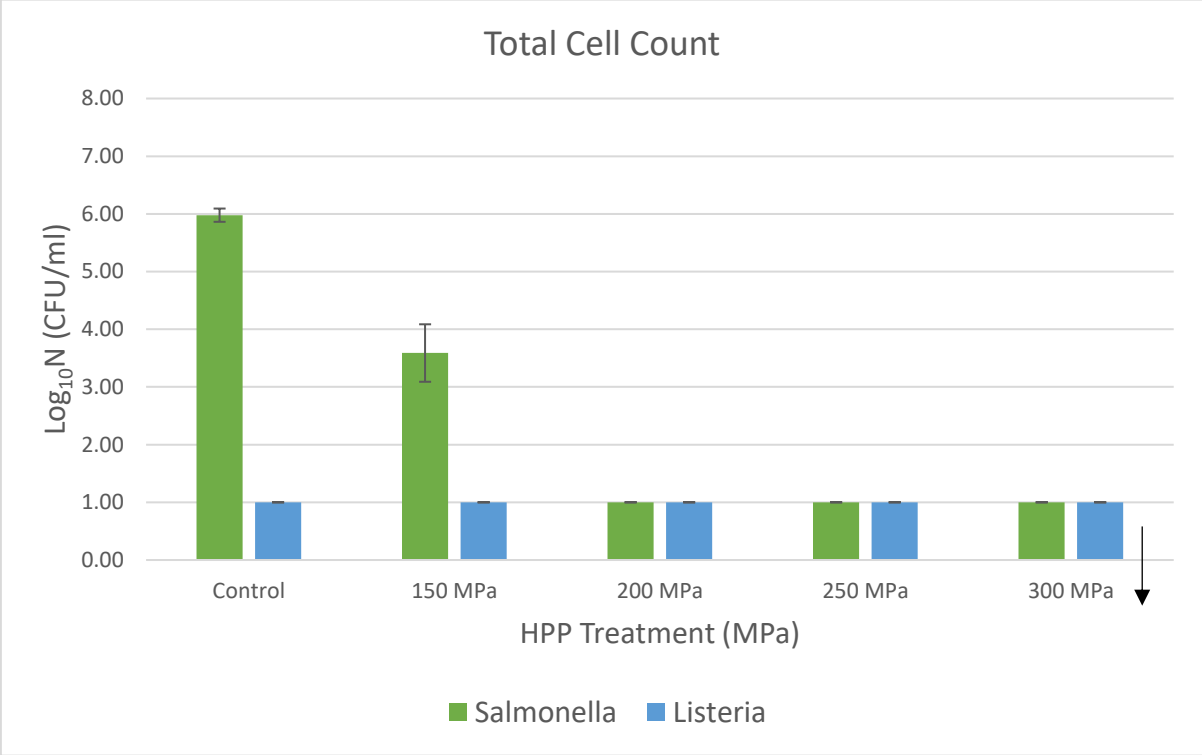

Figure 9. Total count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after the HPP treatment (arrows indicate a cell count below the detection limit)

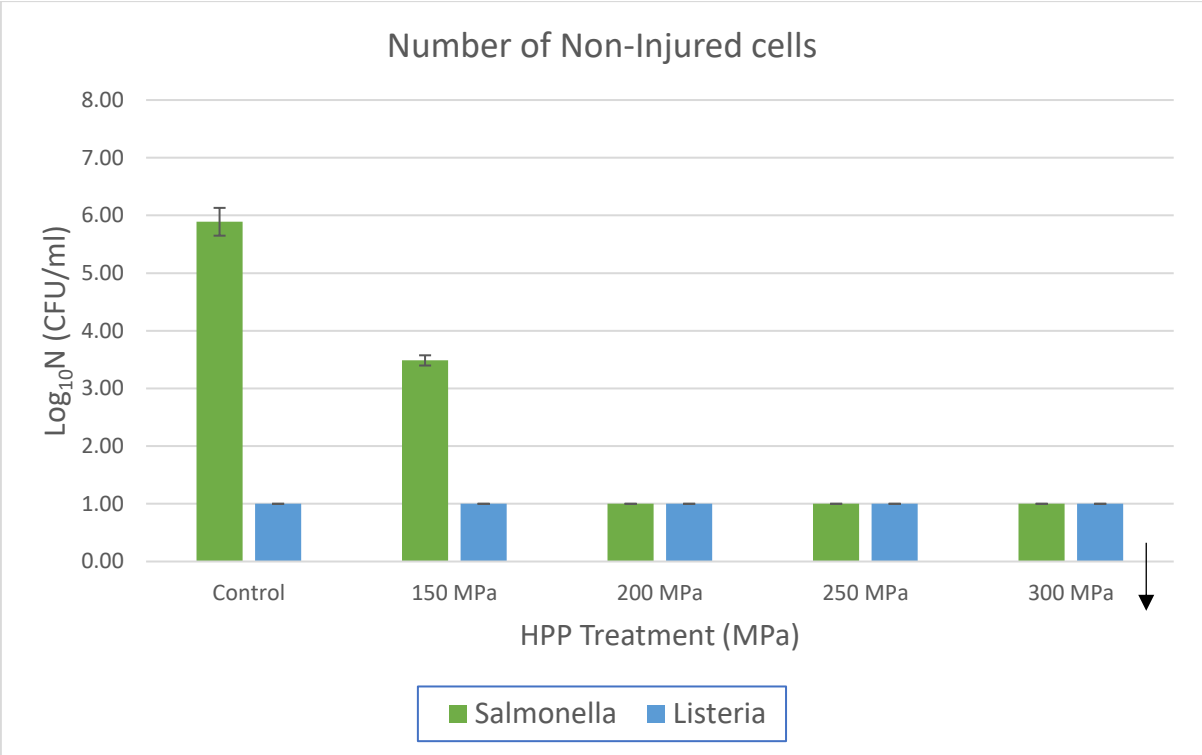

Figure 10. Non-injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after the HPP treatment (arrows indicate a cell count below the detection limit)

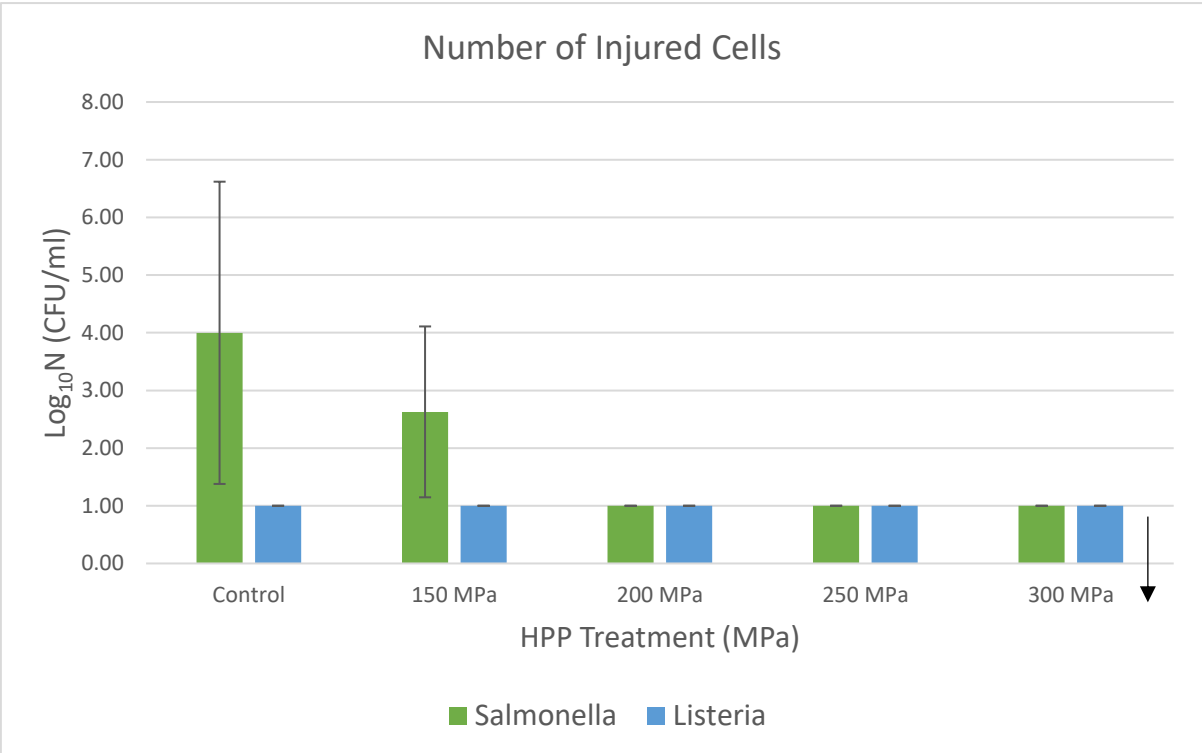

Figure 11. Injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after the HPP treatment (arrows indicate a cell count below the detection limit)

5.3. Survival of *Salmonella* Hartford and *Listeria monocytogenes* after HHP treatment in smoothie

The survival of total cell count, non-injured, and injured cells of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in a smoothie after the HPP treatment at different pressures (150, 200, 250, and 300 MPa) is shown in Figures 12–14. *Salmonella* had the highest survival rate for both total cell count and injured cells at 200 MPa (total cell count: 3.1 ± 0.68 log CFU/mL, injured cells: 2.0 ± 1.661 log CFU/mL). *Salmonella* had the best survival rate in non-injured cells at 150 MPa (5.3 ± 0.108 log CFU/mL). The greatest survival rate for *Listeria* occurred at 150 MPa for non-injured cells, injured cells, and total cell count (5.5 ± 0.064 log CFU/mL, 5.5 ± 0.559 log CFU/mL, 5.9 ± 0.239, respectively). The greatest reduction in *Salmonella* was observed at the same pressure treatment (250 MPa) for the total cell count and non-injured cells, whereas the lowest survival rate was observed for injured cells at 150 MPa. For *Listeria*, samples treated with 250 MPa showed the lowest survival rate across all cell types. For both strains, the largest reduction values were obtained when the cell count for both pathogens was reduced below the detection limit.

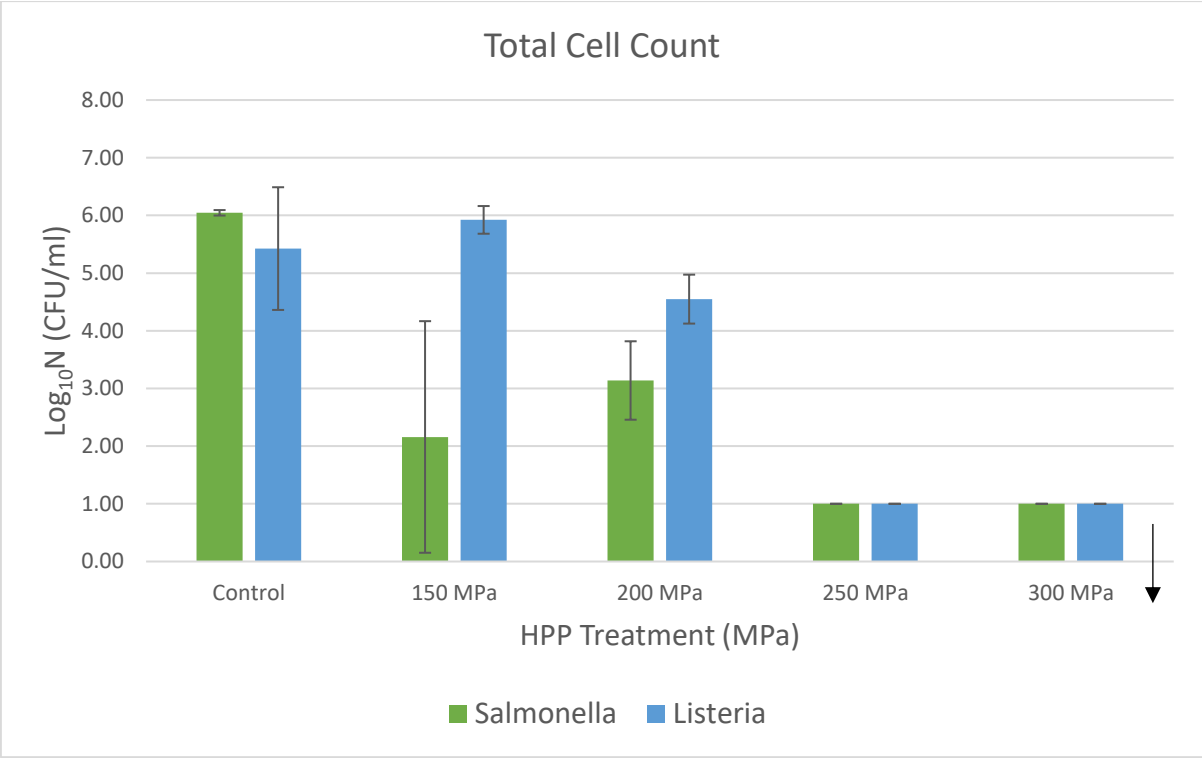

Figure 12. Total count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in smoothie after the HPP treatment (arrows indicate a cell count below the detection limit)

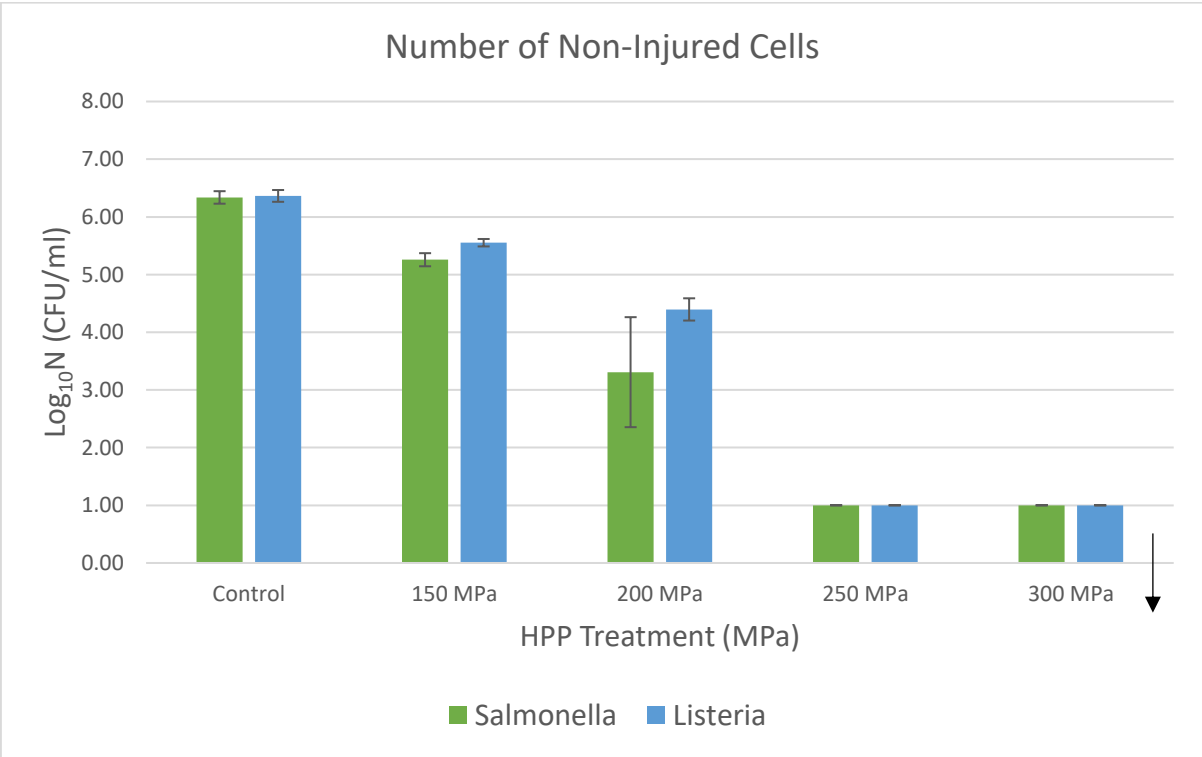

Figure 13. Non-injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in smoothie after the HPP treatment (arrows indicate a cell count below the detection limit)

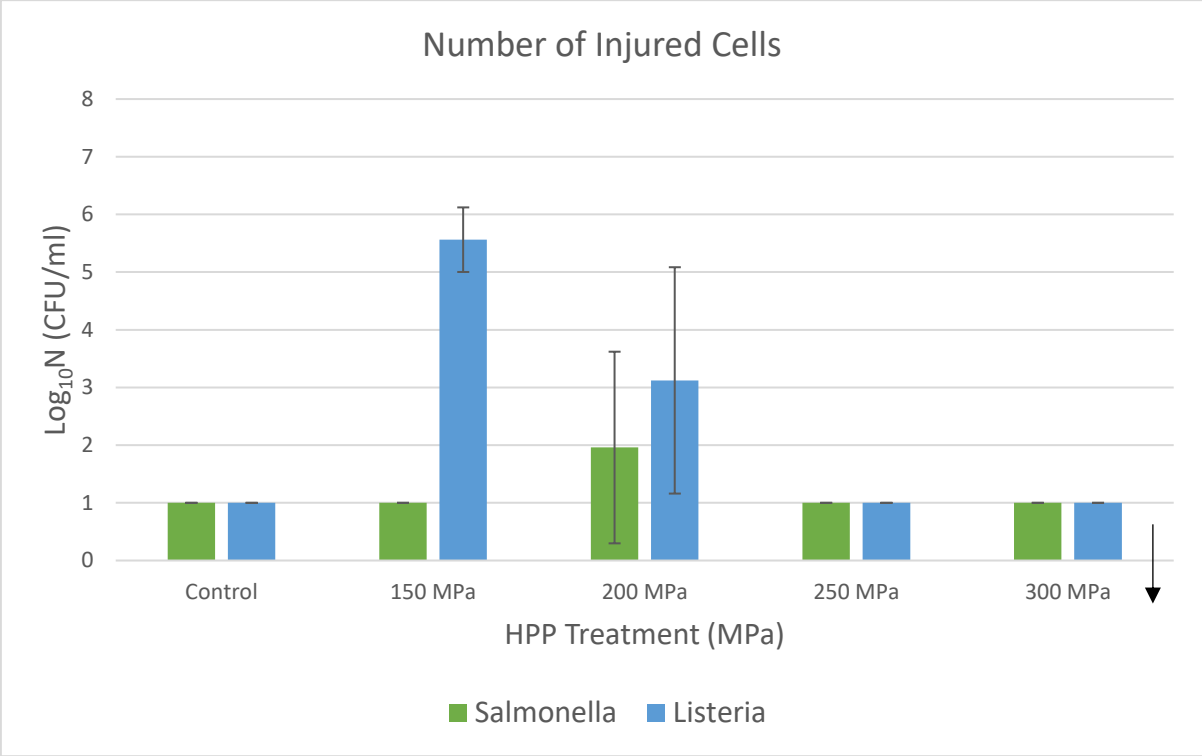

Figure 14. Injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in smoothie after the HPP treatment (arrows indicate a cell count below the detection limit)

5.4. Survival of *Salmonella* Hartford and *Listeria monocytogenes* after 200 MPa HHP treatment at different times in distilled water

Figures 15-17 show the survival of total count, non-injured, and injured cells of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water after HPP treatment at the same pressure but for different lengths of time (200 MPa for 5, 10, and 15 minutes). At 200 MPa/ 5 min, *Listeria* had the highest survival rate for total cell count, non-injured, and injured cells (total cell count: 6.9 ± 0.268 log CFU/mL, non-injured cells: 6.5 ± 0.034 log CFU/mL, and injured cells: 6.7 ± 0.268 log CFU/mL). The highest reduction in *Listeria* was reported for all categories of counted cells at the same pressure treatment (200 MPa/15

min) – total cell count (2.3 ± 0.053 log CFU/mL), non-injured cells (1.3 ± 0.301 log CFU/mL), and injured cells (2.3 ± 0.026 log CFU/mL). The highest survival rate *Salmonella* had at 200 MPa/5 min for all three types of cells (total cell count: 4.48 ± 0.288 log CFU/mL, non-injured cells: 3.95 ± 0.604 log CFU/mL, and injured cells: 4.22 ± 0.155 log CFU/mL). The lowest reduction values for *Salmonella* were obtained when the cell count was reduced below the detection limit. For *Listeria*, the largest reduction values were obtained when the cell count was reduced below the detection limit.

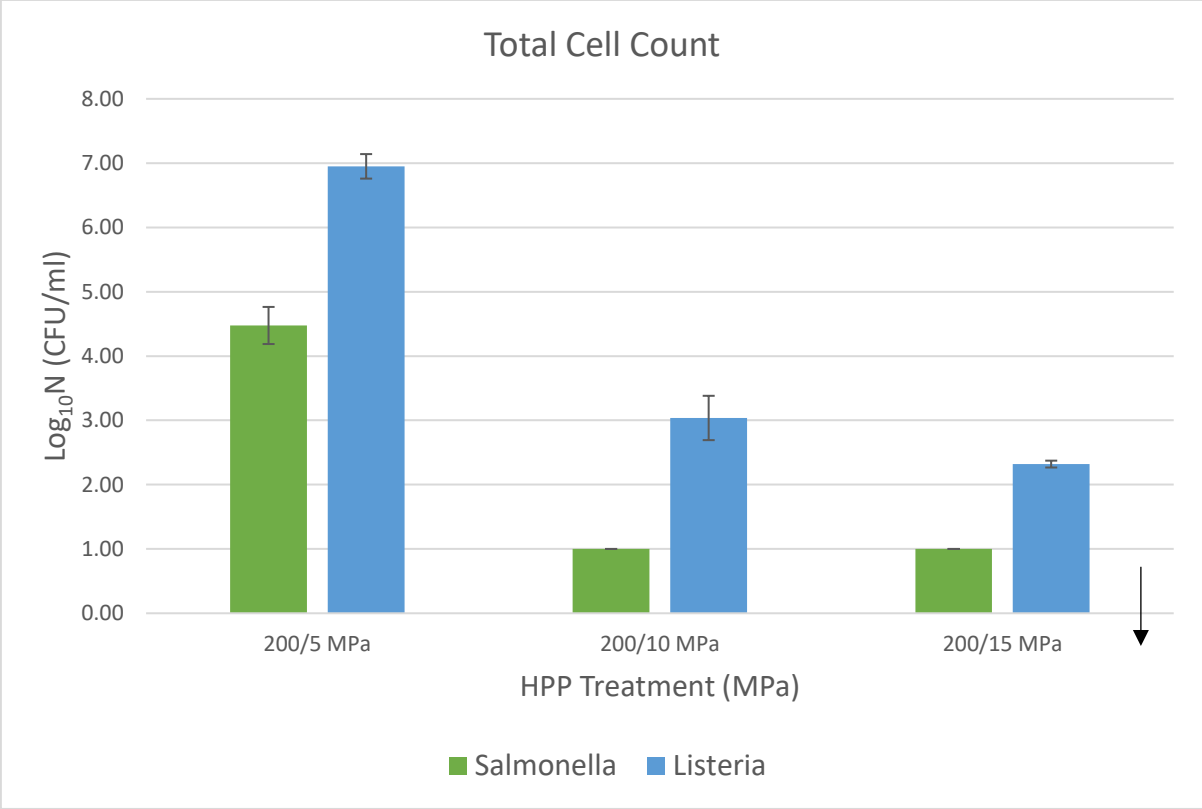

Figure 15. Total count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

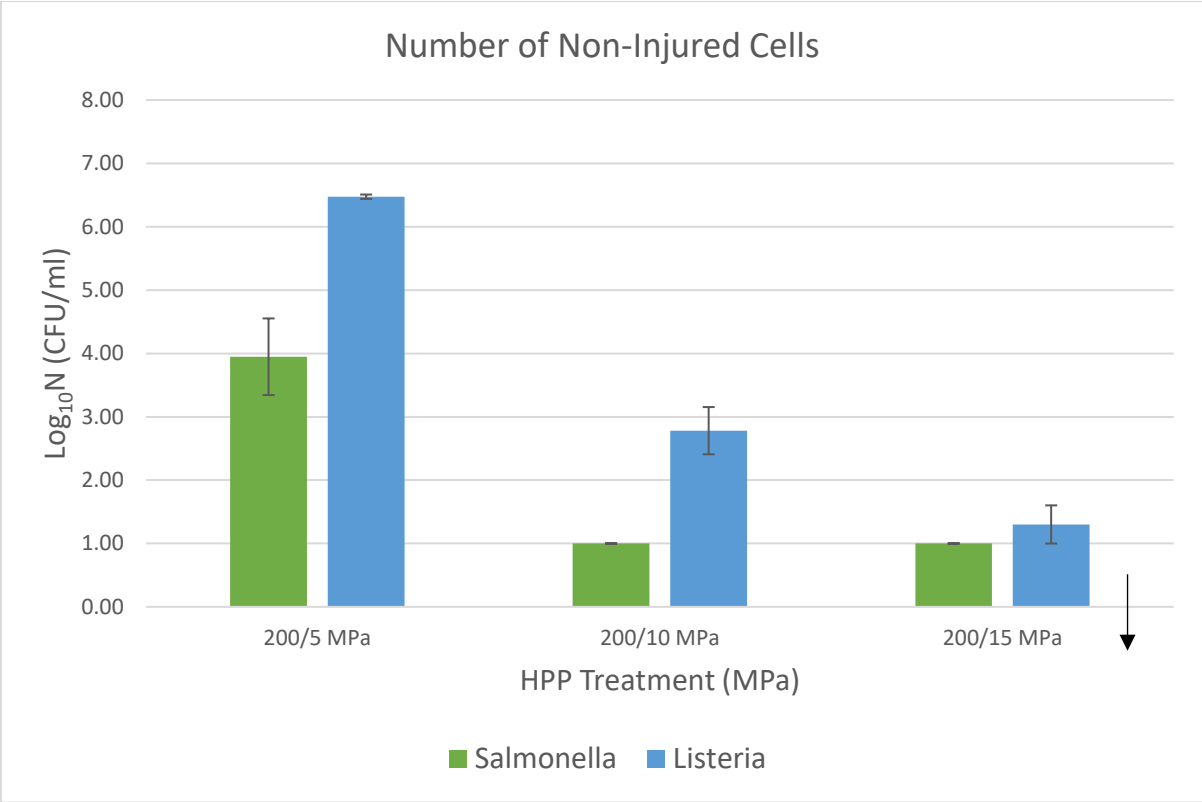

Figure 16. Non-injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

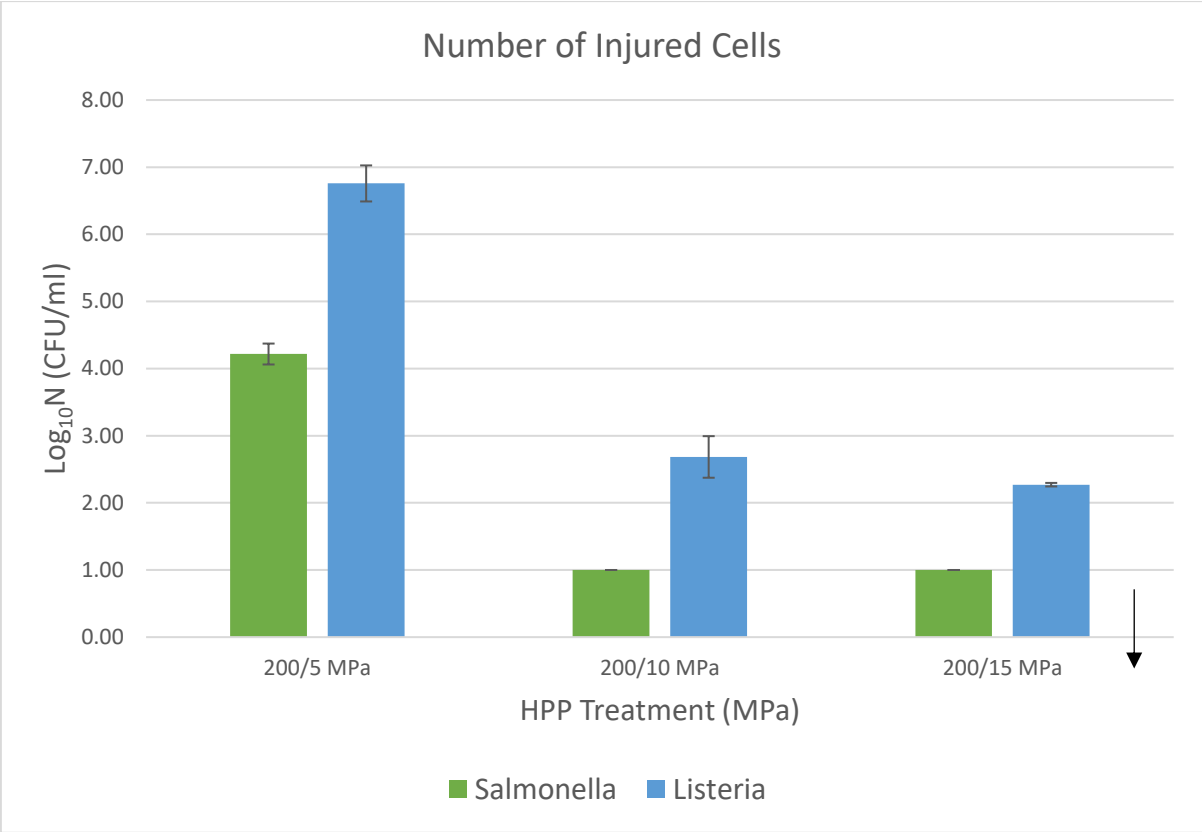

Figure 17. Injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in distilled water after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

5.5. Survival of *Salmonella* Hartford and *Listeria monocytogenes* after 200 MPa HHP treatment at different times in strawberry puree

Figures 18-20 show the survival of total cell count, non-injured, and injured cells of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in the strawberry puree after HPP treatment at the same pressure but for different times (200 MPa for 5, 10, and 15 minutes). The number of cells was reduced below the detection limit after even the shortest (5min) HPP treatment for both, *Salmonella* and *Listeria*.

As observed in the previous experiment with different HHP pressure values the low pH value in combination with HHP treatment resulted in significant microbial destruction.

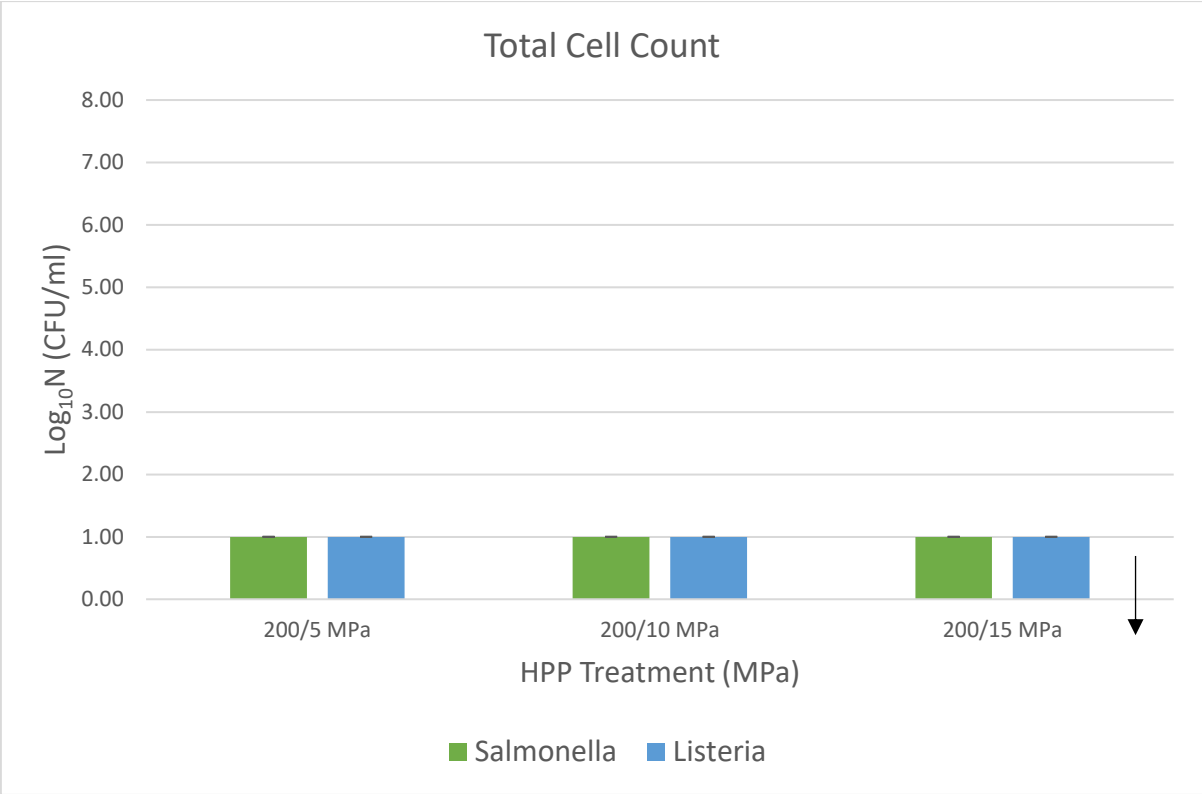

Figure 18. Total count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

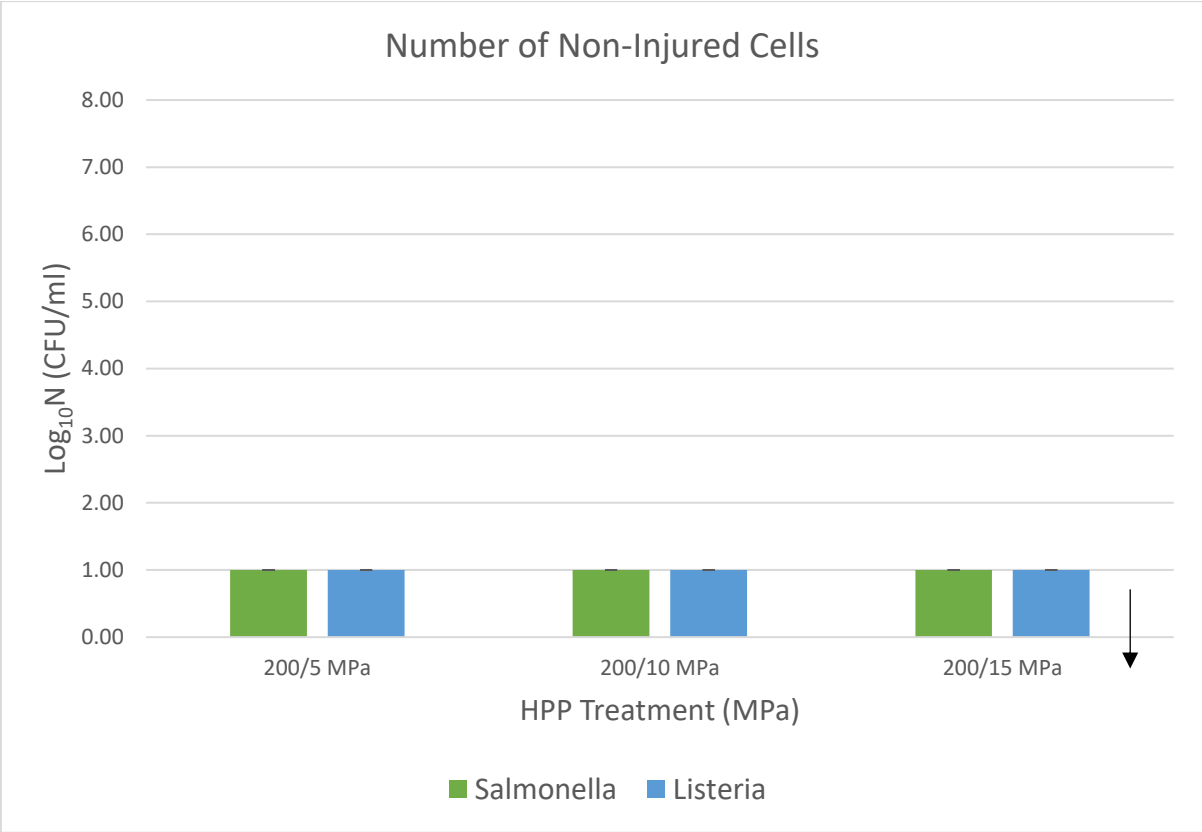

Figure 19. Non-injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

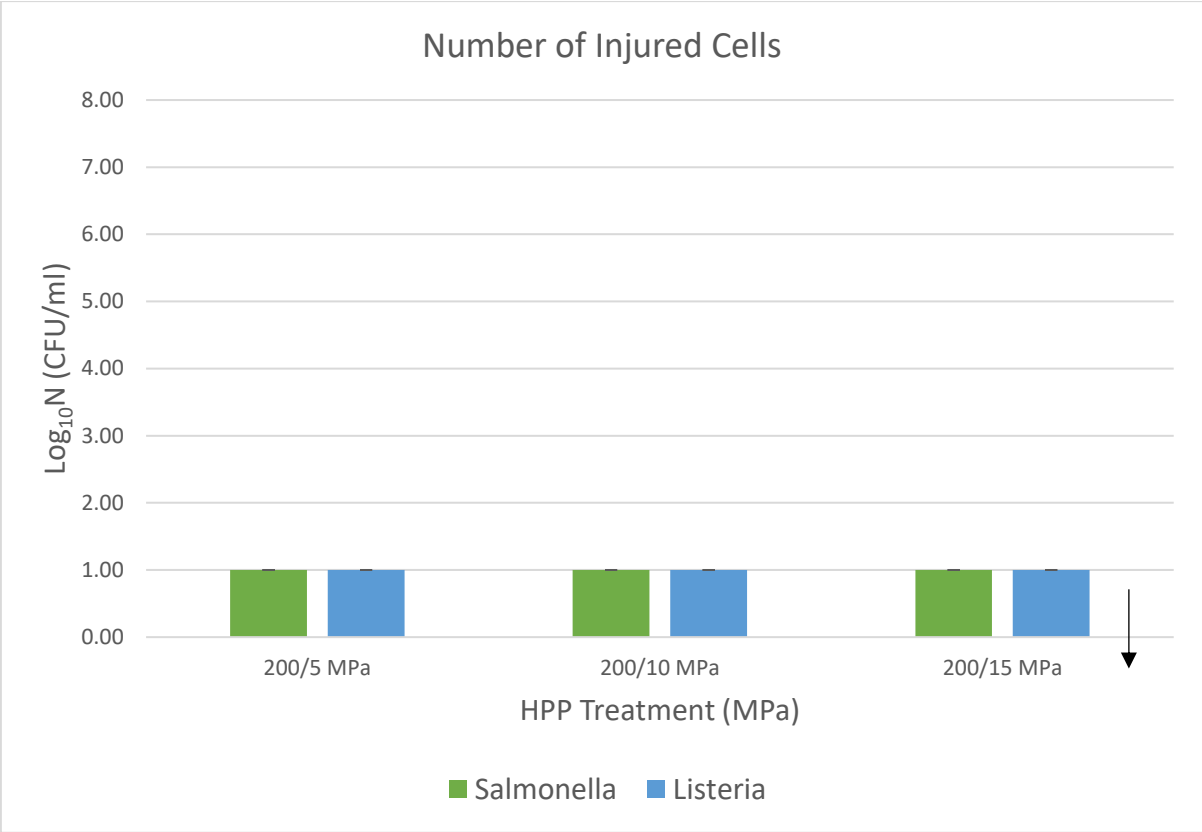

Figure 20. Injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in strawberry puree after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

5.6. Survival of *Salmonella* Hartford and *Listeria monocytogenes* after 200 MPa HHP treatment for different times in smoothie

Figures 21-23 show the survival of total cell count, non-injured, and injured cells of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in a smoothie after HPP treatment at the same pressure but for different lengths of time (200 MPa for 5, 10, and 15 minutes). At 200 MPa/10 min, *Listeria* had the highest survival rate for the non-injured cells (4.6 ± 0.187 CFU/mL), while the highest rate for the total cell count and injured cells was at 200 MPa/15 min (total cell count: 4.9 ± 0.075 log CFU/mL, injured cells: 4.7 ± 0.119 log CFU/mL). *Salmonella* had the highest survival rate for the total count and number of injured cells at 200 MPa/5 min and for the number of non-injured cells at 200 MPa/10 min (total cell count: 3.1 ± 0.680 log CFU/mL, non-injured cells: 1.6 ± 0.187 log CFU/mL, injured cells: 1.9 ± 1.661 log CFU/mL).

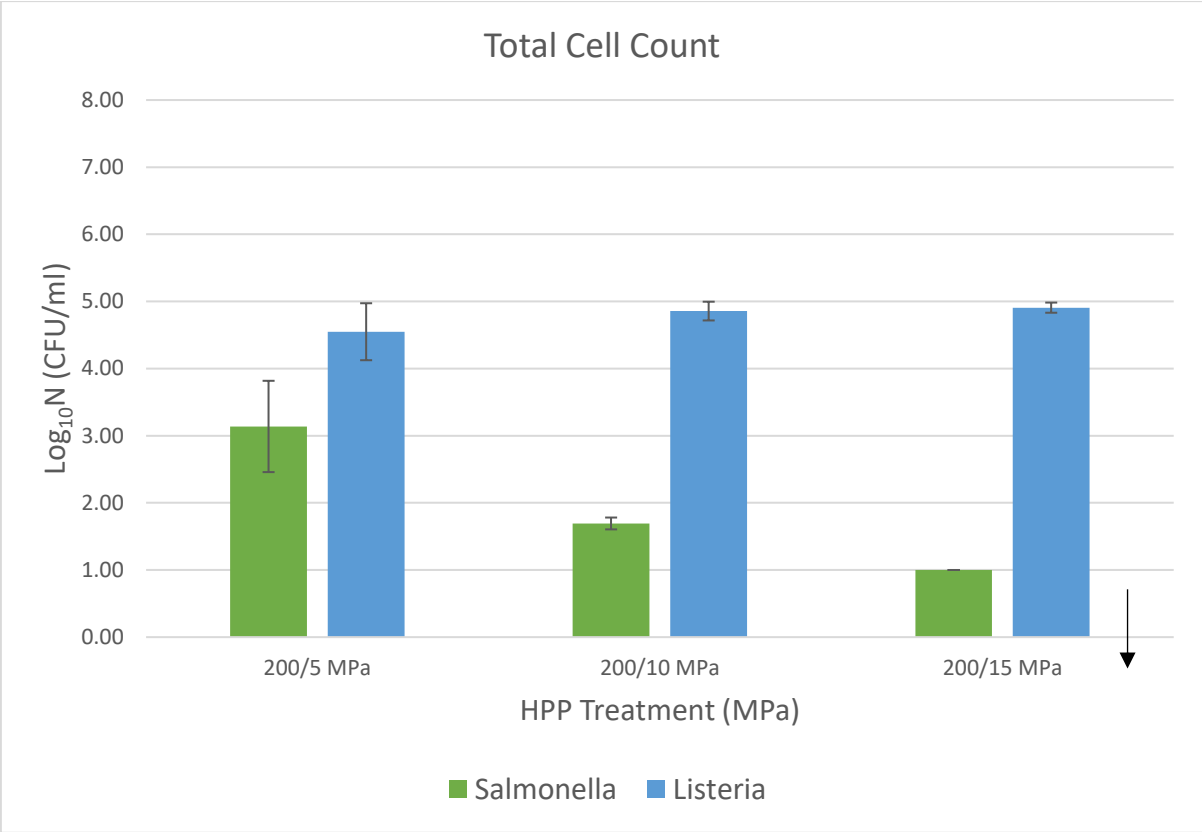

Figure 21. Total cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in smoothie after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

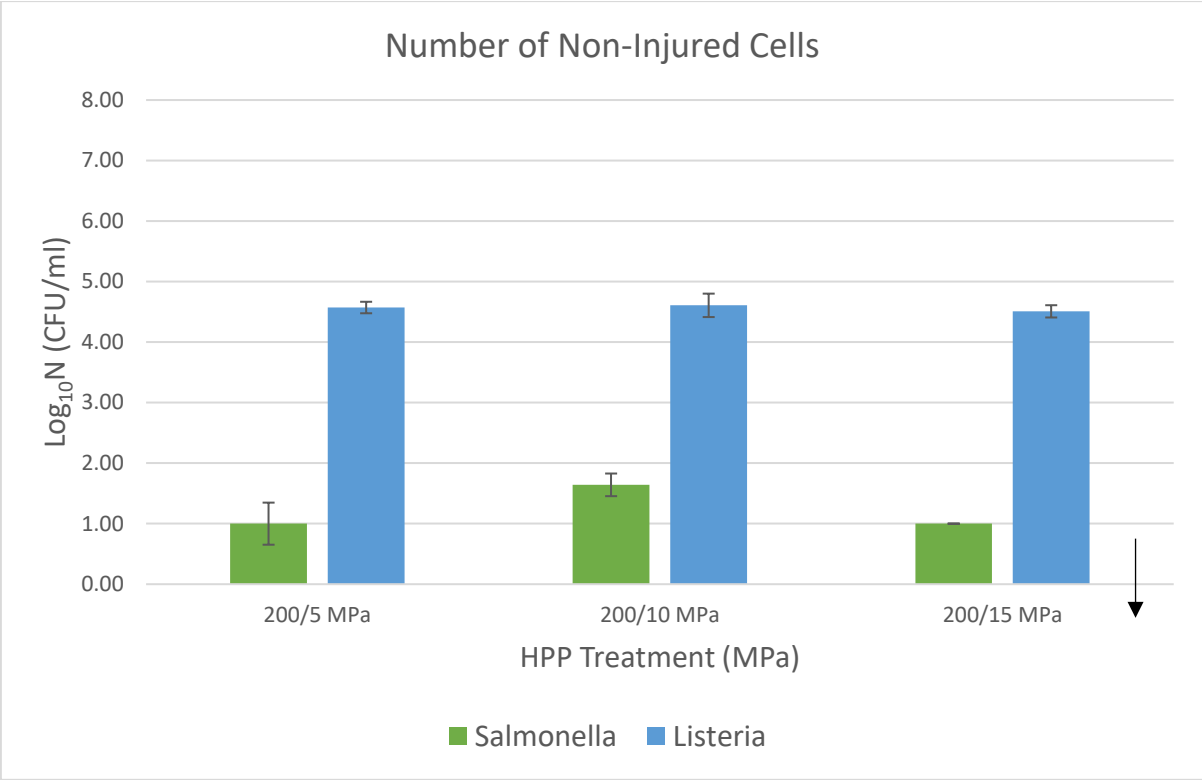

Figure 22. Non-injured cell count of *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in smoothie after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

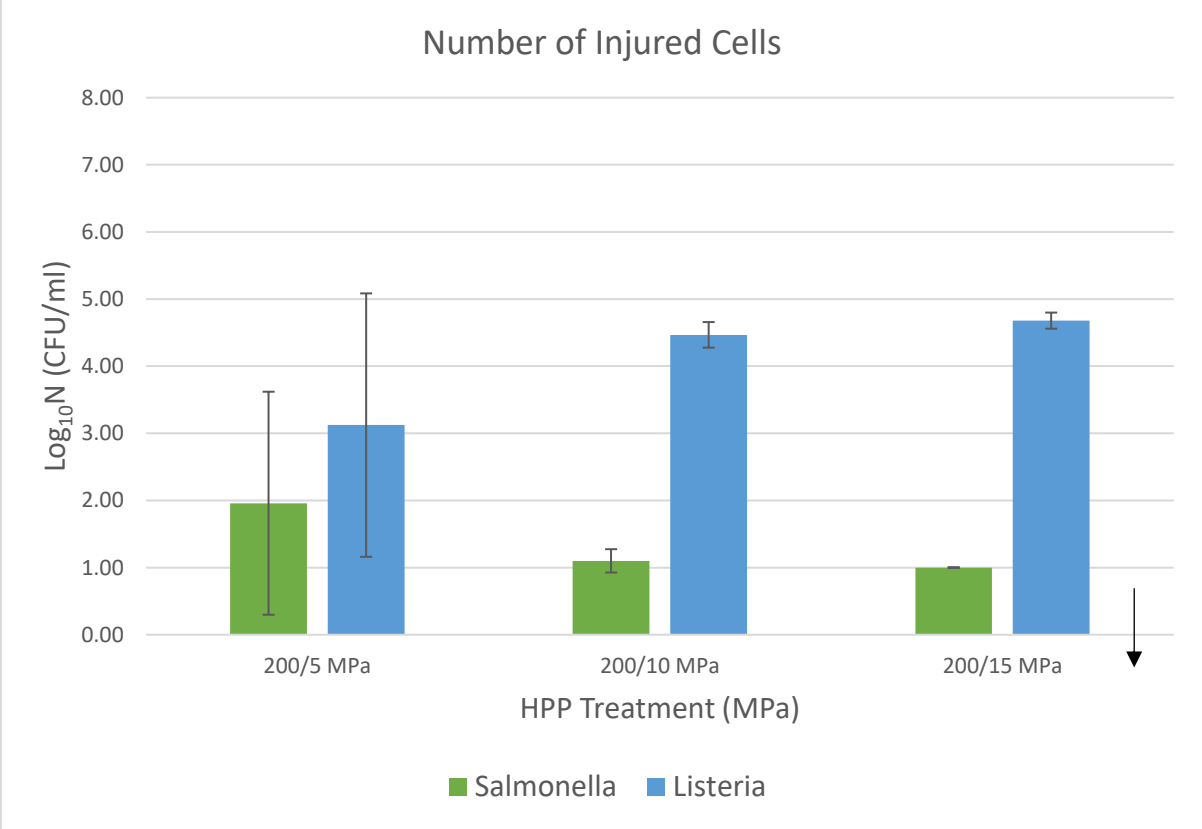

Figure 23. Injured cell count of *Salmonella enterica* serovar Hartford and *Listeria*

monocytogenes in smoothie after HPP treatment with different duration (arrows indicate a cell count below the detection limit)

In general, the results suggest that HPP treatment is effective in reducing the number of *Salmonella* and *Listeria* count in the samples, but the effectiveness of the treatment varies depending on the specific pressure used and the type of microorganism being targeted. The larger reduction in *Salmonella* counts compared to *Listeria* counts may be due to differences in cell structure and sensitivity to pressure.

Mostly the highest bacterial survival for both Salmonella and Listeria in Distilled water, Smoothie, and the strawberry puree was in a range of pressure that is between 150 MPa and 250 MPa. Listeria did not survive in Strawberry matrice, although this bacterial strain can grow and survive in a wide range of environmental conditions, including in food processing plants and refrigerated food products, it cannot grow in strawberry puree. There are a few reasons for this. First, strawberries have a low pH (around 3.0-3.5). However, it is important to note that while Listeria may not be able to grow in strawberry puree, the pathogen can still be present on the surface of the fruit if it has been contaminated during harvesting, processing, or handling. This is why it is important to follow proper food safety practices, such as washing and properly storing fruits and vegetables, to reduce the risk of foodborne illness.

When the pressure treatment was applied for a longer time (10 and 15 mins) its effectiveness was enhanced.

It is also important to note that the reduction in cell counts observed for each HPP treatment in this study may not be sufficient for complete inactivation of the microorganisms and further studies may be needed to determine the optimal HPP treatment conditions for complete microbial inactivation. Furthermore, the safety of foods treated with HHP technology can be increased by combining it with the change of other ecological factors, such as reduced storage temperature, low pH, and the addition of natural antimicrobial compounds.

Somolinos *et al.*2008, studied the Relationship between Sublethal Injury and Microbial Inactivation by the Combination of High Hydrostatic Pressure and Citral or tert-Butyl Hydroquinone. Where the *Listeria monocytogenes*, *Escherichia coli*, and *Saccharomyces*

cerevisiae were exposed to pressure from 200 to 400 MPa at different times (0.5 to 20 min). Results showed that the extent of inactivation and sublethal injury depended on the pH and the composition of the treatment medium. *Listeria monocytogenes* showed the greatest extent of sensitization at pH 4.0, where 6 log10 cycles of inactivation were achieved after 3 min at 300 MPa compared with 3 log10 cycles when the bacterium was pressurized at pH 7.0 for 20 min and the maximum proportion of sub lethally injured cells (99.99% of the survivors, equivalent to a 4-log difference in count on selective and nonselective media) was observed when *L. monocytogenes* was pressurized at pH 7.0 for 15 min at 300 MPa. Jordan *et al.* 2001 reported a five and two log cycle reduction in viable cell numbers of *L. monocytogenes* NCTC11994 in apple and orange juice, respectively after treatment at 500 MPa at 20°C for 5 min, Alpas and Bozoglu, (2003), carried out a study where the goal was to compare high- pressure resistance of *Listeria monocytogenes* strains at 25°C and 50°C at 350 MPa, as well as to use high pressure (250 MPa and 350 MPa) at 30°C and 40°C to inactivate the relatively most pressure resistant strain inoculated in pasteurized apple, apricot, cherry, and orange juices. *L. monocytogenes* was discovered to be the relatively most pressure-resistant strain and raising the pressure from 250 MPa to 350 MPa at 30°C resulted in an additional three to four-log cycle drop in viability, with viable cells remaining after 5 minutes. When 350 MPa was applied at 40°C for 5 minutes, the cell population of all fruit juices was reduced by more than eight log cycles.

Those findings accord with our results where *Listeria* did not grow in low pH matrices (Strawberry puree). Also, the smoothie needed less pressure treatment (250 MPa) for the destruction of all types of *Listeria* (total, injured, and non-injured cells) which can be due to the pH and composition of this food.

Gouvea et al., (2020), conducted research where they investigated the effects of high-pressure processing (HPP) on microbial inactivation in açaí juices with variable pH and soluble solids content (SSC). To investigate the effect of pH and SSC on the inactivation of *Salmonella* spp. by HPP, the pH of açaí juice samples was altered to a range of 4.0 to 5.5, and SSC was modified to a range of 2.9 to 14.9°Brix. With rising pH and SSC, HPP`s ability to deliver a 5-log reduction in the population of Salmonella spp. was diminished. The juices with pH 4.0 and 2.9°Brix showed ≥6-log reductions immediately after HPP, while the juice with 8.9°Brix showed a 5-log reduction. The juices (pH 4.0-14.9°Brix and pH 4.5-2.9°Brix) also showed a ≥6-log reduction in *Salmonella* spp. concentration after one week of

refrigerated storage (7 °C). These findings indicated that a less intense process (below commonly recommended commercial conditions - 600 MPa/3 min) could be used for açaí juice, ensuring required safety, as well as additional microbial inactivation verified during refrigerated storage.

6. Summary

High Hydrostatic Pressure (HHP) is a food preservation method that uses pressure to eliminate bacteria and enzymes in food. *Salmonella enterica* and *Listeria monocytogenes* are harmful bacteria that can cause foodborne illness, with *Listeria monocytogenes* being particularly dangerous due to its high mortality rate. This work aimed to determine the effectiveness of high hydrostatic pressure (HHP) treatment in reducing or eliminating *Salmonella enterica* serovar Hartford and *Listeria monocytogenes* in different food matrices to improve food safety. Additionally, the study sought to assess the extent of injured cells caused by the HHP treatment and optimize treatment parameters such as pressure level and treatment time.

In conclusion, high-pressure processing (HPP) is an effective method for reducing the number of Salmonella and Listeria in various food matrices, but the degree of effectiveness depends on the specific pressure used and the type of microorganism. Salmonella is generally more sensitive to pressure than Listeria, which may be due to differences in cell structure. However, in strawberry puree, Listeria did not survive despite its ability to grow and survive in a wide range of environmental conditions, likely due to the low pH of the puree. Longer pressure treatment times enhanced the effectiveness of HPP. It is important to note that the reduction in cell counts may not be sufficient for complete inactivation of microorganisms, and further studies are needed to determine optimal treatment conditions. Combining HPP with other ecological factors such as low pH and natural antimicrobial compounds can further increase food safety.

This work has certain limits with the duration and the means at our disposal, the number of bacterial strains used, and the limited variety of investigated foods. However, the data collected in the framework of this study constitute a source of information to be exploited.

Further study on the following points should be taken into consideration:

- The Synergic combination effect of HPP pressure treatment and other bacterial destructing treatments like heating, Citral or tert-Butyl Hydroquinone, pH adjustment of the smoothies, and strawberry puree on bacterial survival;
- Larger range of HPP duration treatment effect on the inactivation of *Salmonella* and *Listeria* strains on smoothies and strawberry puree;

- Investigation of the relation between food carriers' composition (biochemical and nutritional) on the microbial response to the HPP treatment;
- Exploration of the effect of HPP treatment on the nutritional composition of smoothies and strawberry puree foods.

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