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Enhancing Maternal Health: Cost-effective Alternatives in High Pressure Processing of Fruit Products

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Abstract

The search for efficacious Alternatives to the Current Standard Procedure in the Food Manufacturing Industry is a public health imperative. Recent advances and rapid adoption of high-pressure processing units in food manufacturing is gaining momentum across various industry sectors. The momentum of industrial importance across various sectors of food manufacturing, has led to requiring extensive microbiological hurdle validation studies for efficacious and feasible utilization of the technology. Fruit juices such as apple juice are an important component of maternal and child health diets. In regards to, microorganisms moving through vertical and horizontal gene transfer mechanisms, these systems work in the prevention of natural and anthropogenic pathogens of public health concern. As a result, adapting High Pressure Processing (HPP) in the inactivation of the pathogens is important. In this study, the protocol involves exposure to various times and intensity levels of elevated hydrostatic pressure. HPP is being used to identify the most effective conditions for vector (microorganism) inactivation. There was a large area of effectiveness of the HPP technology eradicating microorganisms in toxin-producing *Escherichia coli* O157:H7 (STEC), Non-O157 Shiga toxin-producing *Escherichia coli* (nSTEC), *Salmonella* serovars, and *Listeria monocytogenes.* Each were effectively eradicated utilizing this HPP technology. These specific microorganisms were selected as they are some of the most common ones that are involved in foodborne illness. Elevated hydrostatic pressure is a non-thermal procedure that exposes pathogens to pressures of up to 80,000 PSI (>550 MPa). Various times (3, 4, and 5 minutes) at pressure intensity levels of 600 MPa, (87K PSI), 550 MPa (79K PSI), 480 MPa (70K PSI), 415 MPa (60K PSI), and 345 MPa (50K PSI) of elevated hydrostatic pressure were investigated at 4°C and for 45°C. This research measured the effect of HPP treatment in eradicating the microbial load with nominal effects on organoleptic and nutritional quality of fruit juices. The results of this study provide support of cost effective and healthy fruit juice options to mothers and their children.

Keywords: High pressure processing, Nonthermal, Food safety, Apples, Consumer acceptability, Maternal health, Shelf life

Introduction

Humans in general have dietary and nutritional requirements based on a number of factors. However, women of child-bearing age, during pregnancy, women breast-feeding after birth, and infant care throughout their lives is of the highest importance and nutritional vulnerability is the highest [1]. Prior to conception, women should have well-balanced healthy diets to create a sustainable environment for the pregnancy journey. Some of the most common things that lack in women's diets during the pregnancy period include, iodine, iron, folate, calcium, and zinc; which causes a number of issues. Some of the issues caused by lack of vital nutrients includes, anemia, preeclampsia, hemorrhage, death in the carrying mothers, still births, low birthweight, wasting, and developmental delays [1]. Well-balanced meals include both the foods and beverages choices that are made by individuals. There are countless foods and beverages available to the general population, resulting in a constant battle involving healthy versus unhealthy choices.

The introduction and consumption of apple juice early into a person's diet provides an overall healthier dietary pattern [2]. This in comparison to those that consume sugar sweetened

beverages which lead to a less healthy diet, and increased obesity to name some of the many health conditions. Even though pediatricians go up until the age of 18; when apple juice is introduced as children are coming off of milk, it is very important to consider nutritional benefits and overall cost effectiveness. In terms of mother's choices, nutritionally apple juice does not equal milk, in some instances it is a more affordable beverage for mothers. Presently, the average price for a gallon of milk ranges between $5 – $9; which varies based on the brand purchased and the retail store it was purchased at. On the other hand, a gallon of apple juice ranges from $3 - $10; which varies based on brand of juice, retail store it was purchased at, and the quantity of fluid ounces available for purchase and consumption.

According to American Academy of Pediatrics, fruit juices do not provide nutritional benefits [3]. However, consumption of one hundred percent fresh fruit juices, and reconstituted fruit juice can be healthy for children over the age of one [3]. It is also important to consider the consequences of immoderate consumption of fruit juice which can lead to malnutrition, overnutrition, undernutrition, diarrhea, flatulence, abdominal distention, and tooth decay. It is important to consider recommended limitations for the consumption of fruit juices according to the American Academy of Pediatrics emphasizes that fruit juices do not provide nutrition for infants. When considering 100% fruit juices, they lack fiber; which is a critical component in nutrition. The recommendations for consumption of juice for children ages 2-3 is no more than 4 ounces per day; and no more than 4–6 ounces for ages 4-5 [4]. A person's diet is providing a direct connection to human disease alleviation. Apples are one of the most prominent fruits throughout the world because of their rich nutritional value. The healthy benefits of apples include cardiovascular disease, cancers (prostate, breast, colorectal, liver) [5]; and chronic diseases which are global health burdens [6]. Numerous techniques are implemented to understand both nutritional and biochemical effects on analyses of diseases [7]. There are a vast number of resources specifically dedicated to this topic to include, the Maternal and Child Health Bureau of the federal government, the Association of State Public Health Nutritionists, United Nations Children's Fund, World Health Organization, and several other organizations. Apples have been used in several research studies due to their adaptability in different environmental conditions with different pathogens. The pathogens used in this study have historical significance in the food and beverage industries. The pathogens of concern used in this study included, *Salmonella*, Shiga-toxin producing *E. coli O157:H7* (STEC), Shiga-toxin producing *E. coli* non-O157:H7 (nSTEC), and *Listeria monocytogenes*.

Foodborne *Salmonella* infections are a significant public health concern in the United States and also worldwide. There are a number of factors that contribute to food poisoning, however; in many cases it is caused by different bacteria that can affect both high risk and healthy individuals. An article from 2023 noted that around 40% of foodborne illness outbreaks are caused by sick workers. From 2017 to 2019, *Norovirus*, *Salmonella*, and *E. coli* were behind 800 outbreaks across 875 restaurants and 25 health department reports [8,9]. Food workers reported working while sick and serving about 300 meals a day, even though they were dealing with vomiting and/or diarrhea [8]. Food workers gave several reasons for why they worked while sick. Some of these included not wanting to risk making others sick because they didn't feel too bad, leaving their workplace short-staffed, fear of losing their job, and not being paid while they're off. Even though 85% of restaurants had policies to prevent sick staff from working, and about 16% required workers to stay home if they had symptoms like vomiting, diarrhea, sore throat, or fever, these policies weren't always followed [8,9]. In reference to high-risk individuals, they are a population termed YOPI; which stands for the Young, Old, Pregnant, and Immunocompromised [10]. *Salmonella* causes approximately 1.35 million infections; resulting in approximately 420 deaths, around 26,500 hospitalizations annually, and food is the most common source [11]. Shiga-toxin producing *E. coli* infections in the United States annually are approximately 265,000 resulting in outbreaks and serious illness [12]. STEC infections are responsible for about 3,700 hospitalizations and 30 deaths annually in the United States [13,14]. Non-O157 Shiga toxin-producing *Escherichia coli* causes approximately 219,000 infections annually [15,16]. *Listeria monocytogenes* is a psychotropic "cold loving" bacterium that is typically associated with luncheon (deli, cold cuts, sandwich) meats. Since this bacterium is psychotropic, it can proliferate to menacing levels in suitable refrigeration conditions [17,18] *L. monocytogenes* has the capability to enter a vast number of different mammalian cell types. *Listeria* (Listeriosis) causes around 1,600 infections, and an estimated 260 result in death to their illness [19]. Also, when addressing foodborne illness is also important to consider life quality after illness in some immunocompromised individuals. The quality-of-life values are in short referred to DALY (Disability-Adjusted Life Year) and QALY (Quality-Adjusted Life Year). They assist in the public health burden calculations; which are two significant factors. Scallan and colleagues in 2011 and Hoffman and colleagues in 2012 provided pathogens of top public health concern [13,20]. Due to the potential presence of these pathogens in food and beverages, between the farm and fork of the consumers; it is important to consider technologies that will eradicate them. Eradicating will eliminate the presence of pathogens while also providing nutritious, appealing, and tasteful products for consumption. Then, there is a choice of thermal and non-thermal technologies and weighing the risks between the two. The technology most suitable for this study was the use of High-Pressure Processing (HPP).

This research study attempted to address some gaps in other research studies, since pressure treated products are slightly

more expensive than traditional commodities for the current standard treatment 600 Megapascal (MPa) (87K Pounds Per Square Inch (PSI)) for 3 minutes. This study explored effective low- pressure and low-cost alternatives to current high pressure processing practices in the market; cost-effective and safe fruit products that can be used in other fruit products for mothers and young children. Presently, the units are designed for commercial use, however; over time they have the potential to be small enough for women to use them in their homes. In their homes they can be used in eradicating harmful bacteria in both foods and beverages for themselves and their children. Women, maternal care, and consumers in general require safe food and beverage products; and HPP provides a great option for microorganism disinfection while retaining natural taste, aroma, and appearance. Currently, there are not any publications that link the impact of HPP correlated with women and maternal health. HPP assists in bridging the gap by providing safe and cost-effective options for juice products for women and their children. The objectives of this study, were to demonstrate the potential use of low-cost effective mechanisms for the production of safe fruit products; as a nutritional source product for maternal and child populations.

Materials and Methods

Historical background of high-pressure processing (HPP)

Significant use of high-pressure dates back to 1899 when Bert Hite, a researcher in an Agriculture Research Station in Morgantown, West Virginia [21] used the application. Hite's intention in using this technology was to pasteurize milk as well as other food products [22]. In the year 1914, Hite demonstrated results that fungus (yeasts) and lactic acid microbes are both demanding constituents in sweetened, fully developed fruit were extra sensitive to pressure alternatively to living things, specifically endospore-forming bacilli correlated alongside of vegetables [22]. HPP can be managed in circulating (ambient) or refrigerated temperatures, which allows for the eradication of thermally originated cooked abnormal flavors that may be the result of other sterilization processes [23].

HPP utilizes water as its medium as it transfers water between pressures ranging from 100 through 1,000 MPa in a confined vessel [24]; in which the outcomes are food products with bacterial devaluation and elongated shelf-life [23]. There was a total of five major components that collectively form the HPP unit, which include a pressure vessel, two end closures, yoke, a pressure-creating device, and instrumentation and controls (pressure measurement, temperature measurement, flow measurement, and level measurement) [24]. HPP appeases inimical pathogenic and vegetative spoilage bacterium alongside of specifically chosen catalysts [24]. The HPP processing temperatures range from 0 to 100 °C [24]. A schematic of the HPP unit used in this study is displayed below in **(Figure 1)** as used in [25]. One of the driving forces behind this research study, was prior HPP studies from other colleagues in meats (ground meat and meat homogenate) treatments between 250 – 650 MPa [26] and beverages (orange juice and raw milk) [25,27].

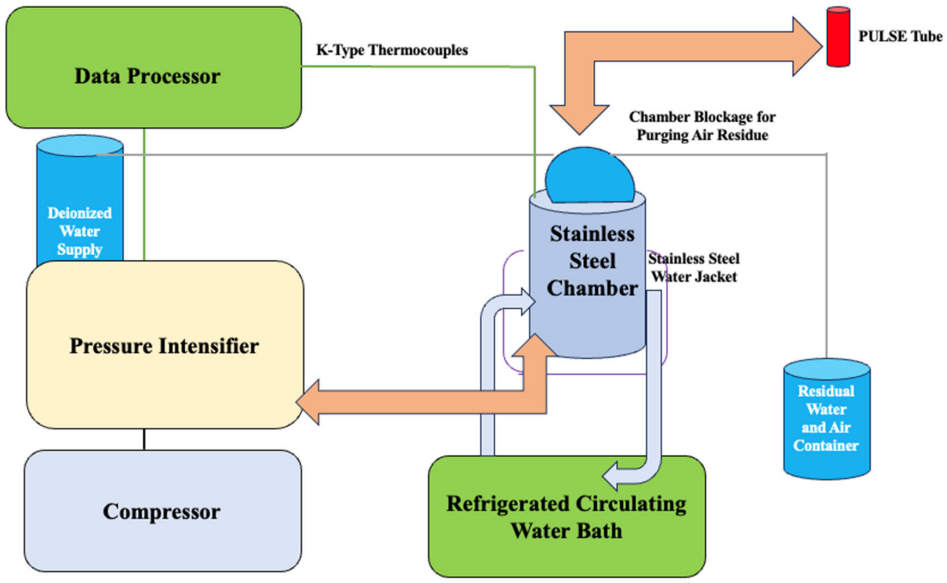

Figure 1. This diagram shows the high-pressure processing unit used in the study. It includes a Barocycler Hub440 that applies different time, temperature, and pressure settings. Apple cider samples were inoculated with various bacteria like Shiga toxin-producing *E. coli* O157, non-O157 *E. coli*, *Salmonella*, and *Listeria*. Each sample was put into individual Barocycler PULSE tubes for treatment. Only one PULSE tube was processed at a time. This diagram setup is based on [25], with a few minor adjustments.

HPP mechanisms and microbial inactivation

Mechanisms and factors affecting inactivation: HPP functions in initiating bacterial cell changes to include, key enzymes and protein synthesis inhibition, cell morphology alterations, microorganism's genetic mechanisms (transcription, translation), cellular function disruption [28-34]. The mechanisms and factors that surround HPP provide beneficial information into its effectiveness. The mechanisms of HPP include cell membrane disruption, protein denaturation, nucleic acid damage, and reversible and irreversible effects. An elaboration of each of the following mechanisms terms, follows **(Table 1)**. Additionally, there are several factors that affect the HPP inactivation process and they include, Pressure Levels, Temperature, Time, Food Matrix, Type of Pathogen, and pH and Water Activity. See **Table 2** for detailed explanations of each mechanism.

Table 1. This table details how HPP affects microorganisms by disrupting cell membranes, denaturing proteins, damaging nucleic acids, and causing both reversible and irreversible changes. These processes collectively demonstrate the mechanisms of HPP.

Mechanisms	Definition	References
Cell Membrane Disruption	HPP primarily affects microbial cells by causing changes in their cell membranes. High pressure can alter the permeability of the cell membrane, leading to leakage of cellular contents and eventually cell death	[35]
Protein Denaturation	Pressure can denature proteins, including those involved in vital cellular functions. When pressure is applied, protein structures are altered, disrupting enzyme activities and other cellular processes essential for microbial survival	[28,35]
Nucleic Acid Damage	High pressure can also affect nucleic acids (DNA and RNA). Pressure can induce conformational changes in these molecules, leading to the inhibition of replication and transcription processes	[35]
Reversible and Irreversible Effects	The impact of pressure can be reversible or irreversible depending on the pressure level and duration. Lower pressures might cause reversible changes, while higher pressures generally lead to irreversible damage	[35,36]

Table 2. This table shows the key factors influencing the HPP inactivation process, including pressure levels, temperature, processing time, food matrix, type of pathogen, and pH and water activity.

Factors	Definition	References
Pressure Levels	The effectiveness of HPP increases with pressure. Typical pressures used in HPP range from 100 to 600 MPa. Higher pressures are generally more effective at inactivating a wider range of pathogens, but they also increase the risk of altering the food's texture and nutritional content.	[35,36] [37,38]
Temperature	Although HPP is primarily a non-thermal process, temperature still plays a role. Elevated temperatures can enhance the effectiveness of HPP by increasing the rate of biochemical reactions and protein denaturation. However, temperature control is crucial to avoid undesirable changes in food quality.	[35,39]
Time	The duration of pressure application affects microbial inactivation. Longer times at high pressures can lead to more effective pathogen destruction. However, excessively long processing times might negatively impact the food's sensory and nutritional properties.	[36]
Food Matrix	The composition and structure of the food matrix influence HPP effectiveness. For example, foods with high fat content or complex structures may provide protection to microorganisms, making them more resistant to pressure.	[35,36]
Type of Pathogen	Different pathogens have varying levels of resistance to pressure. Vegetative cells of bacteria (e.g., *Listeria*, *E. coli*) are generally more susceptible than spores (e.g., *Clostridium botulinum*), which require higher pressures or longer processing times for inactivation.	[40,41]
pH and Water Activity	The pH and water activity of the food product can also impact HPP effectiveness. Lower pH and high-water activity generally make microbial cells more susceptible to pressure.	[42,43]

It is also important to consider, Temperature, Pressure, and Time Context when considering HPP technology. More information regarding these topics will be addressed below. Low Pressure, Short Time: This combination might not be sufficient for inactivating certain pathogens, especially spores or more resilient microorganisms. It may be effective for mildly contaminated foods or non-pathogenic microorganisms [44]. Moderate Pressure, Moderate Time: Commonly used in many HPP applications, this balance can inactivate a broad range of pathogens while minimizing changes to food quality [45]. High Pressure, Long Time: This approach is highly effective against a wide range of pathogens, including heat-resistant spores. However, it might cause significant changes in food texture, flavor, and nutritional content [46, 47]. In summary, HPP's effectiveness in pathogen inactivation depends on a complex interplay of pressure levels, temperature, time, food matrix, pathogen type, and other factors. Understanding these variables helps in optimizing HPP conditions for different food products while maintaining their quality [28,42].

HPP treatment justification

Optimal pressure-time-temperature justifications: The combination of 600 MPa pressure and a 3-minute time parameter is the HPP industrial standard. The choice to use this time-pressure justification is for a number of factors; however, the most important are, pathogen inactivation, nutrient retention, texture and flavor preservation, and efficiency and practicality. See **Table 3** for detailed explanations of each mechanism.

The choice of using 550 MPa for 4 minutes in the High-Pressure Processing industry is influenced by a balance of factors including product preservation and specific product needs. Below are some parameters that justify the use in this research study. Microbial Safety: 550 MPa is still a sufficiently high pressure to inactivate a wide range of microorganisms, including bacteria, yeasts, molds, and viruses. The extended time of 4 minutes compensates for the slightly lower pressure compared to 600 MPa, ensuring that the microbial safety standards are met [36,54]. Nutrient and Quality Preservation: Lowering the pressure to 550 MPa and extending the processing time to 4 minutes can help preserve the integrity of heat-sensitive nutrients, flavors, and textures. This approach can be particularly useful for products where maintaining these quality aspects is critical [55-57]. Operational and Economic Considerations: Equipment and processing conditions might be optimized for different pressure and time settings based on practical considerations, including energy consumption, equipment wear, and cost-effectiveness [58].

The use of 480 MPa for 4 minutes in HPP technology is a strategic choice in research studies. It is selected to achieve a balance between microbial safety, product quality, and operational efficiency. This parameter setting is tailored to specific needs of the food product, aiming to provide effective preservation while considering practical aspects of processing. Below is more specific information regarding this specific pressure and time combination was chosen for this research study. Effective Microbial Inactivation: 480 MPa is lower than pressures like 600 MPa or 550 MPa but is still effective

Table 3. The table outlines the effective HPP settings of 600 MPa for 3 minutes, which inactivate a broad range of microorganisms, including bacteria, yeasts, molds, and viruses, ensuring food safety without high temperatures. This process also preserves nutritional quality by minimizing nutrient degradation, maintains texture and flavor, and offers a practical balance of efficiency, equipment capability, and product quality suitable for various food and beverage types.

Industry Standard Optimal Pressure-Time Justification Parameters	Definition	Pressure and Time	References
Pathogen Inactivation	600 MPa is high enough to inactivate a broad range of microorganisms, including bacteria, yeasts, molds, and viruses, thereby ensuring food safety. This pressure is sufficient to achieve the desired level of microbial reduction without the need for high temperatures.	600 MPa, 3 minutes	[44,47]
Nutrient Retention	Using HPP at 600 MPa for about 3 minutes helps to preserve the nutritional quality of the food. The relatively short processing time at this pressure helps to minimize the degradation of sensitive nutrients like vitamins and antioxidants.		[48,49]
Texture and Flavor Preservation	HPP at this pressure and time minimizes changes in texture and flavor compared to other preservation methods. This is particularly important for maintaining the sensory qualities of fresh-like products.		[50,51]
Efficiency and Practicality	The combination of 600 MPa and 3 minutes is effective for many types of food and beverages. It strikes a practical balance between processing efficiency, equipment capability, and product quality.		[52,53]

at inactivating a wide range of microorganisms, including bacteria, yeasts, molds, and viruses. The extended processing time of 4 minutes compensates for the lower pressure, ensuring that microbial safety standards are met [35,59]. Preservation of Quality: Lower pressures, such as 480 MPa, can be less harsh on the texture, color, and flavor of certain foods. The 4-minute processing time helps ensure that these quality attributes are maintained while still providing sufficient microbial reduction [39,60]. Energy and Equipment Efficiency: Lower pressure settings can reduce energy consumption and wear on equipment. For some products, 480 MPa might be a more economical choice, balancing effective preservation with cost-efficiency in terms of energy and operational costs [58]. Target Microbial Load: Some products might have a lower initial microbial load, allowing for effective inactivation with lower pressure and longer time. This can be particularly useful for products with less aggressive microbial challenges [61,62].

415 MPa for 5 minutes in HPP represents a specific choice designed to meet particular processing and quality objectives. 415 MPa for 5 minutes in HPP is chosen to effectively inactivate microorganisms while preserving the sensory and nutritional qualities of the food product. This parameter setting offers a balance between safety, quality, and operational efficiency, tailored to meet the specific requirements of different food products. Microbial Safety: At 415 MPa, microbial inactivation is still achievable, though it might be less intense compared to higher pressures. The longer duration of 5 minutes compensates for the lower pressure, ensuring effective reduction of pathogens and spoilage organisms while meeting safety standards [63,64]. Quality Preservation: Lower pressure settings like 415 MPa can be gentler on the food's texture, color, and flavor. The extended processing time helps ensure that these quality attributes are preserved while still achieving effective microbial control. This can be particularly important for products that are sensitive to pressure-induced changes [65,66]. Economic Considerations: Processing at 415 MPa requires less energy compared to higher pressures. This can result in cost savings in terms of energy consumption and reduced wear on equipment, making it a more economical choice for certain applications [67]. Flexibility and Optimization: This combination allows for flexibility in processing different types of food products. It's a compromise between achieving effective microbial inactivation and maintaining product quality, particularly when dealing with products that have varying sensitivities to pressure [68].

Using 345 MPa for 5 minutes in HPP is chosen to provide effective microbial inactivation while minimizing impact on product quality and operational costs. It is a practical choice for products that require a balance between safety, quality, and cost-effectiveness [69]. Microbial Inactivation: At 345 MPa, microbial inactivation is still effective, though it may be less aggressive than at higher pressures. The extended time of 5 minutes compensates for the lower pressure, ensuring sufficient reduction of pathogens and spoilage organisms, although it might be used for products with less stringent microbial control needs [70,71]. Product Quality: Lower pressures like 345 MPa are often gentler on food products, helping to preserve texture, flavor, and color more effectively than higher pressures. The longer processing time of 5 minutes helps to ensure that these quality attributes are maintained while achieving effective microbial control [72,73].

Energy and Cost Efficiency: Processing at 345 MPa consumes less energy and places less stress on equipment compared to higher pressures. This makes it a more cost-effective option, particularly for large-scale or economically sensitive production runs [74]. Operational Flexibility: This pressure and time setting provides a good balance between microbial inactivation and preserving product quality. It allows for flexibility in processing a range of food products that might be sensitive to higher pressures [75].

Temperature justifications in HPP: In HPP, temperatures like 4°C and 45°C are used to optimize the effectiveness of the process and maintain the quality of the food product [75]. Why these specific temperatures were chosen: *4°C (Refrigerated Temperature)* is important for three major factors, preservation of quality, microbial load management, and food safety.

- Preservation of Quality: 4°C is a common temperature used for refrigeration. At this temperature, food products are kept cool, which helps in preserving their freshness, color, texture, and nutritional value during the HPP process [76].
- Microbial Load Management: Lower temperatures can reduce the microbial load prior to HPP by slowing microbial growth and activity. This can make the high-pressure treatment more effective by ensuring that the microorganisms are less active or in a more stable state [76, 77].
- Food Safety: Pre-cooling the product to 4°C ensures that the food remains in a stable state during processing, minimizing any risk of spoilage or quality degradation [28, 77].

45°C (Moderate Temperature) is important to consider three major factors, Enhanced Microbial Inactivation, Optimal Processing Conditions, and Improved Efficiency.

- Enhanced Microbial Inactivation: At moderate temperatures like 45°C, the effectiveness of HPP can be enhanced. While HPP itself does not rely on heat, the combination of pressure and temperature can improve the inactivation of certain microorganisms and enzymes. This can be particularly useful for foods that are more resistant to pressure alone [78].
- Optimal Processing Conditions: For some products, maintaining a temperature of around 45°C can help achieve the desired balance between microbial safety and

product quality. It can ensure that the food is processed efficiently while still preserving its taste, texture, and nutritional content [78].

- Improved Efficiency: Heating the product to 45°C can help in reducing the time required to achieve microbial inactivation. This can make the overall processing more efficient and economical, especially for high-throughput systems [47].

Bacteria strains and methodology

Five strain habituated *Salmonella* serovars (ATCC® numbers 13076, 8387, 6962, 9270, 14028); four strain habituated *Listeria monocytogenes* serovars (ATCC® numbers 51771, 51779, 13932, and 20011L2625); six strain habituated Shiga Toxin-producing *Escherichia coli* serovars (ATCC® numbers BAA 2196, 2193, 2192,2219, 2215, and 2440); and *Escherichia coli* Non O157 serovars (ATCC ® numbers O26, O45, O103, O111, O121, and O145) will be used for inoculation of apple juice. The bacterial strains are Centers for Disease Control and Prevention (CDC) outbreak strains purchased from American Type Culture Collection (ATCC).

For each strain, a loopful from frozen glycerol stock was aseptically transferred into 10 ml Tryptic Soy Broth plus 0.6% yeast extract (TSBYE) (Difco, Becton, Dickinson and Company, Sparks, Md.), (BeanTown Chemical), and then incubated 20-24 hours at 37°C. One loopful of the above-mentioned overnight suspension was streak plated onto the surface of Tryptic Soy Agar plus 0.6% yeast extract (TSAYE) (Difco, Becton, Dickinson and company, Sparks, Md.), and incubated at 37°C (degrees Celsius) for 24 hours. The plates were stored up to a month at 4°C prior to the experiment. Five days prior to the experiment, each strain was activated by culturing a single colony from the above-mentioned plates stored at 4°C into 10 ml TSBYE, after incubation at 37°C for 24 hours. A 100 µl (microliter)aliquot will be sub-cultured into 10 ml TSBYE and incubated at 37°C for 24 hours.

Cells were harvested using centrifugal force at 5,000 Revolutions Per Minute (RPM) (5424 x g) (gram) for 15 minutes. After removal of supernatant, in order to remove sloughed cell components, excreted secondary metabolites, and growth media, the cells were washed with 10 ml Phosphate Buffer Saline (PBS), Potential of Hydrogen (pH) 7.4; 0.2 g/L (grams per liter) KH_2PO_4 (Potassium Dihydrogen Phosphate), 1.5 g/L Na_2HPO_4 (Sodium Phosphate Dibasic Dihydrate). $7H_2O$ (Water), 8.0 g/L NaCl (Sodium Chloride), and 0.2 g/L KCl(Potassium Chloride)), and recentrifuged using the above-mentioned time and intensity, and resuspended in 10 mL (milliliter) of Apple Cider. After removal of supernatant, to improve the external validity of the challenge study, prior to experiment, each strain was individually habituated in sterile apple juice for 72 h (hours)at 4°C to allow acclimatization of the pathogen to low temperature and intrinsic factors of the food [79]. For the experiment regarding background microflora, a product without any thermal or non-thermal treatment will be used. The five habituated strains of *Salmonella*, four habituated strains of *Listeria*, six STEC strains, and six habituated strains of *E. coli* non-O157 will then be combined, on the day of the experiment and used as inoculum to conduct the microbiological challenge study.

HPP treatment: The Barocycler Hub440, a machine from Pressure Bioscience Inc. (South Easton, MA, USA), was used to apply different pressure levels (ranging from 50,000 to 87,000 PSI or 345 to 600 MPa) to apple cider samples to eliminate pathogens. The apple cider was placed in a 40 mL chamber, which was temperature-controlled by a stainless-steel water jacket connected to a circulating water bath (Model Refrigerated 1160s) from VWR International (Radnor, PA, USA). Water was used as the pressure transmission fluid in this experiment, and the time it took to increase and release the pressure was not included in the reported pressurization time values. The pressure levels and pressurization times were set manually. The vessel containing bacteria and the food sample (the PULSE tube) was subjected to pressure treatment at different intervals of time, pressure, and temperature. Each experiment was performed twice per treatment. The challenge experiments were carried out in PULSE tubes (up to 2 mL capacity), where the apple cider samples with inoculated pathogens were precisely pressurized at a controlled temperature. The internal pressure, temperature, and compression rate were monitored every 3 seconds using the Barocycler HUB 2.3.11 software from Pressure BioScience Inc. Temperature regulation and monitoring were achieved using k-type thermocouple sensors from Omega Engineering Inc. (Norwalk, CT, USA), which were housed within the chamber wall with thermal paste (Model 5 AS53.5G) from Arctic Silver (Visalia, CA, USA), and connected to the HUB PBI 2.3.11 software.

Methods

Microbiological and pH analyses: The pressurized and control cell suspensions in the PULSE tubes were neutralized using D/E neutralizing broth (Dey-Engley Neutralizing Broth) (Difco, Becton Dickinson), then diluted in a series with 0.1% Maximum Recovery Diluent (MRD) (Difco, Becton Dickinson). The samples will be spread on TSAYE plates to help recover any injured cells, and to count the total aerobic bacteria and *Salmonella* serovars after incubating at 37°C for 48 hours. The non-selective medium is designed to help recover injured cells based on initial tests conducted in the lab.

The pH of the samples was measured at the time of inoculation using a digital pH meter (Mettler Toledo, AG, Switzerland). The experiment was carried out in two separate microbiological replicates, with each serving as a blocking factor in a randomized complete block design. Each block included three independent repetitions for each time/temperature/pressure combination. The data, including the

calculation of CFU/mL and Log CFU/mL, was analyzed using the SAS GLM procedure with Tukey- and Dunnette-adjusted ANOVA, and results will be presented using Microsoft Excel. The statistical analyses was done using the general linear model and mixed procedures in SAS 9.4 software (SAS Inst., Cary, N.C.), with a type 1 error level of 5% (α = 0.05).

Results

Low and moderate temperatures for Shiga toxin-producing *E. coli* O157:H7

In **Figure 2**, the temperature was 4°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The amount of *E. coli* O157:H7 was significantly reduced across all treatments. The most effective reduction was observed at the 345 MPa pressure for 5 minutes, resulting in a 0.86 Log CFU/mL. All treatments at 4°C led to substantial reductions in the *E. coli* O157:H7 counts; which demonstrated the effect of temperature on the pathogen treatment. Higher pressures such as 600 MPa effectively reduced *E. coli* O157:H7 to very low levels; however, at lower pressures such as 345 MPa were more effective. This demonstrated the effectiveness of HPP treatment on *E. coli* O157:H7. Lower pressures and longer treatment time intervals were very effective in reducing *E. coli* O157:H7. In **Figure 3**, the temperature was 45°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The *E. coli* O157:H7 counts were reduced but the effectiveness varied differently in comparison to the 4°C results. The smallest bacterial count was observed at 345 MPa at 5 minutes, which resulted in 2.02 Log CFU/mL. The effectiveness of the pressure and temperatures at 45°C demonstrated an overall reduction; however, at higher temperatures the treatments were less effective. Even though there was significant reduction in *E. coli* O157:H7; the treatments did not reduce the bacterial counts as in 4°C. Both **Figures 2** and **3** show that HPP can reduce *E. coli* O157:H7 counts, the reductions are more significant at the lower temperature (4°C) across all pressure levels. Therefore, to maximize the reduction of *E. coli* O157:H7 in apple cider, it would be better to use lower temperatures combined with high-pressure treatments. The temperatures before and after the treatment temperatures did not change much. The pH measured how acidic or basic the cider was. The pH levels pretty much stayed the same prior to and post treatments at both temperatures. The untreated cider contained around 8.01 on a logarithmic scale of harmful *E. coli* O157:H7. Higher pressure temperatures, especially at lower temperatures (4°C), are more effective at eradicating *E. coli* O157:H7 bacteria in apple cider. Both temperature and pressure play critical roles in how effective these treatments can reduce harmful bacteria.

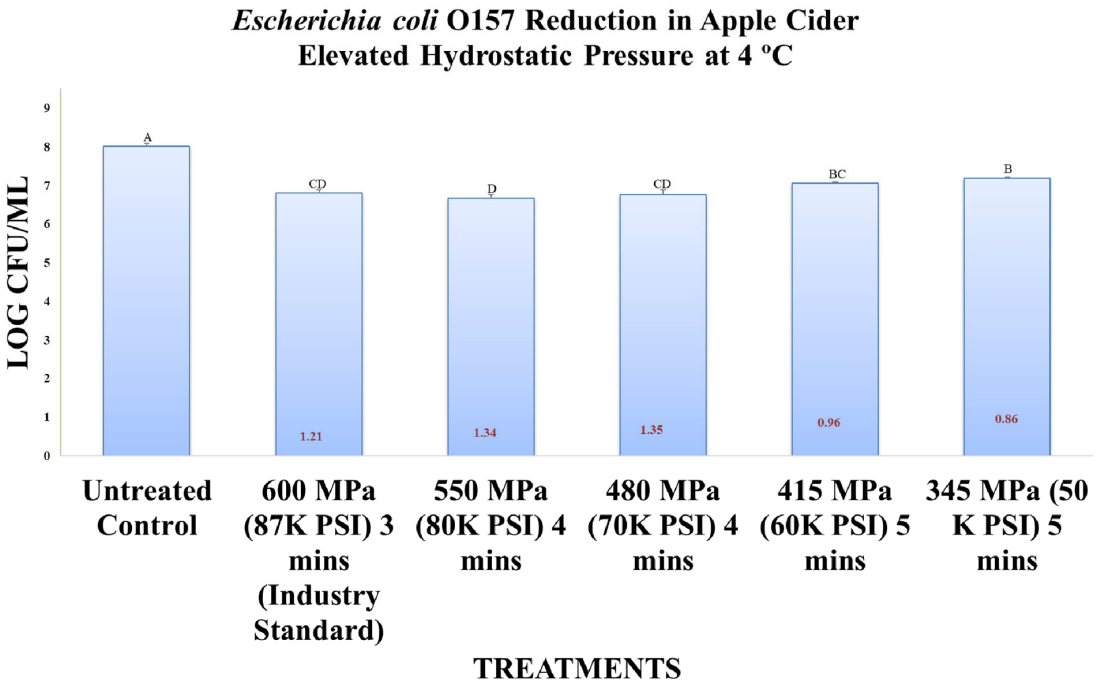

Figure 2. This graph presents the effect of different treatments using elevated hydrostatic pressure on the reduction of *Escherichia coli* O157:H7 in apple cider at a temperature of 4C. The y-axis represents the *Escherichia coli* O157:H7 count in terms of the logarithm of colony-forming units per milliliter (Log CFU/mL), while the x-axis shows the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

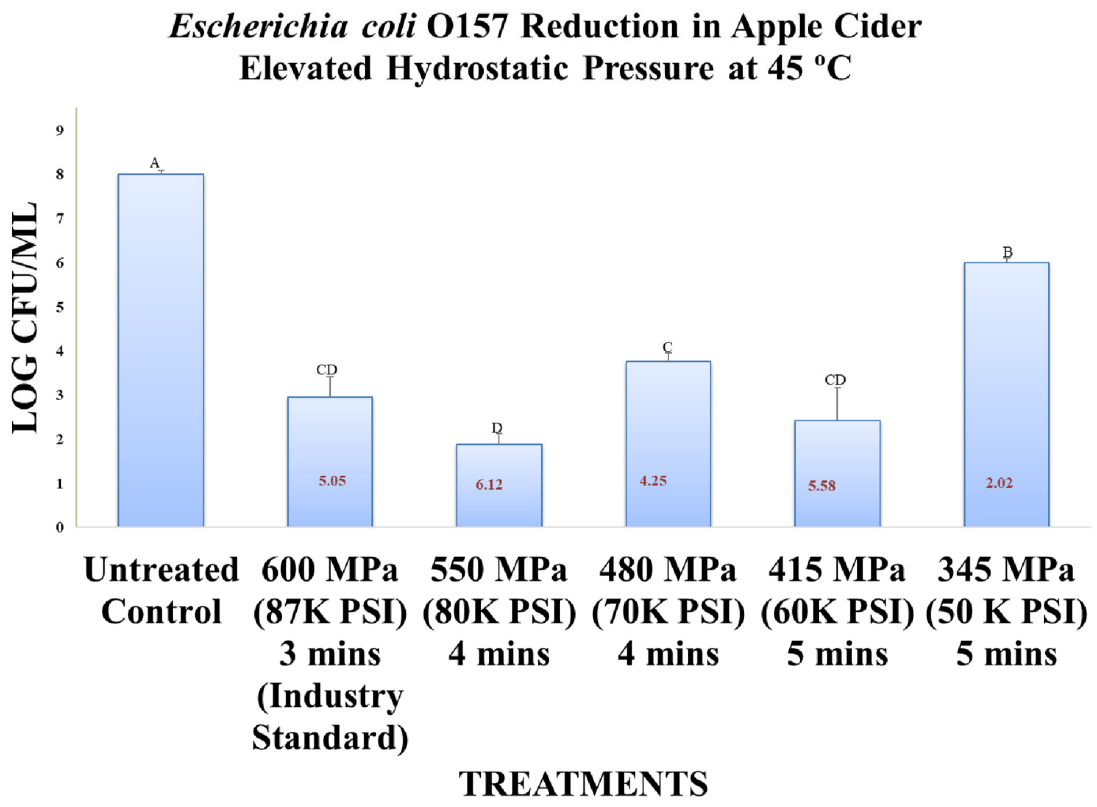

Figure 3. This graph shows the effect of different treatments using elevated hydrostatic pressure on the reduction of *Escherichia coli* O157:H7 in apple cider at a higher temperature of 45°C. The y-axis represents the *Escherichia coli* O157:H7 count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

Low and moderate temperatures for Shiga toxin-producing *E. coli* non-O157:H7

In **Figure 4**, the temperature was 4°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The amount of *E. coli* non-O157:H7 was significantly reduced across all treatments. The most effective reduction was observed at the 345 MPa pressure for 5 minutes, resulting in a 0.64 Log CFU/mL. All treatments at 4°C led to substantial reductions in the *E. coli* non-O157:H7 counts; which demonstrated the effect of temperature on the pathogen treatment. Higher pressures such as 600 MPa effectively reduced *E. coli* non-O157:H7 to very low levels; however, at lower pressures such as 345 MPa were more effective. This demonstrated the effectiveness of HPP treatment on *E. coli* non-O157:H7. Lower pressures and longer treatment time intervals were very effective in reducing *E. coli* non-O157:H7. In **Figure 5**, the temperature was 45°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The *E. coli* non-O157:H7 counts were reduced but the effectiveness varied differently in comparison to the 4°C results. The smallest bacterial count was observed at 345 MPa at 5 minutes, which resulted in 4.42 Log CFU/mL. The effectiveness of the pressure and temperatures at 45°C demonstrated an overall reduction; however, at higher temperatures the treatments were less effective. Even though there was significant reduction in *E. coli* O157:H7; the treatments did not reduce the bacterial counts as in 4°C. Both **Figures 4** and **5** show that HPP can reduce *E. coli* non-O157:H7 counts, the reductions are more significant at the lower temperature (4°C) across all pressure levels. Therefore, to maximize the reduction of *E. coli* non-O157:H7 in apple cider, it would be better to use lower temperatures combined with high-pressure treatments. The temperatures before and after the treatment temperatures did not change much. The pH measured how acidic or basic the cider was. The pH levels pretty much stayed the same prior to and post treatments at both temperatures. The untreated cider contained around 8.01 on a logarithmic scale of harmful *E. coli* O157:H7. Higher pressure temperatures, especially at lower temperatures (4°C), are more effective at eradicating *E. coli* non-O157:H7 bacteria in apple cider. Both temperature and pressure play critical roles in how effective these treatments can reduce harmful bacteria.

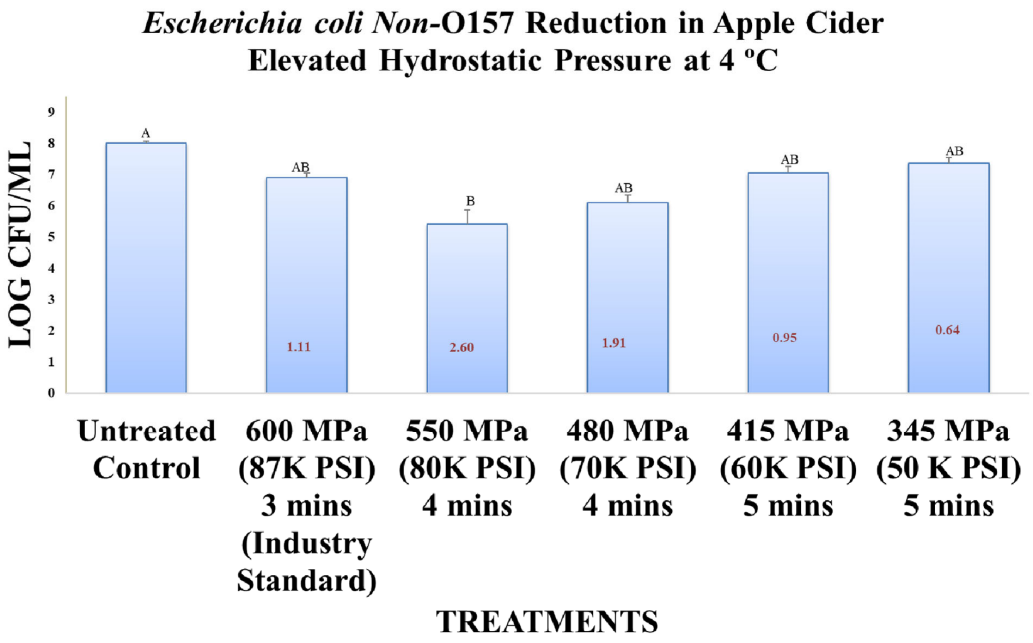

Figure 4. This graph presents the effect of different treatments using elevated hydrostatic pressure on the reduction of *Escherichia coli* non-O157:H7 in apple cider at a temperature of 4C. The y-axis represents the *Escherichia coli* non-O157:H7 count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

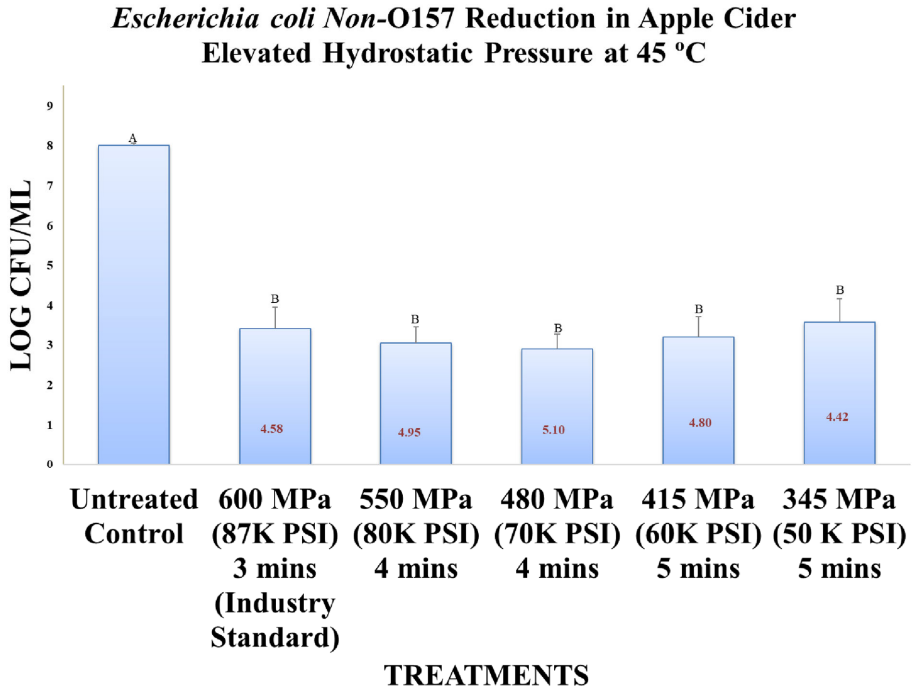

Figure 5. This graph presents the effect of different treatments using elevated hydrostatic pressure on the reduction of *Escherichia coli* non-O157:H7 in apple cider at a temperature of 45C. The y-axis represents the *Escherichia coli* non-O157:H7 count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

Low and moderate temperatures for *Salmonella*

In **Figure 6**, the temperature was 4°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The amount of *Salmonella* was reduced across all treatments. The most effective reduction was observed at the 345 MPa pressure for 5 minutes, resulting in a 2.87 Log CFU/mL. All treatments at 4°C led to reductions in the *Salmonella* counts; which demonstrated the impact of temperature on the pathogen treatments. Higher pressures such as 600 MPa did not effectively reduce *Salmonella* to very low levels; however, at lower pressures such as 345 and 415 MPa were more effective. This demonstrated the effectiveness of HPP treatment on *Salmonella*. Lower pressures and longer treatment time intervals were very effective in reducing *Salmonella* counts. In **Figure 7**, the temperature was 45°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The *Salmonella* counts were reduced and the effectiveness varied differently in comparison to the 4°C results. The smallest bacterial count was observed at 415 MPa at 5 minutes, which resulted in 5.17 Log CFU/mL. All treatments at 45°C led to reductions in the *Salmonella* counts; which demonstrated the impact of temperature on the pathogen treatments. None of the pressures significantly reduced *Salmonella* to very low levels; however, at the lower pressure of 415 MPa was the most effective. This demonstrated the effectiveness of HPP treatment on *Salmonella*. Lower pressures and longer treatment time intervals were very effective in reducing *Salmonella* counts.

Both **Figures 6** and **7** show that HPP can reduce *Salmonella* counts, the reductions are more significant at the lower temperature (4°C) across all pressure levels. Therefore, to maximize the reduction of *Salmonella* in apple cider, it would be better to use lower temperatures combined with high-pressure treatments. The temperatures before and after the treatment temperatures did not change much. The pH measured how acidic or basic the cider was. The pH levels pretty much stayed the same prior to and post treatments at both temperatures. The untreated cider contained around 8.01 on a logarithmic scale of harmful *Salmonella*. Higher pressure temperatures, especially at lower temperatures (4°C), are more effective at eradicating *Salmonella* bacteria in apple cider. Both temperature and pressure play critical roles in how effective these treatments can reduce harmful bacteria.

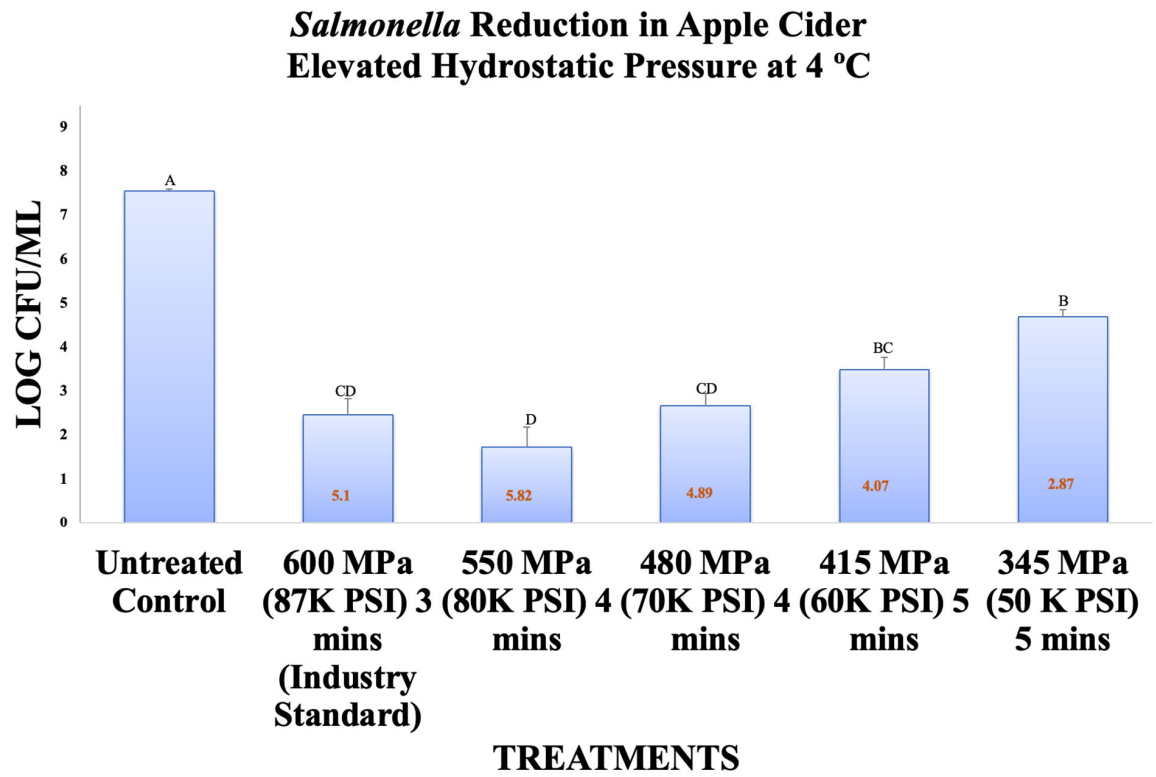

Figure 6. This graph presents the effect of different treatments using elevated hydrostatic pressure on the reduction of Salmonella in apple cider at a temperature of 4°C. The y-axis represents the Salmonella count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

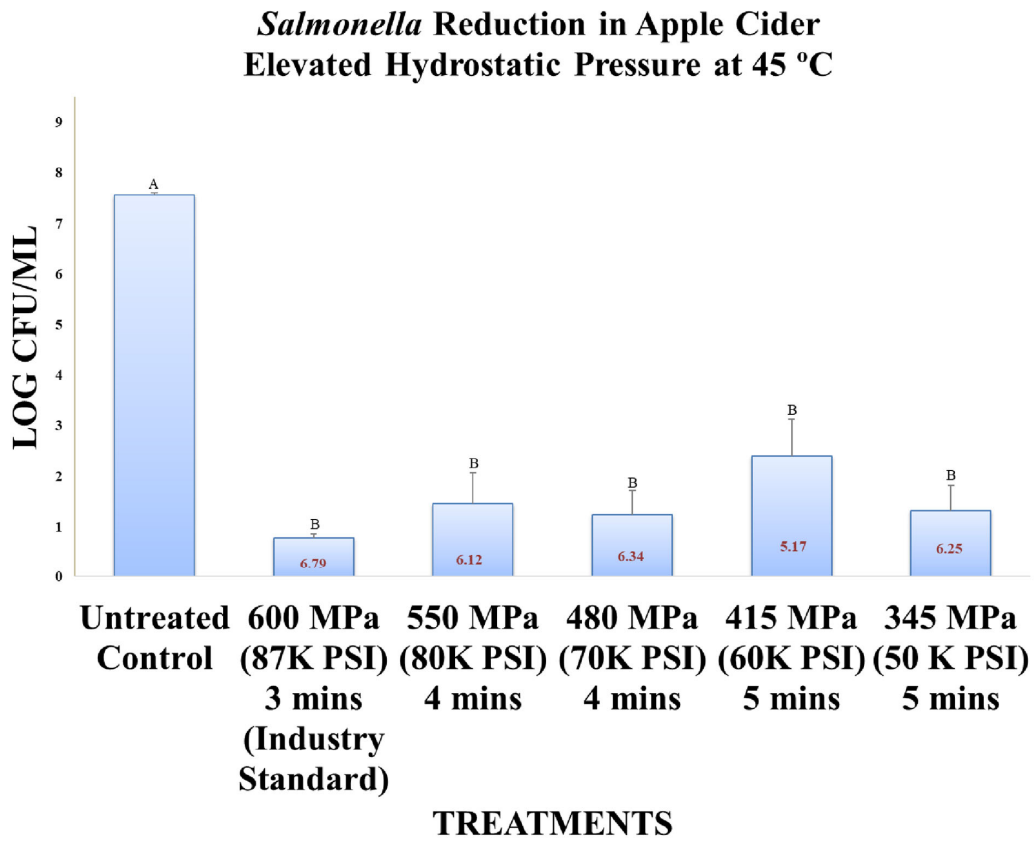

Figure 7. This graph represents the effect of different treatments using elevated hydrostatic pressure on the reduction of Salmonella in apple cider at a temperature of 45°C. The y-axis represents the Salmonella count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

Low and moderate temperatures for *Listeria*

In **Figure 8**, the temperature was 4°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The amount of *Listeria* was reduced significantly across all treatments. The most effective reduction was observed at the 345 MPa pressure for 5 minutes, resulting in a 1.63 Log CFU/mL. All treatments at 4°C led to substantial reductions in the *Listeria* counts; which demonstrated the impact of temperature on the pathogen treatments. Higher pressures such as 600 MPa effectively reduced *Listeria* to low levels; however, at lower pressures such as 345 MPa was more effective. This demonstrated the effectiveness of HPP treatment on *Listeria*. Lower pressures and longer treatment time intervals were very effective in reducing *Listeria* counts. In **Figure 9**, the temperature was 45°C and the treatment pressures ranged between 345 and 600 MPa applied for 3 to 5 minutes. The *Listeria* counts were reduced and the effectiveness varied differently in comparison to the 4°C results. The smallest bacterial count was observed at 550 MPa at 4 minutes, which resulted in 4.91 Log CFU/mL. All treatments at 45°C led to reductions in the *Listeria* counts; which demonstrated the impact of temperature on the pathogen treatments. None of the pressures significantly reduced *Listeria* to very low levels; however, at the higher pressure of 550 MPa treatment was the most effective. This demonstrated the effectiveness of HPP treatment on *Listeria*. Higher pressures and shorter treatment time intervals were very effective in reducing *Listeria* counts. Both **Figures 8** and **9** show that HPP can reduce *Listeria* counts, the reductions are more significant at the lower temperature (4°C) across all pressure levels. Therefore, to maximize the reduction of *Listeria* in apple cider, it would be better to use lower temperatures combined with high-pressure treatments. The temperatures before and after the treatment temperatures did not change much. The pH measured how acidic or basic the cider was. The pH levels pretty much stayed the same prior to and post treatments at both temperatures. The untreated cider contained around 8.01 on a logarithmic scale of harmful *Listeria*. Higher pressure temperatures, especially at lower temperatures (4°C), are more effective at eradicating *Listeria* bacteria in apple cider. Both temperature and pressure play critical roles in how effective these treatments can reduce harmful bacteria.

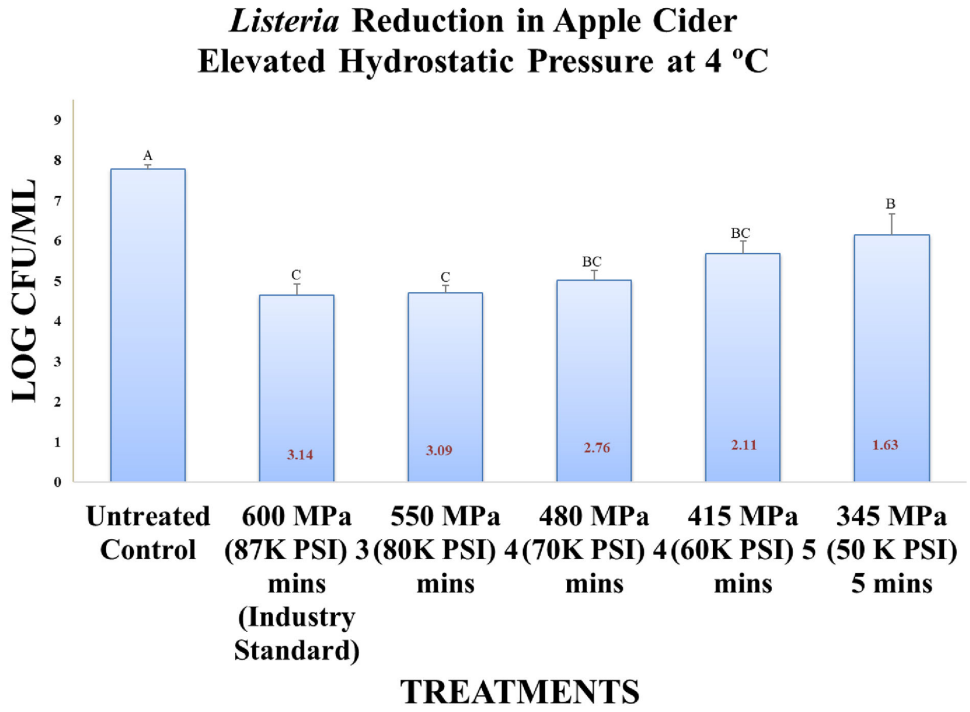

Figure 8. This graph represents the effect of different treatments using elevated hydrostatic pressure on the reduction of *Listeria* in apple cider at a temperature of 4°C. The y-axis represents the *Listeria* count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

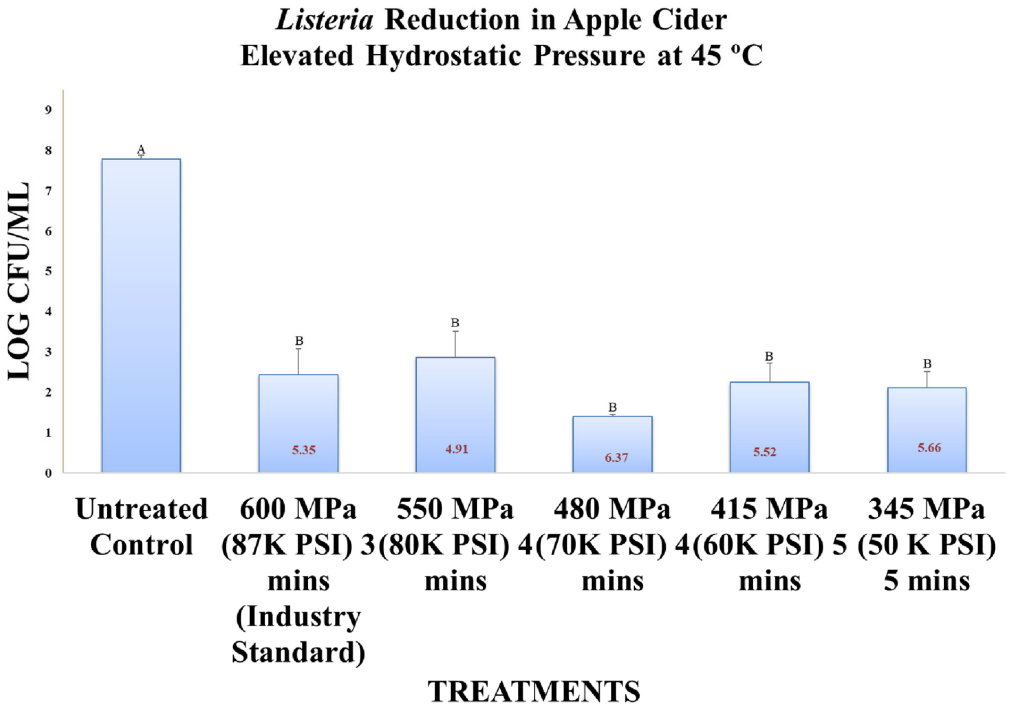

Figure 9. This graph represents the effect of different treatments using elevated hydrostatic pressure on the reduction of Listeria in apple cider at a temperature of 45C. The y-axis represents the Listeria count in terms of the logarithmic of colony-forming units per milliliter (log CFU/mL), while the x-axis lists the different treatments with varying pressure levels (measured in megapascals, MPa, and pounds per square inch, PSI) and duration (measured in minutes).

Discussion

The results from the study depicted in **Tables 1** and **2** provide valuable insights into the effectiveness of HPP on reducing various bacterial pathogens, including *E. coli* O157, non-O157 *E. coli*, *Salmonella*, and *Listeria* in apple cider under different temperature and pressure conditions. Below the temperature and pressure effects on bacterial reduction will be discussed in detail. **Figures 2** and **3** highlight the reduction of *E. coli* O157 at 4°C and 45°C under pressures ranging from 345 to 600 MPa applied for 3 to 5 minutes. At the lower temperature of 4°C, *E. coli* O157 counts were significantly reduced across all pressure levels, with the most substantial reduction observed at 345 MPa for 5 minutes, resulting in a 0.86 Log CFU/mL decrease. Higher pressures like 600 MPa were also effective, but interestingly, lower pressures combined with longer treatment times were more efficient in reducing bacterial counts. Conversely, at 45°C, while reductions were observed, the effectiveness was diminished compared to the results at 4°C, indicating that lower temperatures are more conducive to pathogen reduction when using HPP. **Figures 4** and **5** demonstrate similar trends for non-O157 *E. coli*. At 4°C, the most significant reduction was observed again at 345 MPa for 5 minutes, resulting in a 0.64 Log CFU/mL decrease. Higher pressures were effective, but the same pattern emerged, where lower pressures with longer treatment times were more beneficial. At 45°C, the effectiveness decreased, with the smallest bacterial count reduction observed at 345 MPa for 5 minutes, resulting in a 4.42 Log CFU/mL decrease. This suggests that, like *E. coli* O157, non-O157*E. coli* is more effectively reduced at lower temperatures. **Figures 6** and **7** focus on the reduction of *Salmonella* under the same conditions. At 4°C, a significant reduction was noted at 345 MPa for 5 minutes, with a decrease of 2.87 Log CFU/mL.

Interestingly, higher pressures, such as 600 MPa, did not effectively reduce *Salmonella* to very low levels, highlighting that lower pressures combined with longer treatment times are more effective. At 45°C, the effectiveness varied, with the best reduction observed at 415 MPa for 5 minutes, resulting in a 5.17 Log CFU/mL decrease. However, no pressures significantly reduced *Salmonella* to very low levels, indicating that lower temperatures might be more critical for effective reduction.

Figures 8 and **9** show the reduction of *Listeria* under similar conditions. At 4°C, a significant reduction was observed at 345 MPa for 5 minutes, resulting in a 1.63 Log CFU/mL decrease. Higher pressures like 600 MPa effectively reduced *Listeria* to low levels, but lower pressures combined with longer treatment times proved to be more effective. At 45°C, reductions were less effective, with the most significant reduction observed at 550 MPa for 4 minutes, resulting in a 4.91 Log CFU/mL decrease. This again suggests that lower temperatures are more conducive to reducing *Listeria* effectively. The study underscores the importance of temperature and pressure in the effectiveness of HPP treatments in reducing harmful bacteria in apple cider. Across all pathogens tested, lower temperatures (4°C) combined with high-pressure treatments consistently resulted in more significant reductions of bacterial counts. The data suggest that, to maximize the safety of apple cider, lower temperatures should be used in conjunction with high-pressure processing, as this combination appears to be most effective in reducing harmful pathogens such as *E. coli*, *Salmonella*, and *Listeria*. Additionally, the consistency of pH levels before and after treatments indicates that these HPP conditions do not significantly alter the acidity of the cider, maintaining its quality while effectively reducing bacterial contamination.

HPP nutritional values

High pressure purification treatment, also known as high pressure processing (HPP), is a method used to extend the shelf life of food products, including apple juice, by inactivating microorganisms and enzymes that cause spoilage. The way to address the nutritional value of apple juice after HPP treatment [46, 80-81]. There are six major factors to consider when discussing nutritional values of HPP technology. The six factors include, Retention of Nutrients, Preservation of Bioactive Compounds, Microbial Safety and Quality, Impact on Macronutrients, Shelf-Life Extension, and Consumer Perception.

Two major things to consider when discussing Retention of Nutrients includes Vitamins and Minerals. To elaborate on vitamins, HPP typically retains most of the vitamins in apple juice. For example, vitamin C (ascorbic acid), which is sensitive to heat, is better preserved under HPP compared to traditional thermal pasteurization [82,83]. Minerals such as potassium, calcium, and magnesium are generally stable and remain unaffected by HPP [48,80]. The Preservation of Bioactive Compounds consists of Polyphenols and Enzymes. Polyphenols: Apple juice contains polyphenols like flavonoids and phenolic acids, which have antioxidant properties. HPP helps in maintaining these bioactive compounds better than heat treatments, thus preserving the juice's antioxidant capacity [84,85]. Enzymes: While some natural enzymes in apple juice may be inactivated to extend shelf life, essential enzymes that contribute to the nutritional and sensory properties of the juice are better preserved under HPP [86].

Under the theme of Microbial Safety and Quality, there is Microbial Reduction and Sensory Characteristics. Microbial Reduction: HPP effectively reduces pathogenic microorganisms (like *E. coli*, *Salmonella*, and *Listeria*) and spoilage organisms without significantly affecting the nutritional profile of the juice [46,87]. Sensory Characteristics: The taste, color, and aroma of apple juice are better preserved under HPP compared to thermal methods, ensuring that the juice remains appealing to consumers while retaining its nutritional quality [88,89]. Dietary Fiber, Sugars, and

Carbohydrates are the major themes impacting macronutrient content. Sugars and Carbohydrates: The natural sugars and overall carbohydrate content in apple juice are generally unaffected by HPP, preserving the energy value of the juice [90,91]. Dietary Fiber: If the apple juice contains pulp, HPP does not significantly alter the dietary fiber content [92].

The theme of shelf-life extension, is also known as longer shelf life which is important in foods and beverages. Longer Shelf Life: By inactivating spoilage organisms and enzymes, HPP extends the shelf life of apple juice without the need for preservatives, ensuring the juice remains safe and nutritious for a longer period [83,93]. The final aspect to consider is Consumer Perception; which is closely associated with Clean Labels. Clean Label: HPP-treated apple juice can be marketed as a "clean label" product, as it typically does not require additives or preservatives, appealing to health-conscious consumers looking for natural and minimally processed options [36,93]. In summary, HPP treatment of apple juice maintains most of its nutritional value, including vitamins, minerals, and bioactive compounds, while extending shelf life and preserving sensory qualities. This makes HPP a favorable method for producing high-quality, nutritious apple juice [46,83].

Table 4. This table displays the impact of hydrostatic pressure treatments at 4°C on various pathogens in apple cider. The pathogens include Shiga toxin-producing *Escherichia coli* (STEC), non-Shiga toxin-producing *Escherichia coli* (nSTEC), *Salmonella* serovars, and *Listeria monocytogenes*. Each column shows the Log CFU/mL (colony-forming units per milliliter) values recorded after applying specific pressure levels (ranging from 345 MPa to 600 MPa) for different durations (3 to 5 minutes). The control (0 PSI) column represents untreated cider, showing initial pathogen levels. The table indicates that lower pressures and longer exposure times generally led to significant reductions in pathogen levels.

High Pressure Processing at 4°C						
0 PSI (control)	**87,000 PSI (600 MPa) 3 mins**	**80,000 PSI (550 MPa) 4 mins**	**70,000 PSI (480 MPa) 4 mins**	**60,000 PSI (415 MPa) 5 mins**	**50,000 PSI (345 MPa) 5 mins**	**Pathogen**
8.01	**1.21**	**1.34**	**1.35**	**0.96**	**0.86**	**Shiga toxin-Producing *Escherichia coli* (STEC)**
8.01	**1.11**	**2.60**	**1.91**	**0.95**	**0.64**	**Non-Shiga toxin- Producing *Escherichia coli* (nSTEC)**
8.01	**5.10**	**5.82**	**4.89**	**4.07**	**2.87**	***Salmonella serovars***
8.01	**3.14**	**3.09**	**2.76**	**2.11**	**1.63**	***Listeria monocytogenes***

Table 5. This table presents the effects of hydrostatic pressure treatments at 45°C on various pathogens in apple cider, including Shiga toxin-producing *Escherichia coli* (STEC), non-Shiga toxin-producing *Escherichia coli* (nSTEC), *Salmonella* serovars, and *Listeria monocytogenes*. The table details the Log CFU/mL (colony-forming units per milliliter) values after applying different pressure levels (345 MPa to 600 MPa) for varying durations (3 to 5 minutes). The control (0 PSI) column shows the initial pathogen levels in untreated cider. The results highlight that while hydrostatic pressure reduced pathogen levels, the effectiveness varied, with lower pressures and longer treatment times being generally more effective at reducing pathogen levels.

High Pressure Processing at 45°C						
0 PSI (control)	**87,000 PSI (600 MPa) 3 mins**	**80,000 PSI (550 MPa) 4 mins**	**70,000 PSI (480 MPa) 4 mins**	**60,000 PSI (415 MPa) 5 mins**	**50,000 PSI (345 MPa) 5 mins**	**Pathogen**
8.01	**5.05**	**6.02**	**4.25**	**5.58**	**2.02**	**Shiga toxin-Producing *Escherichia coli* (STEC)**
8.01	**4.58**	**4.95**	**5.10**	**4.80**	**4.42**	**Non-Shiga toxin- Producing *Escherichia coli* (nSTEC)**
8.01	**6.79**	**6.12**	**6.34**	**5.17**	**6.25**	***Salmonella serovars***
8.01	**5.35**	**4.91**	**6.37**	**5.52**	**5.66**	***Listeria monocytogenes***

Conclusion

Consumers are very particular about the food they choose to eat, and a variety of factors play a significant role in their decisions. One of the key factors is the freshness of the product; people increasingly prefer foods that are natural and free from preservatives and synthetic additives. In recent years, there has been a growing demand for "clean label" products, where consumers seek transparency in the ingredients used, prioritizing products that are minimally processed and made with recognizable ingredients [94]. Food safety and quality are also top priorities for consumers, but these concerns go hand in hand with the expectation that the food will maintain its nutritional value and sensory characteristics. Consumers want food that retains its original texture, color, and nutrient content, while also being free from harmful microorganisms that could spoil the product or cause illness.

This creates a challenge for food producers, as they must balance the need for safety and quality with the preservation of the food's natural attributes. High-Pressure Processing is an innovative technology that addresses many of these consumer demands by effectively eliminating pathogens and spoilage microorganisms without the need for high temperatures or chemical preservatives, thus preserving the food's natural qualities [24,95]. When apple cider undergoes high-pressure processing (HPP), the nutritional value is generally well-preserved. Most vitamins, like vitamin C, and antioxidants remain intact because HPP uses pressure instead of heat, which helps maintain the cider's natural nutrients. However, some minor changes might occur, such as slight alterations in flavor or color, but overall, the health benefits of the cider are retained.

However, HPP-treated products tend to be slightly more expensive than those produced using traditional methods. This cost difference can be a barrier for some consumers, but the benefits of HPP, such as enhanced safety and extended shelf life, may justify the higher price for many. The potential applications of HPP technology extend beyond just packaged goods. For example, if pressure systems could be scaled down for use in restaurants, particularly those that serve raw or minimally cooked meat, HPP could ensure that these dishes are safe to eat. This could significantly reduce the risk of foodborne illnesses associated with raw meat and beverage consumption, offering peace of mind to both restaurant operators and their customers. The overarching goal of this project is to expand the use of HPP technology from large-scale industrial applications to more accessible commercial settings, enabling a wider range of foods and beverages to be treated safely and effectively. By doing so, HPP could become a standard process in the food industry, providing consumers with safe, high-quality products that meet their growing expectations for freshness, naturalness, and nutrition [24].

Foodborne illness in Tennessee

Over the years, there have been numerous outbreaks associated with hamburger consumption, highlighting the risks posed by foodborne pathogens in undercooked or contaminated meat. One particularly notable outbreak occurred close to Nashville, in Chattanooga, TN. This incident underscores the vulnerability of even well-established food safety practices and the ongoing challenges in preventing foodborne illnesses. According to data from the Centers for Disease Control and Prevention (CDC), specifically from the National Outbreak Reporting System (NORS) and the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Tennessee has experienced a significant number of foodborne outbreaks over the years. Between 2009 and 2021, the state reported a total of 636 outbreaks, leading to 25,725 illnesses and 27 deaths directly related to hamburger consumption. These statistics reflect not only the frequency of such incidents but also the severe health implications they can have. The data from the CDC highlight the importance of stringent food safety measures at every stage of the food production and preparation process, from the farm to the consumer's plate [96].

Despite advances in food safety technologies and regulations, outbreaks continue to occur, often due to lapses in handling, cooking, or contamination during processing. This is especially concerning in the case of ground beef, where bacteria present on the surface of meat can be mixed throughout during grinding, making thorough cooking essential to eliminate harmful pathogens like *E. coli* and *Salmonella*. The Chattanooga outbreak serves as a reminder that food safety is a critical issue that affects communities across the country, not just in isolated incidents. It also emphasizes the need for continued public health education on safe food handling practices, the importance of cooking ground beef to the appropriate internal temperature, and the role of regulatory agencies in monitoring and responding to outbreaks. In light of these statistics, it's clear that while progress has been made in reducing the number of foodborne illnesses, much work remains to be done. The goal is to ensure that incidents like the one in Chattanooga become increasingly rare, protecting public health and reinforcing consumer confidence in the safety of the food supply [97].

In 2006, Ryan's Family Steak House in Hixson, TN, became the site of a significant foodborne illness outbreak caused by the dangerous pathogen *Escherichia coli* O157. This particular strain of *E. coli* is known for causing severe gastrointestinal illness, and in some cases, it can lead to serious complications such as hemolytic uremic syndrome (HUS), which can result in kidney failure. The outbreak at Ryan's Family Steak House led to seven confirmed cases of illness, as reported by the Chattanooga-Hamilton County Health Department. The *E. coli* O157 strain is particularly concerning because it can cause

severe symptoms even at very low doses, meaning that only a small amount of contaminated food can result in illness. The pathogen is commonly associated with undercooked ground beef, but it can also be found in other foods such as raw vegetables, unpasteurized dairy products, and contaminated water. In the case of Ryan's Family Steak House, the source of contamination was linked to food served at the restaurant, although the exact food item responsible for the outbreak was not definitively identified [97].

The Chattanooga-Hamilton County Health Department's report of seven cases underscores the seriousness of the outbreak. While the number of reported cases may seem relatively small, it is important to recognize that not all cases of foodborne illness are reported, meaning the actual number of affected individuals could be higher. Additionally, *E. coli* O157 outbreaks can have widespread public health implications, as the bacterium can be easily transmitted from person to person, especially in settings where hygiene practices may not be strictly followed. This outbreak at Ryan's Family Steak House serves as a critical reminder of the importance of food safety in both commercial and home settings. It highlights the need for proper cooking temperatures, especially when preparing ground beef, as well as the importance of strict sanitation practices in restaurants to prevent cross-contamination. The incident also reflects the vital role of local health departments in identifying, investigating, and managing foodborne illness outbreaks to protect public health. Overall, the 2006 outbreak at Ryan's Family Steak House is a case study in the ongoing challenges of preventing *E. coli* O157 and other foodborne pathogens in the food service industry. It also emphasizes the need for continued vigilance in food safety practices to ensure that similar outbreaks do not occur in the future [97].

HPP nutritional costs in juice processing

In 2007, the cost of a commercial HPP unit was about $500,000, which is roughly the same as 2.5 million U.S. dollars today [35]. In an article published in 2022, the costs for HPP units ranged from $500,000 to $4,000,000 U.S. dollars. [75]. HPP machinery initially had a vertical orientation, but with technological advancements, horizontal units have also become available for researchers and producers. A 2022 study also revealed that the cost of HPP pasteurization was about 10.7 cents per liter, with a processing capacity of 3,000 liters per hour (or 792.5 gallons per hour) [75].

Although HPP is making progress in the food and beverage industries, it does have some drawbacks. One of the main limitations is that it requires batch or semi-continuous processing for solid foods [75], limited microbial effectiveness [36], capital equipment expenses are very high [98]. A good point from Nawawi *et al.* (2023) was how optimizing the operational aspects of HPP can make it more cost-effective. Increasing the capacity of processing vessels or reducing the time per cycle can help boost throughput and reduce costs, which could be beneficial for industries looking to adopt HPP but concerned about the expense [99]." In addition, production rates in factories are approaching 40 million pounds per year, which is a good sign that the costs of both investing in and running HPP are likely to keep going down [75]. This is all important as The United States Food and Drug Administration's Center for Food Safety and Applied Nutrition department's regulatory documentation requires a 5-log reduction of pathogens of human beverage concerns [100]. The more industries that adopt this technology, the cheaper that the units will become overall.

Limitations

To conduct the HPP in containers similar to those available in the market, such as 4, 6, or 8 oz pouches, boxes, and other liquid containers used for products like 6 or 12 oz juice servings would be beneficial. This would allow the results to be more directly applicable to real-world conditions and consumer products. However, these systems only allow use of specialized PULSE tubes, which limits the direct translation of findings to commercially available packaging. Another limitation to consider is the potential presence of microorganisms in the processing plants and the large-scale materials used in commercial production. In a controlled laboratory environment, it is easier to manage and monitor microbial contamination, but this may not fully represent the conditions in a large-scale industrial setting. These experiments on apple cider, have a similar density to apple juice. While this allows us to infer some results for apple juice and other similar beverages, the findings might not be directly applicable to all juice types. Different juices have varying compositions, which could influence how they respond to HPP. For example, differences in sugar content, pH, and pulp presence could all impact the effectiveness of the HPP process. Therefore, further research is needed to fully understand how these factors affect HPP outcomes in different juice products.

Conflicts of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results. Content of the current publication does not necessarily reflect the views of the funding agencies.

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