MSC THESIS

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THERMAL TOLERANCE OF FOODBORNE PATHOGENIC BACTERIA IN FRUIT PUREE MATRIX

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1. Introduction

Food safety is a critical concern worldwide as the consumption of contaminated food can lead to severe health consequences, including foodborne illness. The leading causes of foodborne illness are viruses, parasites and bacteria including *Listeria monocytogenes* and *Salmonella enterica*. These bacteria have been found in a variety of food products, including fruit purees and smoothies, which are popular and widely consumed by individuals of all ages, Microorganisms present in the raw fruits and vegetables used to make juice can remain viable, increasing the risk of foodborne illness. Therefore, it is recommended to avoid the purchase of unpasteurized juices unless they have been properly treated with a validated process to destroy harmful microorganisms.

Controlling a wide range of variables is necessary for the manufacture of high-quality, microbiologically safe foods, including the standard of raw ingredients, hygienic circumstances and practices during food processing, the characteristics of completed goods, and the right storage conditions. Foods can attain microbial stability by having microorganisms inactivated or eliminated, having them stop growing, and not being contaminated again.

Listeria monocytogenes and *Salmonella enterica* are two common foodborne pathogens that can survive and grow in fruit puree-based products. *Listeria monocytogenes* is a Gram-positive bacterium that can cause severe infections, particularly in immunocompromised individuals, pregnant women, and neonates. *Salmonella enterica* is a Gram-negative bacterium that can cause gastroenteritis and systemic infections.

The ability of these bacteria to survive and proliferate in food products is influenced by various factors, such as temperature, pH, and water activity. Of these factors, the temperature is considered one of the most critical determinants of bacterial growth and survival in food. Heating is frequently used to inactivate germs that could endanger the safety or quality of finished food products. The heating temperature, heating period, and the heat resistances of microorganisms all affect how well microorganisms are inactivated by heat treatments. The design of the processing equipment, the type of heating media, the size and form of the food, as well as the content and viscosity of the finished product, all have an impact on the heating process. Bacteria have specific temperature ranges at which they can grow and survive, and understanding these ranges is crucial in the development of food safety measures.

The heat resistance of the targeted microorganisms determines the amount of heat required to inactivate microorganisms. The destruction of vegetative cells of bacteria is the common goal of heat treatment. This includes for example *Pseudomonas* species that cause vegetative deterioration as well as pathogenic species such as *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Staphylococcus aureus* and *Listeria monocytogenes*.

In response to stress exposures, foodborne pathogens modify their cellular processes to a state that permits them to retain viability and proliferation under stressful conditions. Bacteria create stress-adaptive responses by sensing environmental changes and reprogramming gene expression to produce stress response proteins that aid in survival difficult settings.

Damage to a variety of targets, such as the cell wall, the cytoplasmic membrane, and ribosomal RNA, as well as denaturation of proteins, such as ribosomal proteins and enzymes involved in cellular metabolism and repair, cause the thermal inactivation of vegetative bacteria cells. The ability of cells to withstand heat is influenced by both their inherent characteristics and by environmental factors that may cause stress-related cell-harnessing reactions. The chances of a cell surviving and recuperating are good if it can repair heat damage (Den Besten et al., 2018).

So, it is essential to determine the thermal tolerance of *Listeria monocytogenes* and *Salmonella enterica* in food products. This information can be used to establish appropriate processing and storage conditions that can help prevent or minimize the growth of these pathogens.

2. Goal of the thesis

The food industry is constantly facing challenges in ensuring the safety of the products it offers to consumers. Among the most serious concerns are foodborne pathogenic bacteria, such as *Listeria monocytogenes* and *Salmonella enterica*, which can cause severe illnesses and even fatalities.

Heat treatment is one of the most used methods for eliminating such microorganisms from food products. However, the efficacy of heat treatment depends on various factors, including the type of matrix or medium in which the bacteria are present.

In this context, the aim of this work is to study the thermal tolerance of *Listeria monocytogenes* and *Salmonella enterica* bacteria in different fruit-based media.

The specific objectives of this work is to determine the effect of mild heat treatment on the number of uninjured cells and total number (injured and intact cells) of surviving *Listeria monocytogenes* and *Salmonella enterica* in liquid foods. To achieve these objectives, the study focuses on three types of matrices, namely, strawberry puree, smoothies, and distilled water. By analysing the thermal destruction kinetics of the targeted bacteria in these matrices, the study aims to calculate the D-values, which show how long it takes for the bacterial population to decrease by one log unit at a particular temperature.

Furthermore, the thesis seeks to extend the investigation by determining the z-value, which reflects the change in temperature required for a 1-log reduction in the D-Value. The calculation of the z-value for *Listeria monocytogenes* and *Salmonella enterica* bacteria in different media would enable a better understanding of the thermal processes needed to ensure the elimination of these pathogenic microorganisms in food.

Overall, this research contributes to the advancement of knowledge on the thermal destruction kinetics of foodborne pathogenic bacteria in fruit-based matrices and provides valuable insights for the development of effective thermal processing strategies in the food industry.

3. Literature review

3.1. Frequency of foodborne diseases

Hippocrates (460 B.C.) observed that there is a significant link between food ingested and human illness. This was one of the earliest accounts of the connection between food consumption and human diseases (Hutt and Hutt, 1984). According to U.S. Center for Disease Control and Prevention (CDC) estimates, 48 million Americans (or 1 in 6) fall ill each year, 128,000 are hospitalized, and 3,000 lose their lives to foodborne illnesses (CDC, 2011a). Biological agents that can result in a foodborne illness occurrence are called foodborne pathogens, and they include bacteria, viruses, and parasites. The emergence of two or more cases of a similar illness brought on by consuming a common meal is known as a "foodborne disease outbreak" (CDC, 2012). The most severe cases typically affect the young, the elderly, pregnant and those with weakened immune systems, and healthy people who have been exposed to extremely high doses of an organism (CDC, 2012). According to the European Food Safety Authority In 2021, 4,005 foodborne disease outbreaks were recorded by 27 member states and the UK (Northern Ireland) (29.8 percent more than in 2020); these outbreaks caused 32,543 cases of illness, 31 fatalities, and 2,495 hospitalizations (EFSA, 2022). The rate of foodborne outbreak reports in the EU per 100,000 people in 2021 was 0.89%. This amounts to a rise of 29.0% from 2020 (0.69 per 100.000 people), and a drop of 18.3% from the years before the COVID epidemic (1.09 per 100.000 population in 2017–2019) (EFSA, 2022) Most foodborne outbreaks (FBOs) in the EU, accounting for 19.3% of all outbreaks, have been linked to *Salmonella*. Additionally, this was linked to most cases (20.8% of cases connected with the outbreak) and hospitalizations (45.0% of outbreak-related hospitalizations). The most common serovar, accounting for 79.7% of all *Salmonella* outbreaks, was *S.* Enteritidis and 12 from 31 deaths were related to *L. monocytogenes* (EFSA, 2022).

In comparison to 2020 and the years prior to the pandemic, outbreaks connected to the eating of "vegetables, juices and other products thereof" increased significantly. When it came to the overall number of instances recorded in FBOs in 2021, this food was only surpassed by "mixed food." (EFSA, 2022).

3.2 Foodborne pathogenic bacteria

Foodborne illnesses are most frequently brought on by bacteria, which come in a range of sizes, varieties, and characteristics. Some pathogenic bacteria are very heat-resistant because they can produce spores (e.g., *Clostridium botulinum, C. perfringens*, *Bacillus* subtilis, *Bacillus* cereus). Some can produce toxins that can withstand heat (e.g., *Staphylococcus aureus, Clostridium botulinum*). The majority of foodborne infectious bacteria are mesophilic, with the best growth temperatures between 20 and 45 °C. However, some foodborne pathogens (psychrotrophic), such as *Yersinia enterocolitica* and *Listeria monocytogenes*, can also grow in the refrigerator or at temperatures lower than 10 °C. (Bacon et al., 2003). Bacteria are most frequently blamed for food poisoning, which affects millions of people worldwide and can even be fatal. According to official statistics, 300 million individuals in China acquire food-borne illnesses on average every year. Microorganisms are thought to have been responsible for 56.1% of food poisoning occurrences in China in 2012 (Bai and Huang, 2014). Despite the fact that there are about 250 different food-borne diseases, bacteria cause two-thirds of outbreaks of these illnesses (Argaw et al., 2015). One of the most pervasive contemporary global public health issues is bacterial food-borne diseases (Addis et al., 2015). Some food-borne poisoning-causing bacteria are more significant than others in terms of the frequency and/or severity of the illness. Food-borne poisoning is caused by a variety of bacteria, both Gram-positive and Gram-negative, which can result in symptoms ranging from gastrointestinal problems to paralysis and death (Le Loir et al., 2003).

According to reports, 69% of cases of bacterial food-borne illness are caused by Gram-negative bacteria (Kebede et al., 2014). According to estimates published by (CDC) we can see from the (Table 1.) The annual numbers of disease waves caused by the most important pathogenic bacteria transmitted by food in the USA (CDC, 2011b).

Table 1. Estimated annual number of episodes of illnesses caused by some of pathogens (bacteria) transmitted commonly by food, United States. (CDC, 2011b).

Pathogen bacteria	**Laboratory confirmed**	**Total, mean (90% CrI)**	**Travel related, percentage**	**Domestically acquired, mean (90% CrI)**	**Foodborne, percentage**	**Domestically acquired foodborne, mean (90% CrI)**
Bacillus cereus	85	63,623 (15,770–147,827)	<1	63,411 (15,721–147,380)	100	63,400 (15,719–147,354)
Clostridium botulinum	25	56 (34–92)	<1	55 (34–91)	100	55 (34–91)
Clostridium perfringens	1,295	969,342 (192,977–2,492,003)	<1	966,120 (192,331–2,483,682	100	965,958 (192,316–2,483,309
ETEC	53	39,781 (53–102,250)	55	17,897 (24–46,215)	100	17,894 (24–46,212)
Listeria monocytogenes	808	1,662 (582–3,302)	3	1,607 (563–3,193)	99	1,591 (557–3,161)
Salmonella spp., nontyphoidal	41,930	1,229,007 (772,129–2,008,076)	11	1,095,079 (687,126–1,790,225)	94	1,027,561 (644,786–1,679,667)
Staphylococcus aureus	323	241,994 (72,584–531,398)	<1	241,188 (72,352–529,509)	100	241,148 (72,341–529,417
Streptococcus spp. group A	15	11,257 (15–78,104)	<1	11,219 (15–77,875)	100	11,217 (15–77,875)

3.3 Foodborne pathogenic microorganism in fruit juices, purees, and smoothie

A variety of micronutrients, vitamins, minerals, and antioxidant chemicals are present in fruit juices. Fruit juices that have not been treated are susceptible to rapid microbiological, enzymatic, chemical, and physical degradation. A variety of microorganisms from the typical microflora or those introduced during the processing of product can be found in juice generated from healthy fruits (Kalia and Gupta, 2006). Makers and customers may have issues if fruit juices are infected with pathogenic and spoilage microorganisms that can develop and live in an acidic environment (Ferrario et al., 2015; Bayındırlı et al., 2006).

Salmonella, *Escherichia coli* O157:H7, and the parasitic *Cryptosporidium*, can all be spread by unprocessed fruit juice (Kalia and Gupta, 2006). While *E. coli* O157:H7 and *Cryptosporidium parvum* are both thought to be connected to apple juice, *Salmonella* is often a bacterium that appears in acidic juices (Danyluk et al., 2012). Enterohemorrhagic serotype *E. coli* O157:H7 is now known to be one of the leading causes of foodborne illness worldwide (Duffy et al., 2008). It was reported that *E. coli* O157:H7 may grow in apple juice with a low pH and that the bacterial dose is estimated to be 10-100 cells (Burnett and Beuchat, 2000). *E. coli* O157:H7 in apple juice and *E. coli* O111 both play major roles in many outbreaks (Danyluk et al., 2012). The utmost heat- and acid-resistant pathogen found in fruit drinks was revealed to be *E. coli* O157:H7 (Mazzotta, 2001; Baskaran et al., 2010). These bacteria may trigger anything from moderate, non-bloody diarrhoea to hemorrhagic colitis and hemolytic uremic syndrome (HUS), which is marked by hemolysis, hemolytic anemia, thrombocytopenia, and severe renal failure. It can also lead to possible lethal serious kidney damage in kids. (Karmali, 2004; Duffy et al., 2008).

Since the FDA mandates that juice operations adhere to a hazard analysis, unpasteurized juices must be regarded as potentially dangerous food and develop into a public health issue, leading to modifications in the fresh juice industry establishments across the country (Danyluk et al., 2012). Moreover, enterotoxigenic *E. coli* and *Shigella flexneri* were associated in incidents involving unpasteurized orange juice (Danyluk et al., 2012).

Due to its low thermal resistance, *Salmonella* is also a disease-causing agent connected to unpasteurized fruit juices. According to a study, processing sour juice can increase the ability of *Salmonella* spp. to survive in the human digestive system, potentially raising the danger of a

Salmonella outbreak from juice (Yuk and Schneider, 2006). Moreover, Typhi and Typhimurium serovars of *Salmonella* were linked to two outbreaks involving unpasteurized apple juice and one involving home-made watermelon juice (Danyluk et al., 2012). Food poisoning, intestinal infectious illnesses, and other health issues are all caused by *Salmonella* Typhimurium. In some nations, like China, *Salmonella* is estimated to be the root of over 80% of foodborne bacterial outbreaks (Ma et al., 2016; Duan et al., 2016).

One of the most famous outbreaks related to *Salmonella enterica* serotype Hartford (*Salmonella* Hartford) was an outbreak associated with unpasteurized orange juice among persons visiting a theme park in Orlando, Fla, USA, in 1995 (Cook et al., 1998).

Listeria has been categorized as a source of infection for above 70 years, but it wasn't until the 1980s that it started to pose a threat to food goods. It is frequently said to as a resilient organism that can endure challenging circumstances. *L. monocytogenes*, along with *Salmonella enterica* and *E. coli* O157:H7, was identified as a bacterial pathogen relevant to juice safety, while not being directly linked to outbreaks of foodborne diseases from juices (Gabriel and Nakano, 2009). And rather, because of its propensity to thrive and spread on surfaces of machinery and in unfavorable environments, as well as because of its psychoactive behavior, this bacterium should be taken into consideration as a worry in fruit juices (Caggia et al., 2009). *L. monocytogenes* was able to endure both harsh storability and cooling in apple, orange, and white grape juices. Hence, steps must be taken to get rid of and avoid *L. monocytogenes* in the area where fruit juice is processed in order to guarantee the juice's safety and quality (Piotrowski, 2003). Food poisoning from *Staphylococcus aureus* is brought on by eating items tainted with the organism's enterotoxins. *Staphylococcus aureus* can multiply and produce heat-resistant enterotoxins due to improper handling. Brutal vomiting and nausea are the major symptoms (Argudı´n et al., 2010). A smaller proportion of samples of freshly made juices (sweet lime, orange, and carrot) contained *Staphylococcus aureus* (Aneja et al., 2014). Further, of the 150 samples of fruit juices sold on the street in India, 73.3% was infected with *S. aureus* and 48.6% contained *Shigella* spp (Reddi et al., 2015).

A summary of outbreaks connected to fruit juices is summarized in Table 2.

Table 2. Outbreaks of human foodborne illness caused by various bacteria linked to juices in the USA and Canada between 2012 and 2016 (Krug et al., 2020).

Product	Year	Pathogen	Location in USA	Cases (Deaths)
Unpasteurized	2012	*E. coli* O157:H7	US (MI)	3 (0)
Unpasteurized	2013	*Cryptosporidium*	US (IA)	10 (0)
Unpasteurized	2013	*Cryptosporidium*	US (OH)	8 (0)
Unpasteurized	2013	*S.* Typhimurium	US (PA)	10 (0)
Unpasteurized	2014	*E. coli* O157:H7	Canada (ON)	3
Unpasteurized	2014	*Campylobacter jejuni*	US (AZ)	6 (0)
Unpasteurized	2015	*E. coli* O157	US (MN)	2 (0)
Unpasteurized	2015	*E. coli* O45	US (MI)	2 (0)
Unspecified	2015	*E. coli* O111	US (CA)	15 (0)
Unpasteurized	2016	*E. coli* O157:H7	US (KS)	56 (0)

In addition, with a blender, a variety of fresh fruits and vegetables are turned into smoothie drinks. Previous research has shown that they may also pose a threat to the development of certain harmful bacteria. As we know, the smoothie consists of fresh vegetables, fruits, and ready-to-eat plants, so it is vulnerable to contamination with all the pathogenic bacteria that were previously mentioned, in addition to non-pathogenic bacteria, which plays the role of evidence of contamination and vegetables, workplaces, preparation, and the workers like Coliform bacteria (Krahulcová et al., 2020).

In this thesis, I will work and talk about two of the most important pathogenic bacteria in the field of food, namely *Listeria* monocytogenes and *Salmonella* enterica so that we can know more about their characteristics, growth characteristics, and heat tolerance conditions.

3.3.1 *Listeria monocytogenes*

3.3.1.1 Characteristics

Listeria monocytogenes is a Gram-positive, small, rod-shaped, (Figure 1.), facultative anaerobe that is motile by means of a single flagellum. This pathogen is beta-hemolytic when cultured on blood agar (Todar, 2021). This pathogen is both microaerophilic and a facultative anaerobe. It is a non-sporulating bacterium, but it is quite hardy in that it is heat, freezing, drying, and halotolerant (Rees et al., 2017). Additionally, *Listeria monocytogenes* is neutrophile and psychrotroph. This means that it can grow in a wide range of pH levels, but it prefers neutral conditions, and it can grow at low temperatures, including in refrigerated environments (Sibanda et al., 2022).

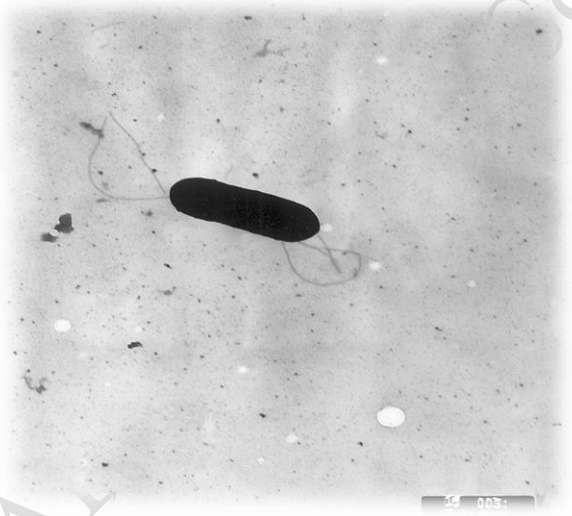

Figure 1. *Listeria monocytogenes* by electron microscopic (TEM) (Internet 1)

Except for a few uncommon strains, *Listeria monocytogenes* is catalase-positive, oxidase-negative, and esculin-positive. Many carbohydrates are fermented by *Listeria* without creating gas. *L. monocytogenes* strains that generate lecithinase are D-xylose negative. Typically, they are L-rhamnose positive (Bergis et al., 2021).

Listeria monocytogenes grows best at 37°C (Table 3.), although it can tolerate a large range of temperatures (0–45°C); it is relatively resistant to NaCl (growth at 10%; survival at 20–30%); and it can tolerate a wide variety of pH conditions (pH 4.6–9.2) (Rees et al., 2017).

Table 3. *Listeria monocytogenes* in a lab setting: growth characteristics (variable according to the strain) (Anonymus, 2020).

limit	Temperature (°C)	pH	NaCl (%)	Water Activity (a_w)
Minimum	-1.5	4.0	–	0.90
Optimum	30–37	6.0–8.0	–	0.97
Maximum	45	9.6	10	–

According to the European Union there are specific physicochemical parameters (pH, a_w, inhibitors, and storage temperature) of ready-to-eat (RTE) foods that are not believed to support the growth of *L. monocytogenes* such as low pH (less than 4.4), low water activity (a_w less than 0.92), and low pH combined with low a_w (pH≤5.0 with the a_w≤0.94). However, it is important to note that these factors alone do not guarantee the absence of *Listeria monocytogenes* (Rees et al., 2017).

Listeria monocytogenes is spread mostly through three different channels: contact with animals, hospitalized newborns getting sick from each other, and foodborne illness. The majority of human instances of listeriosis are caused by the latter two sources (Blackburn et al., 2009).

3.3.1.2 Subtyping of *Listeria monocytogenes*

The genetic diversity of *L. monocytogenes* is demonstrated by the strains' division into four evolutionary lineages (I-IV), 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 4ab, 4b, 4c, 4d, 4e, and 7), and four significant molecular serogroups based on PCR testing (IIa, IIb, IIc, and IVb). Serotype 4b (serogroup IV b), which is now less frequently identified in food, used to be the most prevalent serotype in clinical cases involving humans. Serotype1/2a (serogroup IIa) has been often related to human disease and has been the most abundant serotype in food and environmental samples over the past decade, producing large outbreaks in Europe and North America (Bergis et al., 2021 & Rees et al., 2017).

The majority of *L. monocytogenes* clinical isolates that have been linked to human illness appear to come from lineages I and II, which contain serotype 1/2a (lineage II) and serotypes 1/2b and 4b (lineage I). In contrast, lineage II strains are ubiquitous in farm and natural contexts and are frequently isolated from foods. Lineage II strains are frequently recovered from instances of animal listeriosis in addition to human disease (serotype 1/2a). Very rarely and mostly from animal sources, lineage III and IV strains are isolated. Such genetic analysis has not, however, yet provided an explanation for why 95% of human isolates are of serotypes 1/2a, 1/2b, or 4b. In fact, serotype 4b strains have been linked to numerous significant outbreaks of foodborne disease and account for 33-50% of human listeriosis cases globally. This has given rise to the theory that this serotype's clones are more virulent than those of other strains (Rees et al., 2017).

3.3.1.3 Illness

According to estimates, *Listeria monocytogenes* causes around 1,600 infections per year in the United States, leading to more than 1,500 hospitalizations and 260 related fatalities (Scallan et al., 2011). *Listeria monocytogenes* is a significant foodborne pathogen that can cause serious illness, particularly in vulnerable populations such as pregnant women, the elderly, and immunocompromised individuals. Bacteria that are invasive have spread outside of the intestines (gut). When *Listeria* have expanded outside of the intestines, invasive listeriosis results. Within two weeks of consuming *Listeria*-contaminated food, symptoms of an invasive infection typically appear: for pregnant women like fever, flu-like symptoms, such as muscle aches and fatigue. For the individuals who are not pregnant typically experience the following symptoms: fever, flu-like symptoms, such as exhaustion and muscular aches, headache, rigid neck, confusion, loss of equilibrium and seizures (CDC, 2022). invasive disease severity in pregnant women Pregnant women typically experience only moderate symptoms. Some pregnant women never experience any symptoms. Unfortunately, infections during pregnancy frequently result in miscarriage, stillbirth, early delivery, or infections of the baby that are potentially fatal (CDC, 2022). The severity of illness can depend on factors such as the individual's immune status, the dose of bacteria consumed, and the strain of *Listeria monocytogenes* involved (Lecuit, 2007). Listeriosis seldom affects healthy adults, especially when less than 10^3 cfu/g or ml of food or drink is consumed (Tompkin, 2002). *Listeria monocytogenes* can reproduce and spread inside host cells like macrophages. *Listeria monocytogenes* can enter the bloodstream and lead to more serious sickness

in circumstances where the host immune system is overtaxed or impaired (Drevets et al., 2008). In susceptible populations like pregnant women, early detection and treatment of *Listeria monocytogenes* infection are essential. *Listeria monocytogenes* infection is often treated with antibiotic therapy using penicillin, ampicillin, or trimethoprim-sulfamethoxazole, and these therapies have been demonstrated to be helpful in lowering mortality and morbidity and antimicrobial resistance must be managed and monitored going forward. (Lecuit, 2007; Heidarzadeh et al., 2021). In the case of pregnant women, prompt treatment of *Listeria monocytogenes* infection is essential to prevent transmission to the fetus, which can result in serious illness or even fetal death. Antibiotic treatment has been shown to reduce the risk of transmission to the fetus and improve maternal and neonatal outcomes (Wang et al., 2021).

3.3.1.4 Disease sources and reservoirs

Listeria monocytogenes has a large number of reservoirs in nature and industrial environments, and it has been isolated from various sources including asymptomatic humans, animals, plants, and environmental samples. *L. monocytogenes* has been isolated from a wide range of sources including soil, water, sewage, vegetation, and animal feces. It has also been found in many food processing environments, such as food contact surfaces, equipment, and drains. In addition, *L. monocytogenes* has been isolated from various animal species, including domesticated pets and agricultural animals, as well as from numerous wild animals, such as birds, rodents, and marine mammals (Kathariou et al., 2002; Linke et al., 2014). due to the widespread presence of *L. monocytogenes*, it is estimated that 2-6% of healthy individuals are asymptomatic fecal carriers of the bacterium (Rees et al., 2017). Similarly, *L. monocytogenes* has been isolated from various types of food, including dairy products, meats, seafood, and vegetables, which can serve as sources of infection for humans (Ferreira et al., 2014). *L. monocytogenes* has been found in a variety of foods sold in stores. There are numerous ways for it to enter the food chain because it can naturally be found in close proximity to numerous food items that are both of plant and animal origin. However, *Listeria* has also been found to be able to colonize food production equipment and facilities, which has resulted in the cross-contamination of food products inside the industrial environment. This is due to its ability to grow under low temperature and low nutrition circumstances. Although *L. monocytogenes* counts are frequently relatively low in these items, it is of particular concern in

refrigerated RTE foods with an extended shelf-life since *L. monocytogenes* might potentially multiply during chilled retail and home storage (Rees et al., 2017).

3.3.1.5 Infection dose

it is important to know that the infectious dose can vary widely depending on the specific food product and the individual's susceptibility to the bacteria and many different factors like the age and health of the individual consuming the contaminated food, and other environmental factors. It is conceivable that less than 1,000 cells may spread disease to susceptible people in circumstances involving raw or insufficiently pasteurized milk, for instance (FDA, 2012). According to estimates, *L. monocytogenes* has a very minimal risk of spreading disease when present in food at concentrations lower than 100 CFU/g, and healthy adults need not be concerned if there are less than 1000 CFU. More severe controls are in place for food supplied to healthcare facilities because it is believed that this level will make immunocompromised individuals ill (Rees et al., 2017).

3.3.1.6 Stress protein synthesis

Along the food value chain, *L. monocytogenes* faces numerous physical and chemical stresses that prevent it from growing and surviving as a foodborne pathogen (Wiktorczyk-Kapischke et al.,2021). Foodborne pathogens adapt their cellular processes to a state that enables them to maintain viability and growth in stressful situations in response to stress exposures (Vorob'eva, 2004). Sensing environmental changes and reprogramming gene expression to produce stress response proteins that help bacteria survive in challenging environments lead to the development of stress adaptive responses (Guerreiro et al., 2020). This ability of *L. monocytogenes* to adapt to stress is what allows it to colonize and persist in a variety of niches found in environments that are used for processing food, as well as to overcome hurdles associated with food processing and preservation (Bucur et al., 2018). The complex process of stress protein synthesis in *Listeria monocytogenes* entails the activation of particular genes and the production of specialized proteins. High temperature, low pH, and oxidative stress are only a few of the challenges that these proteins help the bacterium withstand (Sibanda et al., 2022).

3.3.1.7 Acid tolerance response

The complicated sensing system that *Listeria monocytogenes* has is an opportunistic pathogen with an unclear mechanism. To adapt to low pH conditions, *Listeria monocytogenes* has an Acid Tolerance Response (ATR) mechanism. To assist the bacteria, survive and thrive in acidic environments, the ATR involves the stimulation of numerous stress response genes and proteins, such as chaperones, transporters, and enzymes. The expression of virulence genes, which are essential for infection and survival within host cells, is likewise regulated by ATR, (Cotter et al.,2000). The molecular mechanisms underlying ATR *in L. monocytogenes* have been the subject of numerous research, for example, (Cotter et al.,2000). The sigma factor B is connected to the ATR and controls the production of genes involved in stress response, according to a different study by (O'Byrne et al., 2008).

3.3.1.8 Thermal rolerance of *Listeria monocytogenes*

Since ancient times, food manufacturing and preservation have used thermal treatments and temperature control as a means of preventing or limiting contamination and the spread of food-borne infections. However, *L. monocytogenes'* inherent capacity to survive and actively proliferate at temperatures between - 0.4 and 45 °C limits the effectiveness of thermal therapy against this pathogen (Bucur et al., 2018). To inactivate vegetative microbial cells of food-spoilage bacteria and food-borne diseases, mild thermal treatments (100 °C) are frequently used in the food processing industry. As long as food products are properly packaged and stored, these procedures provide food safety and a longer shelf life (Van et al., 2010). Nonetheless, the capacity of sublethally injured pathogenic bacteria to recover and thrive during post-processing storage continues to be the main issue with thermal processing of foods. Due to its ability to proliferate across a wide temperature range, *L. monocytogenes* is particularly relevant in this regard (Bucur et al., 2018). While *L. monocytogenes* does not exhibit particularly strong heat tolerance, it has been demonstrated to be more heat tolerant than other non-spore-forming pathogens like *Salmonella* and *E. coli* (Bucur et al., 2018). Several factors, like the age of the bacterial cells, test and growing settings, previous environmental challenges, or food components, can affect *L. monocytogenes*' resistance to heat (Doyle et al., 2001). Prior to a thermal challenge, *L. monocytogenes* cells subjected to sublethal stressors can become noticeably more heat resistant.

The scientists discovered that *L. monocytogenes* cells adapted to heat stress after being exposed to a temperature of 48°C for 30 min. Moreover, exposure to this minor stress for a brief period of time had no impact on the ability to grow (Shen et al., 2014). Acidity is a variable that may affect the thermotolerance of microorganisms. Fruit juices' acid-adaptation has been found to significantly boost *L. monocytogenes*' resistance to a subsequent heat treatment (Mazzotta, 2001). Moreover, compared to the exponential growth phase, the stationary phase of *L. monocytogenes* exhibits much higher D60 °C values (2.2 min) and greater heat tolerance (0.6 min) (Bucur et al., 2018). The expression of genes from particular heat-shock regulons, such as class I and class III heat-shock genes and genes of the SigB-dependent class II stress response, is involved in *L. monocytogenes*' response to 48°C at the molecular level. Moreover, the expression of recA, an SOS response activator linked to DNA repair, was upregulated (Veen et al., 2007). Heat-shock proteins (HSPs), which operate as intracellular chaperones and whose expression is boosted when denatured proteins build up in the cytoplasm, are produced by the class I heat-shock genes (*grpE, dnaK, dnaJ, groEL,* and *groES*). HSPs have the function of stabilizing and assembling partially unfolded proteins to stop them from aggregating in stressful situations. The first gene of the *dnaK* operon encodes the HrcA repressor, which in turn controls the expression of class I heat-shock genes under physiological growth circumstances at ambient temperature (Bucur et al., 2018). Under stressful circumstances, such as high temperature, ATP-dependent proteases (ClpC, ClpP, and ClpE) are needed for the destruction of misfolded proteins. The CtsR repressor, which is produced by the first gene in the *clpC* operon, negatively regulates these proteases (Nair et al., 2000).

3.3.2 *Salmonella enterica*

3.3.2.1 Characteristics

Salmonellae are rod-shaped (Figure 2.), facultatively anaerobic, Gram-negative bacteria that move by means of peritrichous flagella and are typically 2 to 5 microns long and 0.5 to 1.5 microns wide. *Salmonellae* are important pathogens for both humans and animals and are members of the Enterobacteriaceae family (Andino and Hanning, 2015). The majority of *Salmonellae* are catalase-

positive, oxidase-negative, lactose fermenters, and hydrogen sulfite makers. Salmonella can also be recognized by its ability to decarboxylate lysine, hydrolyze urea, and thrive exclusively on citrate as a carbon source, among other biochemical traits (Jensen et al., 2000; Abulreesh, 2012). *Salmonella* is not a fastidious microbe, it can grow on plain glucose-salts medium, and it can grow more quickly in media with high levels of supplements (Tajkarimi, 2007). The pH range for *Salmonella* growth is 4.1 to 9.0 (Abushelaibi et al., 2003).and the minimum pH value for its growth is 4.05 (Jay, 2000). But some can grow at pH of 3.7 (Tajkarimi, 2007). Between 0.96 to 0.999 is its optimum water activity range. Between 0.7 to 0.8, the microorganism dies quickly, and at lower values, more slowly (Tajkarimi, 2007). This microbe can grow between 5 and 45°C, with 35 to 37°C being the optimal temperature for growth (Tajkarimi, 2007). Since some strains of *Salmonella* can survive in temperatures as high as 54°C and as low as 2°C, the bacteria are thought to be mesophilic. (Kazmierczak et al., 2005). *Salmonella* is heat-sensitive and frequently dies at temperatures of 70 °C or higher (Jajere, 2019).

Salmonella can multiply by a factor of two every 20 minutes under ideal conditions of nutrients, water activity, temperature, and pH. Hence, foods that are wet and have a high pH are more likely to encourage the growth of *Salmonella*, especially when temperatures are elevated (over 5°C). To reduce the danger of salmonellosis, it is crucial to keep these items either chilled (< 5°C) or heated to temperatures above 60°C (Abushelaibi et al., 2003).

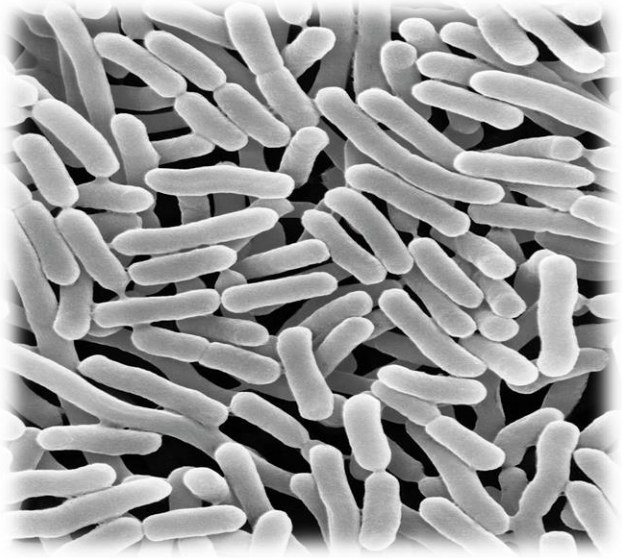

Figure 2. *Salmonella enterica* by microscopic (Internet 2)

3.3.2.2 Taxonomy and serotyping

The *S. enterica* is further divided into six subspecies based on biochemical characteristics and genomic relatedness. Roman numerals are used to identify these subspecies in the nomenclature: I. *S. enterica* subsp. *enterica*, II. *S. enterica* subsp. *salamae*, III. *S. enterica* subsp. *arizonae*, IIIa. *S. enterica* subsp. *diarizonae*, IV. *S. enterica* subsp. *houtenae*, and V. *S. enterica* subsp. *indica*. The most prevalent subspecies of Salmonella, *S. enterica* subsp. *enterica* (I), is primarily linked with mammals and is responsible for 99% of infections in humans and warm-blooded animals. The other five *S. enterica* subspecies and *S. bongori*, on the other hand, are primarily found in cold-blooded animals and the environment, and are uncommon in humans (Jajere, 2019). In addition to the subspecies categorization based on phylogeny, there is also the Kauffman and White classification scheme. This classification system further divides Salmonella into serotypes based on three key antigenic determinants: somatic (O), capsular (K), and flagella (H). The somatic (O) antigen, which is heat-stable and forms the oligosaccharide component of the lipopolysaccharide (LPS) of the bacterial cells, is found near the outer membrane of the cell. A certain *Salmonella* serotype may express more than one O antigen. The heat-labile H antigens are primarily located on bacterial flagella and are implicated in the activation of host immunological responses. The majority of *Salmonella* species include two distinct genes that produce the flagellar proteins. These microorganisms might be diphasic (phase I and II), which would give them the special capacity to express only one protein at a time. Whereas phase II antigens are non-specific and can be found in many other serotypes, phase I H antigens, which are in charge of immunological identification, can be expressed by various serotypes. Most *Salmonella* serotypes do not typically include the surface K antigens, which are heat-sensitive polysaccharides primarily found at the surface of the bacterial capsule. The Vi antigens, which belong to the K antigen subtype, are specific to the serotypes Typhi, Paratyphi C, and Dublin. The word "serovar," which is synonymous with "serotype," has frequently been used in the literature. A specific *Salmonella* serotype's name typically omits the subspecies. For instance, the name Salmonella ser. Typhi or *S.* Typhi is frequently used in literature to refer to *S. enterica* subspecies *enterica* serotype Typhi. More than 2500 serotypes have been found thus far, and more than half of these serotypes are associated with the *S. enterica* subspecies. Each serotype has a distinctive combination of somatic O and flagellar H1 and H2 antigens. The majority of human infections with Salmonella are caused by these serotypes (Jajere, 2019; Switt et al., 2009).

3.3.2.3 Illness

Worldwide, *Salmonella* is a significant contributor to bacterial foodborne diarrhea in people. Most *Salmonella* serotypes are capable of infecting both humans and animals, and some are host-adapted or host-specific. *S.* Pullorum and *S.* Gallinarum are adapted to poultry, *S.* Typhi to human, Infants and young children often have the highest incidence of non-typhoid *Salmonella*, and boys typically have a greater prevalence than girls. Infections with *Salmonella* are most common in the northern hemisphere around August and the southern hemisphere around March (Tajkarimi, 2007). Diarrhea (that can be bloody) is the primary symptom of a *Salmonella* infection in most cases. Fever, stomach cramps, nausea, vomiting, or a headache are other possible side effects. Typically, symptoms appear 6 hours to 6 days after infection and continue 4 to 7 days (CDC, 2019). Most people return to normal within four to seven days without the need for antibiotics. Nonetheless, some individuals with severe diarrhoea can require hospitalization or antibiotic treatment. There are three different syndromes of *Salmonella,* typhoid fever caused by *S.* Typhi, consist of a persistent fever, diarrhea, and stomach pain, and they may cause deadly liver, spleen, pulmonary, and neurological damage. Symptoms of typhoid fever last for two to three weeks. (Percival and Williams, 2014). Enteric fever caused by *S.* Paratyphi A, B, and C having an incubation period that is shorter, 1–10 days (Percival and Williams, 2014). Gastroenteritis symptoms caused by all other kinds of *Salmonella* (Tajkarimi, 2007). Gastroenteritis, an infection of the colon, often develops 18–48 hours following *Salmonella* consumption. The symptoms of gastroenteritis include diarrhea, fever, and stomach pain, usually self-limiting, the infection lasts 2 to 5 days (Percival and Williams, 2014).

3.3.2.4 Reservoirs and transmission

Animals, both domestic and wild, such as poultry, swine, cattle, birds, dogs, rats, tortoises, turtles, and cats, are reservoirs for *Salmonella*. Moreover, humans act as a reservoir, including asymptomatic patients and convalescent carriers. Chronic carriers are uncommon in humans but frequent in birds and other animals. Consuming food, milk, or water contaminated with the feces of infected hosts or meat products can result in a *Salmonella* infection. *Salmonella* Typhi and *S.* Paratyphi only colonize humans and are not widely spread in nature. The presence of these

organisms in humans suggests exposure to human waste. Non-typhoidal *Salmonella* species, on the other hand, are widely spread in nature and intimately related to mammals (Percival and Williams, 2014).

For human infections, fruits and vegetables are not a typical habitat. *Salmonella* contamination in the field or greenhouse is one theory, whereas direct or cross contamination during fruits and vegetables harvest, processing, and preparation as a raw agricultural commodity is another (Bartz et al., 2015). It is well known that bacteria on plant surfaces can join forces to build substantial biofilms. Cleaning and sanitizing the crops is challenging due to the biofilms' tenacity. Several elements are believed to play a role in epidemics involving plant products, such as fruits, nuts, and vine stalk vegetables (Table 4.), which are common sources (Andino and Hanning, 2015).

Table 4. Number of *Salmonella* foodborne outbreaks in the US connected to crop production from 2006 to 2011 (Andino and Hanning, 2015).

Food	Number of Outbreaks	Number of Illnesses
Fruits/nuts	36	2359
Sprouts	21	711
Vine stalk vegetables	21	3216
Leafy vegetables	11	306
Roots	6	172
Grains/beans	5	259
Oil/sugar	1	14
Fungus	1	10

Salmonella appears to be spread most frequently through the ingestion of tainted poultry and meat products. The main cause of meat contamination is when it comes into contact with feces while being slaughtered. *Salmonella* can spread after the meat has been compromised due to poor storage or undercooking. Food and water can become contaminated with *Salmonella* when it is expelled in feces, making it possible for humans to become infected. And one of the transmission methods is person-to-person transmission and faecal–oral transmission (Percival and Williams, 2014). The United States has a 0.1% prevalence of *Salmonella* in people. Between 0 and 30%, the prevalence in animals is typically higher (Tajkarimi, 2007).

3.3.2.5 Infection dose

According on the serotype, the infectious dosage varies. The infectious dosage of non-typhoidal salmonellosis is about 10^3 bacilli. The infectious dosage for enteric fever is roughly 10^5 bacilli when consumed. Individuals with achlorhydria, impaired cell-mediated immunity, or advanced age may contract the infection at a lower infectious dosage. The level of acidity in a person's stomach may also affect the infectious dose (Agency of Canada, 2011). Some contest the idea of a minimal infectious dose and assert that any quantity of *Salmonella* can induce infection, however the likelihood varies with the quantity. It is necessary to take into account factors including the virulence of the *Salmonella* strain, individual tolerances and susceptibilities, and stomach content. *Salmonella* is resistant to stomach acid in foods high in lipids like chocolate, ice cream, or peanut butter, and in these it only takes a few cells to cause an infection (Tajkarimi, 2007).

3.3.2.6 Acid tolerance

The PhoP/PhoQ system controls a number of virulence factors in bacteria, including *Salmonella*. PhoP genes influence the bacterial cell membrane by promoting survival inside the macrophage and boosting resistance to low pH. When exposed to acidic conditions ranging from pH 5.5 to 6.0 (preshock), *Salmonella* triggers an advanced acid tolerance response (ATR), which prepares the cells to adapt to subsequent exposure to pH 4.5 (acid shock), which increases the likelihood of *Salmonella* survival under severely acidic conditions (pH 3.0 to 4.0). *RpoS* sigma factor and *Pho*PQ, two acid shock proteins, are necessary for the ATR process. It has been demonstrated that

RpoS and PhoPQ offer defense against inorganic acids, while *RpoS*, the iron regulatory protein *Fur*, and the adaptive response protein Ada offer significant resistance to the stress of organic acids (Andino and Hanning, 2015).

3.3.2.7 Thermal tolerance of *Salmonella enterica*

Salmonella strains can withstand heat treatment and the environment of food processing due to a number of variables (Andino and Hanning, 2015). For instance, prior to thermal treatment, preexposure to stress may improve the ability to survive throughout processing. In particular, it was discovered that *S.* Senftenberg could live in broiler litter for up to 24 hours at 80°C (Chen et al., 2013). It is well known that when microorganisms are exposed to heat, they respond differently depending on their previous development environment, with stationary phase cells demonstrating more heat resistance than log phase cells (Dawoud et al., 2017). Additionally, it has been demonstrated that stressed cells, such as those that have been subjected to temperatures that are just a little above an organism's ideal growth range (also known as heat shocked cells), those that have been grown on scarce carbon sources, those that have been desiccated, and those that have been starved before heat treatments, have higher levels of thermal tolerance (Dawoud et al., 2017).

When exposed to higher than ideal temperatures, misfolded and unfolded proteins accumulate in significant amounts in the cytoplasmic membrane and the outer membrane as a protective response. This, in turn, triggers the expression of heat shock proteins (HSPs) through the regulation of the heat shock factor σ^H (Dawoud et al., 2017). Proteins involved in heat shock are *DnaK, DnaJ, GrpE, ClpP, HscAB,* σ^H *and* σ^{32} *, FourU, TlpA, HtrA, RpoS*^{a}*, FkpA, SurA*^{a}*, H-NS* (Dawoud et al., 2017). Chaperones, proteases, and tiny heat shock proteins are produced to induce the creation of HSPs (s-HSPs). They play a protective role by refolding saved proteins, eliminating harmed proteins, and fixing deteriorating protein aggregation (Dawoud et al., 2017).

When heat stress is detected, sigma factors are expressed, which starts the adaptive regulation of genes There are two distinct thermal stress responses in cells: the cytoplasmic thermal stress response, which is governed by the heat shock sigma factor, σ^H or σ^{32}, and the extracytoplasmic thermal stress response, which is controlled by the extracytoplasmic function sigma factor, σ^E or

σ^{24}. also known as extreme heat stress sigma factor, are the two sigma factors that are typically expressed. Sigma factors are a sizable collection of genes that express proteins with essential processes connected to the RNA polymerase holoenzyme complex that serve as instructions for core RNA polymerases to recognize their promoters and start transcription (Dawoud et al., 2017). The alternate sigma factors $\sigma^{32/H}$ and $\sigma^{24/E}$ control heat shock reactions. These two components, which are encoded by the *rpoH* and *rpoE* genes, are the third and fourth subgroups of sigma factors, respectively. *RpoH* controls both the transcription of heat shock genes and its own translation. The translation of the *rpoH* gene is prevented when the temperature is within the range that is ideal for microbial development. With rising temperatures (42°C), the stem III and I of the *rpoH* mRNA secondary structure are freed, facilitating ribosomal binding and improving translation efficiency (Dawoud et al., 2017). Around 100 genes have been shown to be regulated by sigma factors related to the response to heat stress. More than 30 of them are under the control of sigma factor $\sigma^{32/H}$, the majority of which are linked chaperones and proteases (Dawoud et al., 2017). *Salmonella*'s ability to adapt to heat shock can have a variety of additional impacts, including an increase in virulence potential through gene regulatory systems. Thermal stress causes defensive reactions in *Salmonella* and can alter the levels of gene expression in virulence genes.

One of the cellular stress reactions is cross-protection that happens when a first, non-fatal injury brought on by exposure to an environmental stress strengthens resistance to a second, subsequent stress (Gruzdev et al., 2011). This phenomenon, whereby insufficient cooking and heat treatments during food processing could actually boost *Salmonella* and other foodborne bacteria' capacity for cross-protection and thermal resistance by enhancing virulence, has been attributed to (Dawoud et al., 2017).

Knowing the kinetics of thermal inactivation of the target microorganism is important to calculate the decimal-reduction (D) time and associated temperature (z) in bacteria in order to create an efficient thermal inactivation of foodborne pathogens (Stringer and Metris, 2018). The intrinsic properties of the product, particularly in respect to nutrient composition, pH, and water activity, determine these kinetics parameters (Cebrian et al., 2017).

4. MATERIALS AND METHODS

4.1 Strawberry puree, smoothie, and sterile distilled water

Commercially available frozen strawberries were purchased from a Lidl store in Budapest, Hungary. The strawberries were subsequently homogenized using a blender within the Department of Food Microbiology, Hygiene and Safety at the Hungarian University of Agriculture and Life Sciences.

A smoothie was prepared using a commercially available mixture of frozen fruits, which included strawberries (37.2 %), banana (24.1%), avocado (13.3%), as well as almond milk (25.4%) that was also obtained from the Lidl store.

Distilled water was prepared within the laboratory setting and subsequently subjected to autoclaving at 121 °C for a duration of 15 minutes.

4.2 Bacterial strains

Strains of *Salmonella enterica* serotype Hartford and *Listeria monocytogenes* CCM4699 were acquired from the local laboratory of the Department of Food Microbiology, Hygiene and Safety, at the Hungarian University of Agriculture and Life Sciences.

The bacterial strains were stored in the freezer of the department. Before the experiments, the strains were recultivated on TSA medium. For each experiments strains were subcultured on TSA agar and incubated at a temperature of 37 °C for one day to obtain a fresh and active strain (Figure 3.), prior to their use in the experiments.

Figure 3. fresh strains for *Salmonella enterica* and *Listeria monocytogenes*

4.3 Media, diluent solution

XLD Agar (Xylose Lysine Deoxycholate Agar) is a differential and moderately selective medium that is utilized for the isolation and differentiation of Gram-negative enteric pathogens, with particularly *Salmonella*, in food samples (Taylor, 1968). The media was prepared following the instructions provided by the manufacturer. Specifically, 56.7 grams of the agar powder were dissolved in one liter of distilled water and heated slightly, without autoclaving.

Palcam *Listeria* Selective Agar is a selective agar medium utilized for differential diagnosis as well as for the isolation and detection of *Listeria monocytogenes* from fecal and biological specimens, food samples, and heavily contaminated environmental materials (Van Netten et al., 1989). To prepare the selective agar for the isolation and detection of *Listeria monocytogenes*, 68.8 grams of the agar powder were dissolved in one liter of distilled water according to the manufacturer's instructions. The agar was sterilized by autoclaving at 121 °C for a duration of 15 minutes. The media was then cooled down to 50 °C, and the dissolved contents of two vials of Palcam Listeria Selective Supplement (Cat No. 91986) were added. The supplement was acquired from Sigma-Aldrich and manufactured in Spain. 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.

Trypticase Soy Agar (TSA), called Soybean-Casein Digest Agar Medium by the United States Pharmacopeia is a general-purpose nonselective growth medium that supports the growth of most Gram-negative and non-fastidious Gram-positive bacteria as well as many yeasts and moulds (Interenet X). The culture medium was prepared by adding 30 grams of Tryptic Soy Broth (TSB) and 15 grams of bacteriological agar to one liter of distilled water. The mixture was sterilized by autoclaving at a temperature of 121 degrees Celsius for 15 minutes.

The diluent solution 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. The resulting solution was then distributed into 9-ml tubes.

After the solution was distributed, the tubes were sterilized by autoclaving at a temperature of 121 °C for 15 minutes.

4.4 Heat treatment experiment

Heat treatments were 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 directly before the heat treatments. The cell count was adjusted using a DEN-1BMcFarland Densitometer (Biosan). Samples were prepared in Eppendorf tubes (1,5 ml of matrix/tube). Separate tubes were prepared for each sampling time. Three replications were performed for each time period to ensure data accuracy and reproducibility. As a control, three replicate samples were prepared without any heat treatment to ensure that any observed effects were not due to factors other than the heat treatment. Heat treatment was carried out in a water bath at three temperatures: 55, 65 and 75 °C. At 55 °C samples were taken at 0, 2.5, 5, 7.5, and 10 minutes. Samples were taken at 65 °C at 0, 1, 2 and 4 minutes, while at 75 °C 0, 0.5, 1 and 1.5 minutes, respectively.

Following the heat treatment ten-fold serial dilutions were prepared from the samples. The appropriate members of the dilution series were inoculated to agar plates using the TAL method.

With the help of the TAL method the number of injured cells (non-intact cells) that result from heat treatment can be determined. Enumeration of injured *Listeria monocytogenes* and *Salmonella* Hartford was performed using the thin agar layer method (Kang and Fung, 2000). 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 with TSA agar. Intact cells grow well both in selective and non-selective media (Palcam or XLD agar), but injured cells can form colonies only on non-selective media (TSA agar). In the TAL method injured cells can resuscitate and grow on the top layer of the non-selective TSA agar while selective agents of the bottom agar (XLD or Palcam agar) diffuse from the selective agar to TSA layer. Then colonies form typical reactions with the selective components of the selective agar, which enable differentiation of pathogen from the background microflora of a food sample. The number of injured cells was calculated by subtracting the number of viable cells on the selective agar alone from the number of viable cells on the overlaid selective agar.

Inoculation was done by spread plating (0.1 ml inoculum/Petri dish) or by the "drop" method.

To culture the bacteria with the "drop" method 10-10 µL of the appropriate dilution were pipetted on the sterile agar surfaces (Figure 4.).

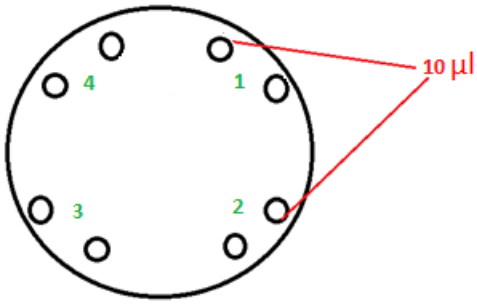

Figure 4. Method of dropping onto Petri dishes containing growth media.

5. RESULTS AND DISCUSSION

5.1 Heat treatment at 55 °C in sterile distilled water

5.1.1 Survival of *Salmonella enterica* at 55 °C in sterile distilled water

The number of surviving *Salmonella* cells was monitored after heat treatment at 55 °C on XLD agar for the number of non-injured bacteria and on a TSA agar for total number of bacteria. The number of injured cells was determined by the difference between these values.

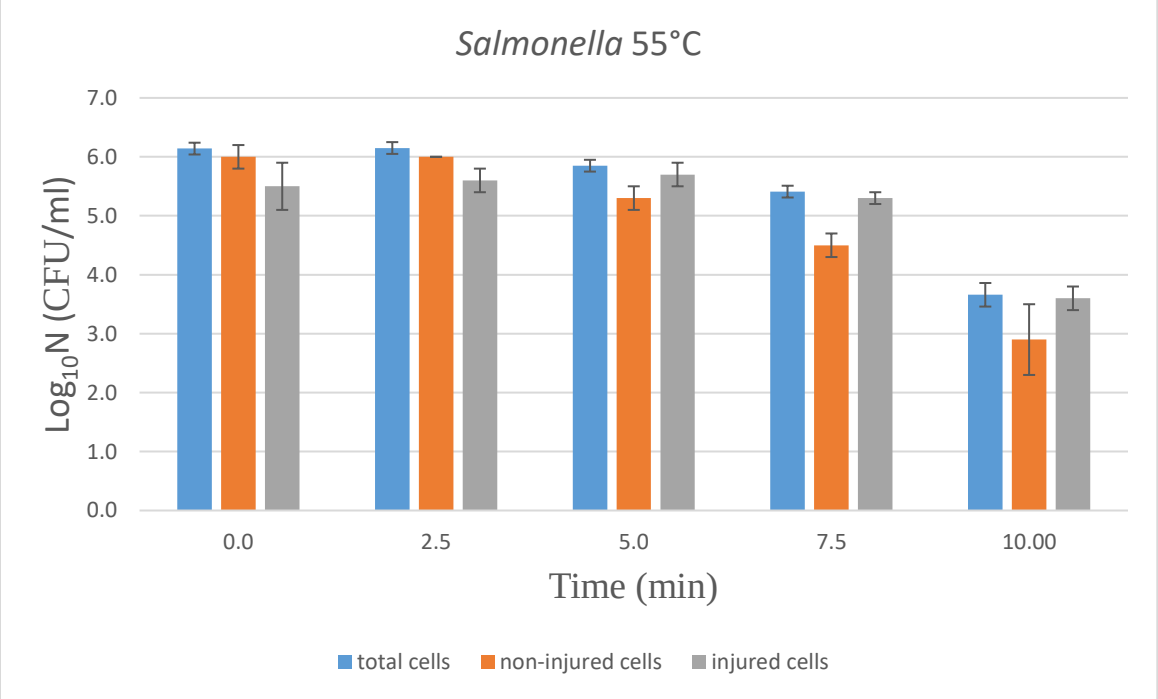

Figure 5. Number of survivals of *Salmonella enterica* after 55 °C - heat treatment in sterile distilled water

The results of the experiment showed that the total cell count decreased with increasing duration of heat treatment (Figure 5.). At 0 minutes (the control), the total cell count was 6.1 log CFU/ml, whereas at 10 minutes of heat treatment, it decreased to 3.7 log CFU/ml. This suggests that the heat treatment was effective in reducing the viability of *Salmonella*. The non-injured cell count also decreased with increasing duration of heat treatment, indicating that some of the cells were

not able to survive the heat treatment. At 0 minutes (no-heat treatment), the non-injured cell count was 6 log CFU/ml, whereas at 10 minutes of heat treatment, it decreased to 2.9 log CFU/ml. The injured cell count, on the other hand, increased with increasing duration of heat treatment. Although the absolute number of injured cells decreased with increasing treatment time, the proportion of injured cells in relation to the total microbial count at that time point increased gradually, i.e., as the heat treatment time progressed, the proportion of damaged cells in the population increased more and more.

The standard deviation of the total cell count increased with increasing duration of heat treatment, indicating that the variability in the results also increased. This could be because the heat treatment was more severe at longer durations and had a greater effect on some cells than on others.

Overall, the results of the experiment suggest that *Salmonella* is sensitive to heat treatment and that longer durations of heat treatment are more effective in reducing the viability of the bacteria. However, since the proportion of damaged cells increased with treatment time, it should be kept in mind that damaged cells can regenerate after insufficient heat treatment, which could have implications for food safety and the prevention of foodborne illness.

To determine the D value for a given temperature, a logarithmic plot of the surviving microorganisms (total cell) against the corresponding heating times was created, and a regression line (y=mx+b) was generated (Figure 6.). The slope of this line was utilized to calculate the D value using the formula D = -1/m. To fit a regression line, at least three heating times were required. The minimum heating time required to achieve a one-log cycle reduction in the bacterial population at 55 °C, known as the D_{55} value, was determined to be 4.39 min. This value can be employed in the food industry to guarantee food safety by calculating the heat treatment time needed to attain a specific level of reduction in the *Salmonella* population. To compare the D-value obtained in this experiment a study conducted by Dufort and co-workers (2017) found a D_{54} value of 3.79 minutes for *S. enterica* in acidified tomato purée as the test matrix. It is important to note that this difference in the results could be due to several factors, including the use of a slightly different temperature, and a different experimental medium.

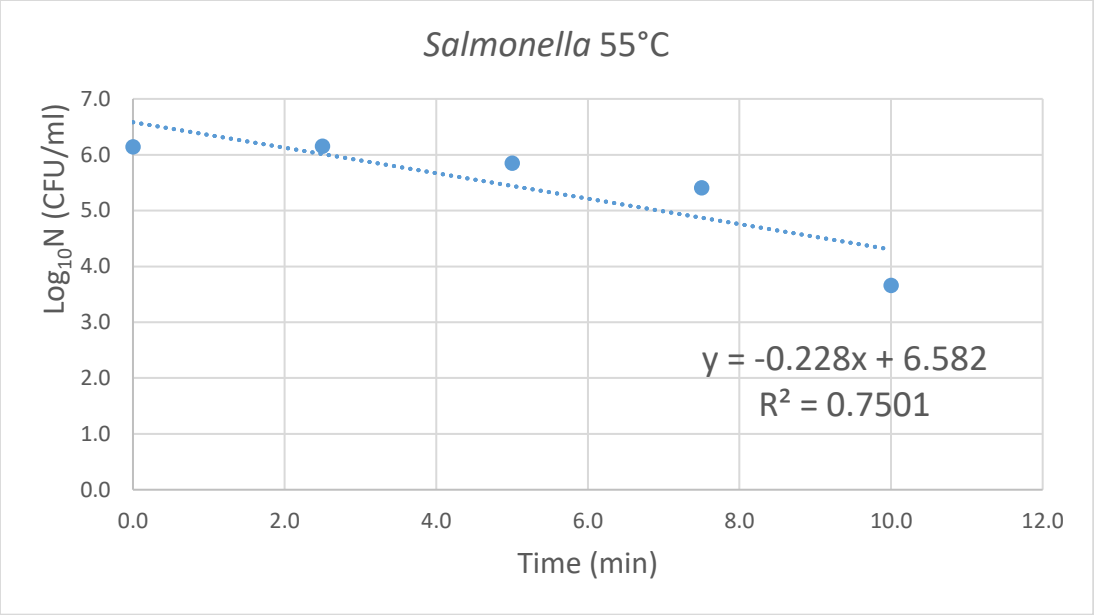

Figure 6. Survival curve (considering total cell counts) of *S. enterica* at 55 °C heat treatment in sterile distilled water

5.1.2 Survival of *Listeria monocytogenes* at 55 °C in sterile distilled water

Listeria survival was monitored after heat treatment at 55 °C on Palcam *Listeria* selective agar for the number of non-injured bacteria and on a TSA agar for total number of bacteria. The difference between these values resulted in the number of injured cells.

The results of the experiment showed that as the exposure time to heat increased, the total number of *Listeria* cells decreased (Figure 7.). After exposure to heat for 2.5 minutes, the total number of cells decreased to 6.5 log CFU/ml. The non-injured cells were 6.4 log CFU/ml, while the injured cells were 5.8 log CFU/ml. This suggests that some cells were able to survive the heat treatment, but their ability to grow was impaired. Further increasing the exposure time to heat for 5 minutes resulted in a more significant decrease in the total number of cells to 5.6 log CFU/ml. The number of the non-injured cells were 5.1 log CFU/ml, while the injured cells were 5.4 log CFU/ml. This indicates that more cells were injured, and only a small number were able to survive the heat treatment. After exposure to heat for 7.5 minutes, the total number of cells decreased dramatically to 3.4 log CFU/ml, and the number of non-injured cells were only 2.4 log CFU/ml, while the injured cells were 3.3 log CFU/ml. This shows that most of the cells were unable to survive the heat treatment. Finally, after exposure to heat for 10 minutes, the total number of cells further

decreased to 3.3 log CFU/ml. The number of non-injured cells were only 2.4 log CFU/ml, while the injured cells were 3.3 log CFU/ml. This indicates that the heat treatment was lethal to most of the *Listeria* cells, and only a few were able to survive.

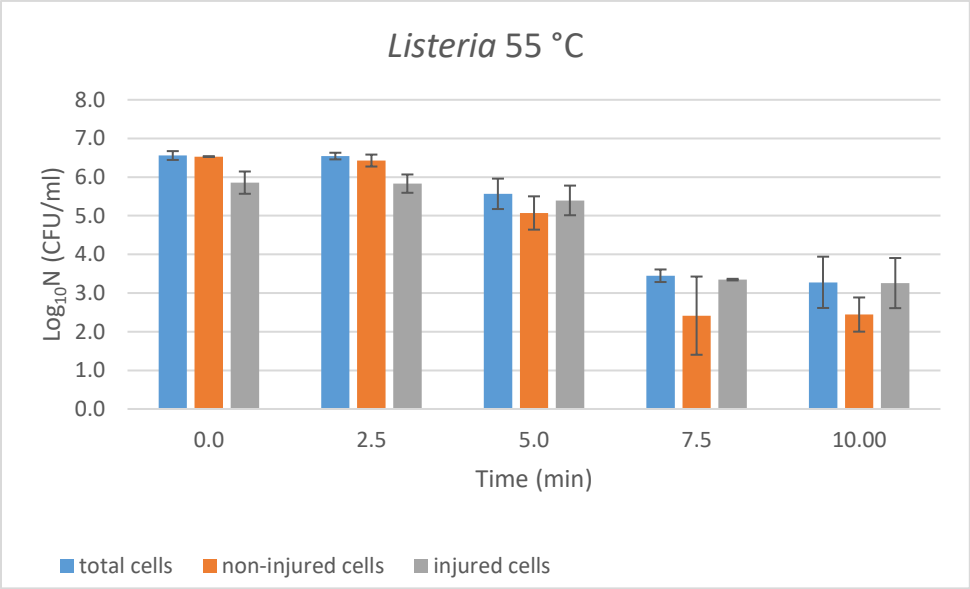

Figure 7. Number of survivals of *Listeria monocytogenes* at 55 °C heat treatment in sterile distilled water

Overall, the results of this experiment demonstrate that *Listeria* has a low tolerance to heat, and exposure to a temperature of 55 degrees Celsius for more than 5 minutes could significantly reduce the total number of cells. The injured cells that were able to recover and grow again on TSA agar suggest that under the right conditions some cells may be able to recover from heat stress and may represent a potential source of contamination.

The regression line (y=mx+b) was generated (Figure 8.), and the D_{55} value was determined to be 2.59 min.

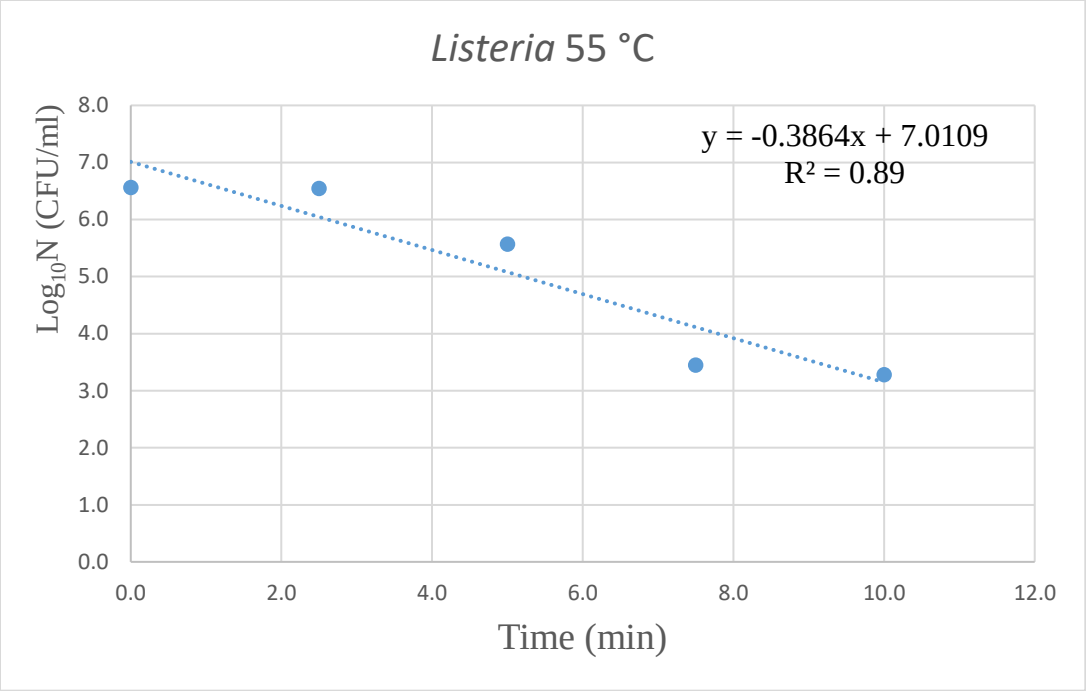

Figure 8. Survival curve (considering total cell count) of *L. monocytogenes* at 55 °C heat treatment in sterile distilled water

5.2 Heat treatment at 65 °C in sterile distilled water

5.2.1 Survival of *Salmonella enterica* at 65 °C in sterile distilled water

Salmonella Survival was monitored after heat treatment at 65 °C on XLD agar for the number of non-injured bacteria and on a TSA agar for the number of the total bacteria. The number of injured cells was determined by the difference between these values.

The results showed a clear trend in the reduction of bacterial survival with increased exposure to heat. For the non-injured cells, the $\log_{10}$N values remained constant for the 1-minute treatment (Figure 9.), However, for the 2 and 4-minute treatments, there is a significant decrease in the number of non-injured cells, indicating that they have been killed or damaged by the heat treatment.

For the injured cells, the $\log_{10}$N values decreased with increasing exposure time to heat treatment, indicating that a longer exposure time leads to fewer injured cells that under optimal conditions

might later recover and grow again. This finding suggests that the extent of injury to the bacterial cells increases with increasing exposure time to heat treatment.

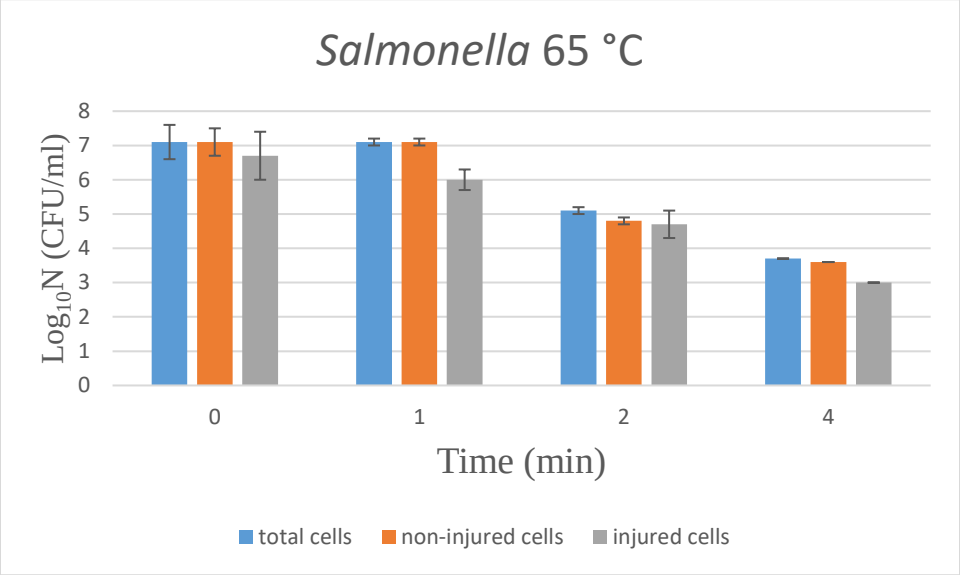

Figure 8. Number of survivals *of Salmonella enterica* at 65 °C in sterile distilled water

The regression line (y=mx+b) was generated (Figure 9.), and the D_{65} value was determined to be 1.1 min.

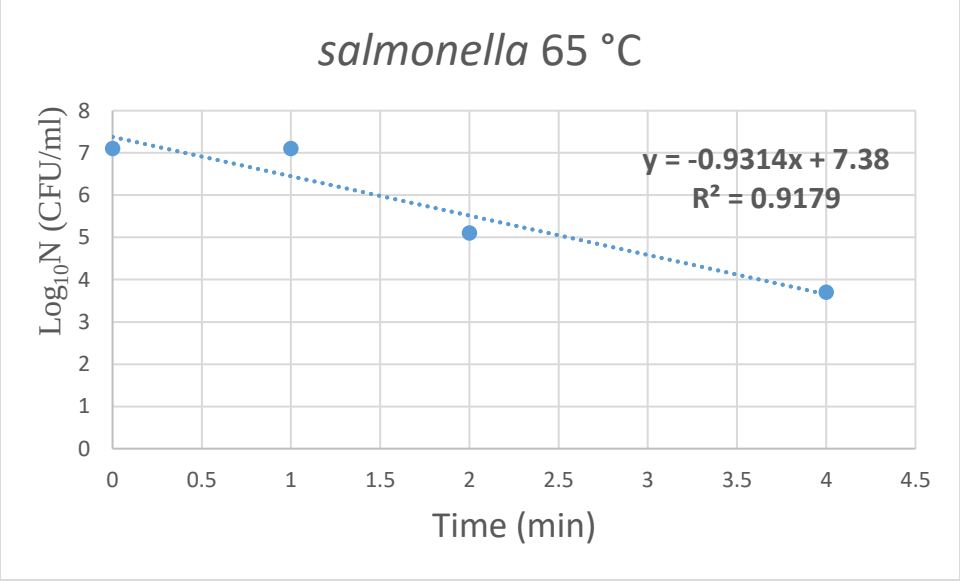

Figure 9. Survival curve (considering total cell count) of *S. enterica* at 65 °C heat treatment in sterile distilled water

5.2.2 Survival of *listeria monocytogenes* at 65 °C in sterile distilled water

Listeria survival was monitored after heat treatment at 65 °C on Palcam *Listeria* selective agar for the number of non-injured bacteria and on a TSA agar for the number of total bacteria. The difference between these values resulted in the number of injured cells.

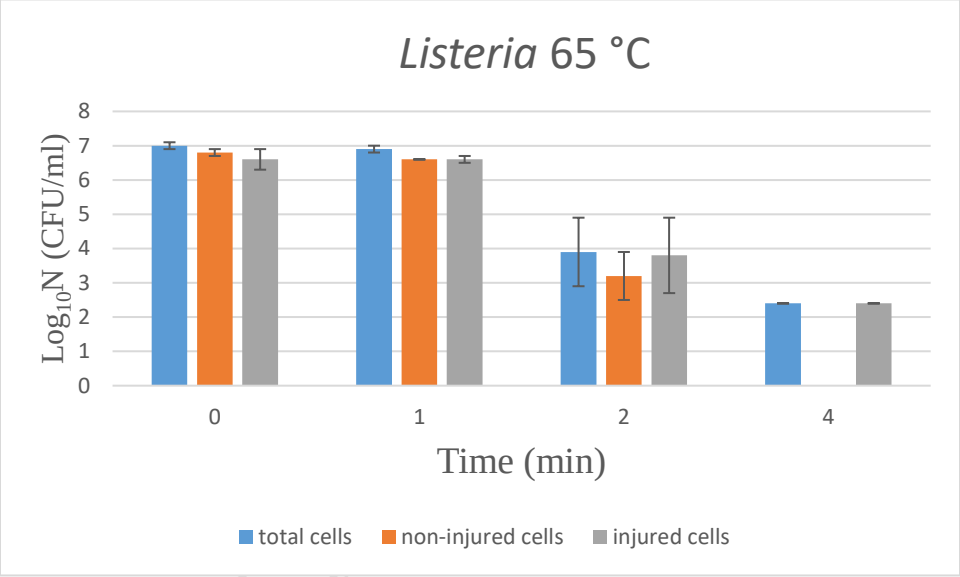

Figure 10. Number of survivals *of Listeria monocytogenes* at 65 °C in sterile distilled water

For the 1-minute heat treatment, the $log_{10}N$ of total cell count decreased to 6.9 log CFU/ml, which is a slight reduction compared to the control sample (Figure 10.). The number of non-injured cells remained almost the same as the control sample, while the injured cells showed no significant change in their $log_{10}N$ value.

However, the 2-minute heat treatment showed a significant reduction in the log CFU/ml of total cell count to 3.9 log CFU/ml, which indicates that the majority of the bacteria were killed. The number of non-injured cells were severely affected to a $log_{10}N$ value of 3.2 log CFU/ml, while the injured cells showed a $log_{10}N$ value of 3.8 log CFU/ml with high standard deviation of the injured cells.

The 4-minute heat treatment showed a further reduction in the $\log_{10}$N of total cell count to 2.4 log CFU/ml, which indicates that most of the bacteria were killed. Non-injured cells were not detected, indicating that all intact cells were killed at this temperature and all survival cells were injured.

The results of the experiment indicate that *Listeria monocytogenes* is sensitive to heat treatment, and even a short exposure of few minutes can reduce the total cell count. However, a longer exposure of 4 minutes was able to kill all the non-injured cells, resulting significant number of injured cells that are not able to grow on selective media, so they can escape detection and false positive samples during microbiological investigations.

The regression line (y=mx+b) was generated (Figure 11.), the D_{65} value, was determined to be 0.79 min.

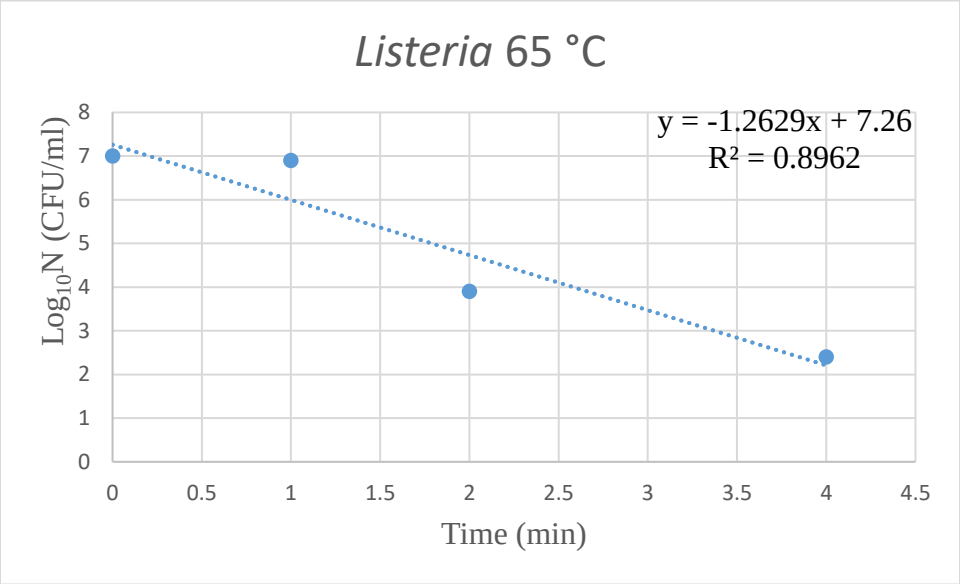

Figure 11. Survival curve (considering total cell count) of *L. monocytogenes* at 65 °C heat treatment in sterile distilled water

5.3 Heat treatment at 75 °C in sterile distilled water

5.3.1 Survival of *Salmonella enterica* at 75 °C in sterile distilled water

Salmonella survival was monitored after heat treatment at 55 °C on XLD agar (Figure 12.), for the number of non-injured bacteria and on a TSA agar (Figure 13.) for the number of total bacteria. The number of injured cells was determined by the difference between these values.

Figure 12. Growth of *S. enterica* on XLD agar after 1-min heat treatment at 75 °C

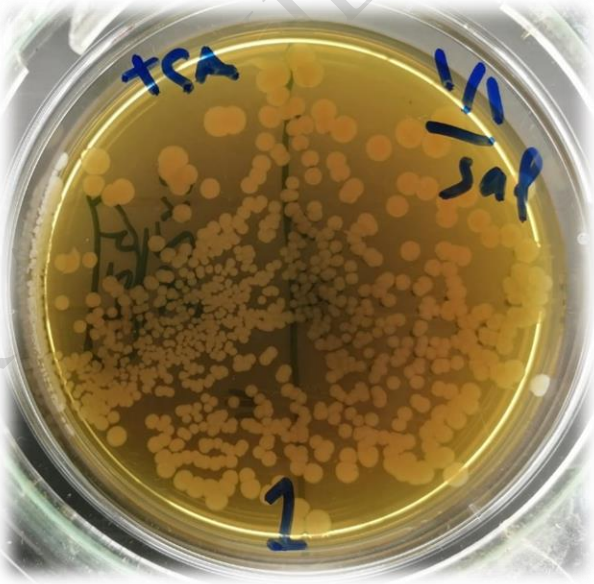

Figure 13. Growth of *S. enterica* on TSA agar after 1-min heat treatment at 75 °C

At 0.5 minutes of heat treatment, the number of total cells decreased, and the number of injured cells increased significantly (Figure 14.), indicating that the bacteria were injured by the heat

treatment. However, the non-injured cells remained relatively constant, indicating that some cells were able to survive the heat treatment.

At 1 minute of heat treatment, there was a drastic reduction in the number of total cells, non-injured cells, and injured cells. This result suggests that the heat treatment was severe enough to kill most of the bacteria, including those that were initially able to survive the 0.5-minute heat treatment. At 1.5 minutes of heat treatment, no growth was observed, indicating that the heat treatment was sufficient to kill all the bacteria, including the cells that were initially able to survive the 1-minute heat treatment.

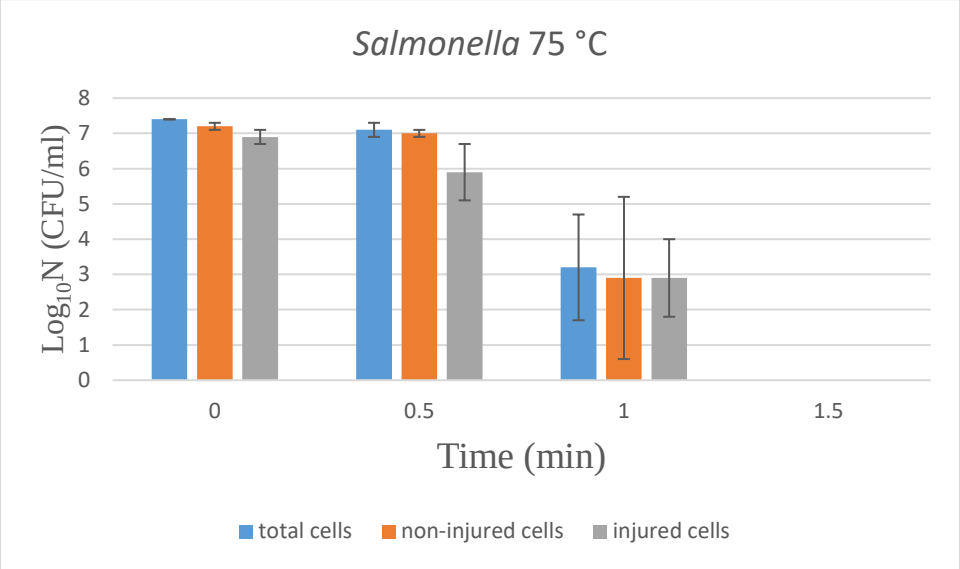

Figure 14. Number of survivals *of Salmonella enterica* at 75 °C in sterile distilled water

The regression line (y=mx+b) was generated (Figure 15.), and the D_{75} value was determined to be 0.24 min.

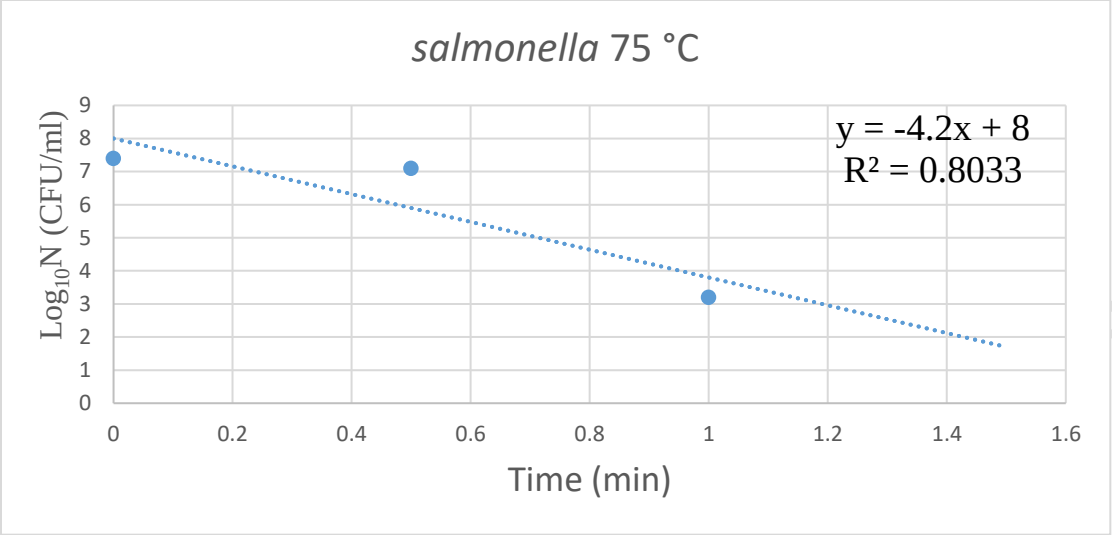

Figure 15. total cell count of *S. enterica* at 75 °C heat treatment in sterile distilled water

A heat resistance curve was plotted based on the results of the three heat treatment experiments. Z value was calculated using the formula z = -1/m, which was derived from the regression line's negative slope over the $\log_{10}$ of D values at the appropriate temperatures. The Z-value is a measure of the temperature sensitivity of bacteria and indicates the increase in temperature required to achieve a one log reduction in D value.

The regression line (y=mx+b) was generated (Figure 16.), and the z value was determined to be 4.6 °C.

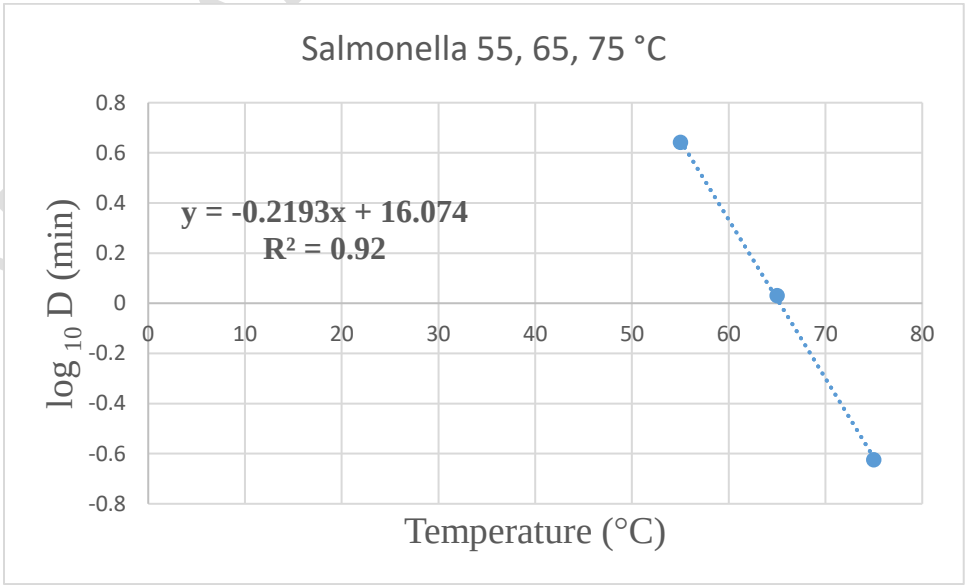

Figure 16. z value for *S. enterica* in sterile distilled water

5.3.2 Survival of *Listeria monocytogenes* at 75 °C in sterile distilled water

Listeria survival was monitored after heat treatment at 75 °C on Palcam *Listeria* Selective agar (Figure 17.) for the number of non-injured bacteria and on a TSA agar for the number of total bacteria (Figure 18.). The difference between these values resulted in the number of injured cells.

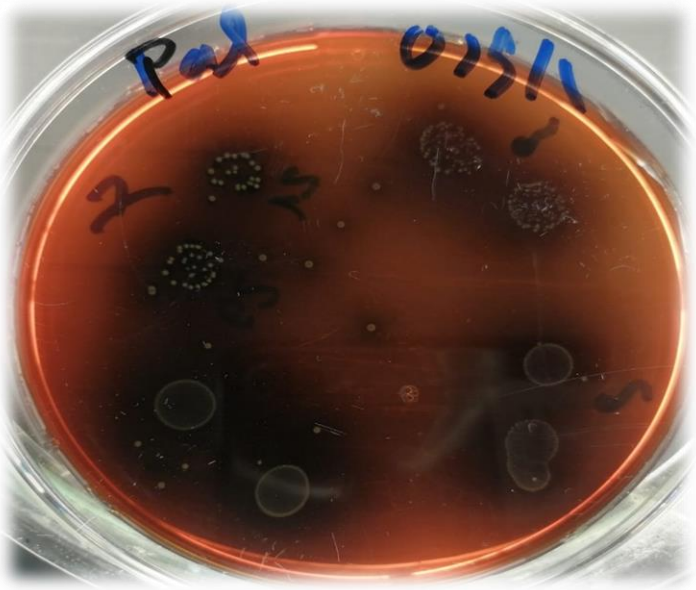

Figure 17. Growth of *L. monocytogenes* on Palcam agar after 0.5-min heat treatment at 75 °C

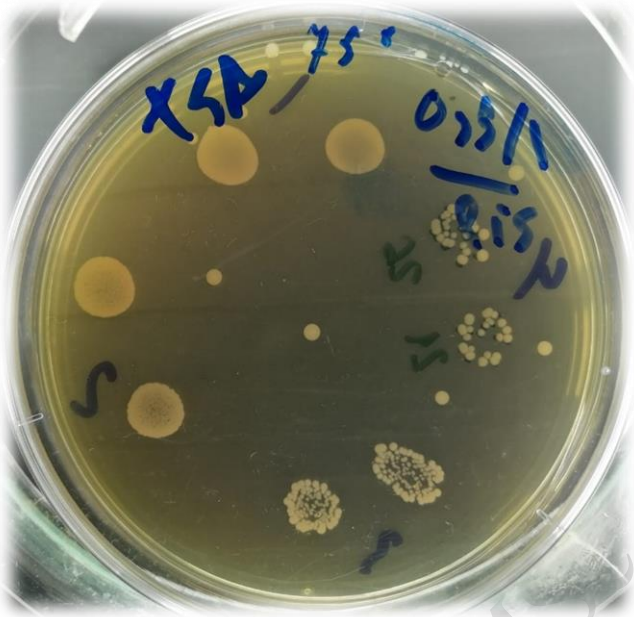

Figure 18. Growth of *L. monocytogenes* on TSA agar after 0.5-min heat treatment at 75 °C

The results of the experiment indicate a clear effect of heat treatment on the survival of *Listeria monocytogenes* by the log CFU/ml values for total cells and non-injured cells.

The control samples, which were not exposed to any heat treatment, had the highest log CFU/ml values, with a mean value of 7.5 log CFU/ml for total cells, 7.4 log CFU/ml for non-injured cells, and 6.9 log CFU/ml for injured cells (Figure 18.). The log CFU/ml values for injured cells increased slightly after 0.5 minutes of heat treatment but decreased significantly at 1 and 1.5 minutes.

The standard deviation values for each set of data provide an estimate of the variability within the data set. In general, the standard deviation values were low, indicating that the data were relatively consistent and reliable. Overall, the results of this experiment indicate that *Listeria monocytogenes* is sensitive to heat treatment and that longer exposure to high temperatures results in a greater reduction.

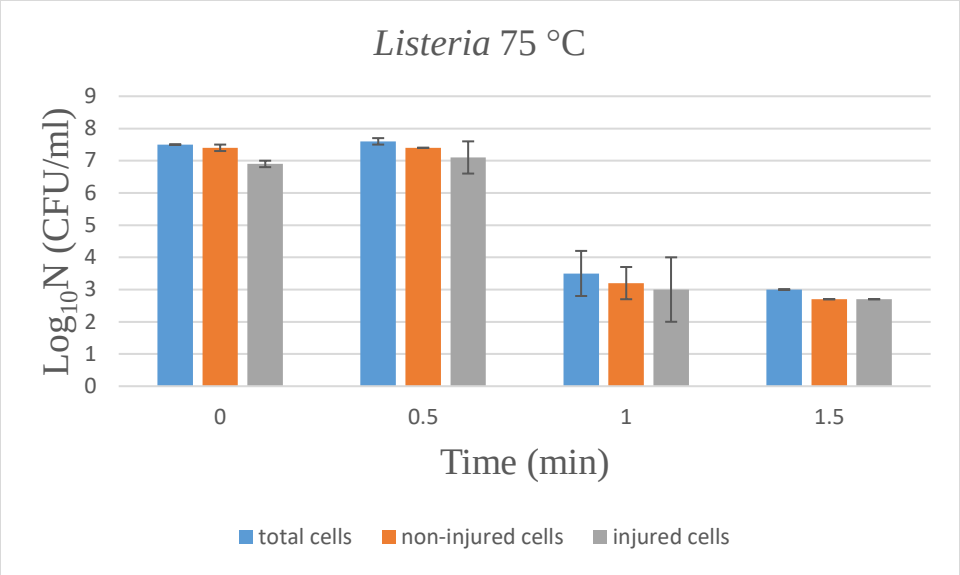

Figure 19. Number of survivals *of Listeria monocytogenes* at 75 °C in sterile distilled water

A heat resistance curve was plotted based on the results of the three heat treatment experiments. The regression line (y=mx+b) was generated (Figure 20.), and the D_{75} value was determined to be 0.28 min.

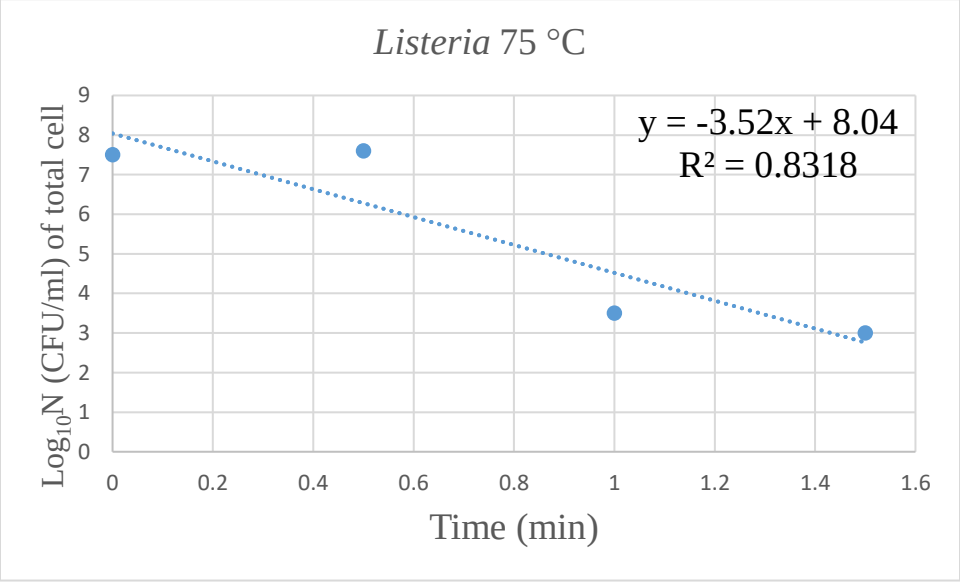

Figure 20. Survival curve (considering total cell count) of *L. monocytogenes* at 75 °C heat treatment in sterile distilled water

A heat resistance curve was plotted based on the results of the three heat treatment experiments. The regression line (y=mx+b) was generated (Figure 21.), and the z value was determined to be 20.83 °C.

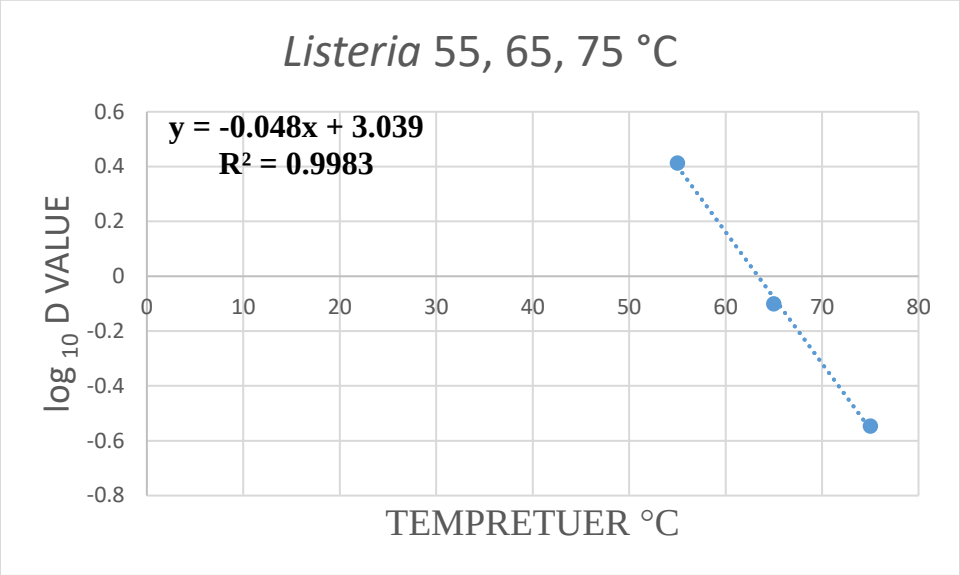

Figure 21. z value for *L. monocytogenes* in distilled sterile water

5.4 Heat treatment at 55 °C in smoothie

5.4.1 Survival of *Salmonella enterica* at 55 °C in smoothie

Salmonella survival was monitored after heat treatment at 55 °C on XLD agar for the number of non-injured bacteria and on a TAL-XLD agar for the number of total bacteria. The number of injured cells was determined by the difference between these values.

At 2.5 minutes of heat treatment, there was no significant reduction in the total cell count compared to the control samples (Figure 22.). However, at 5 minutes of heat treatment, there was a significant reduction in the total cell count. At 7.5 minutes of heat treatment, there was a significant decrease in the number of survivals.

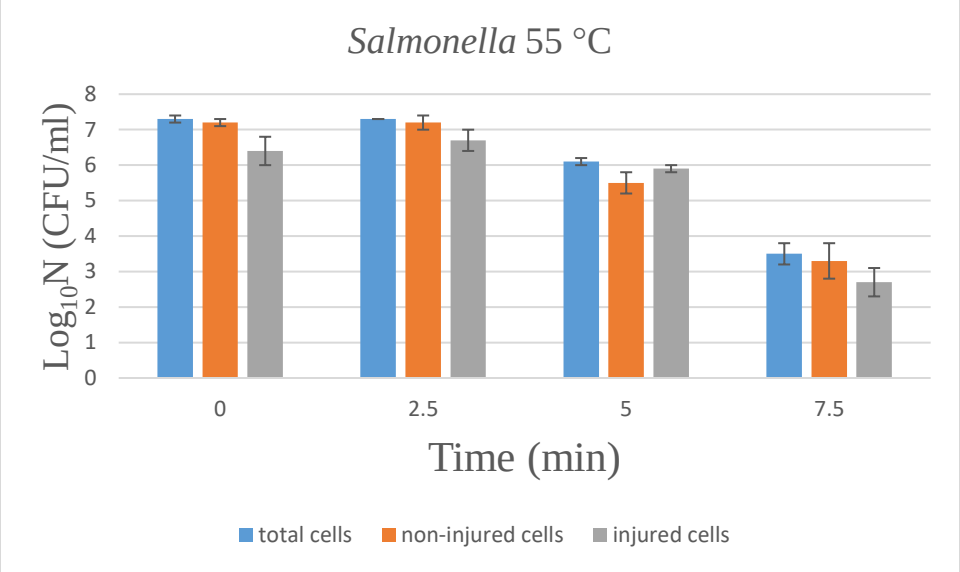

Figure 22. Number of survivals *of Salmonella enterica* at 55 °C in smoothie

The regression line (y=mx+b) was generated (Figure 23.), the D_{55} value was determined to be 1.94 min. To compare the results obtained in this experiment with a previous study by Sharma and co-workers (2005), it was found that the D_{57} value for *Salmonella* Poona 01A3907 was 2.7 minutes, whereas the D_{57} value for *Salmonella* Saphra 97A3312 was 2.0 min. The experiment used cantaloupe juice as the test matrix. However, several factors may have contributed to the observed differences in the D-values. Firstly, Sharma and co-workers (2005) used a slightly different temperature (57 °C) compared to the current experiment (55 °C), which may have influenced the thermal resistance of the microorganisms. Secondly, the two experiments used different strains of *Salmonella*, which may have different thermal resistance characteristics. Finally, the medium used in the two experiments was also different, which may have influenced the growth and survival of the microorganisms.

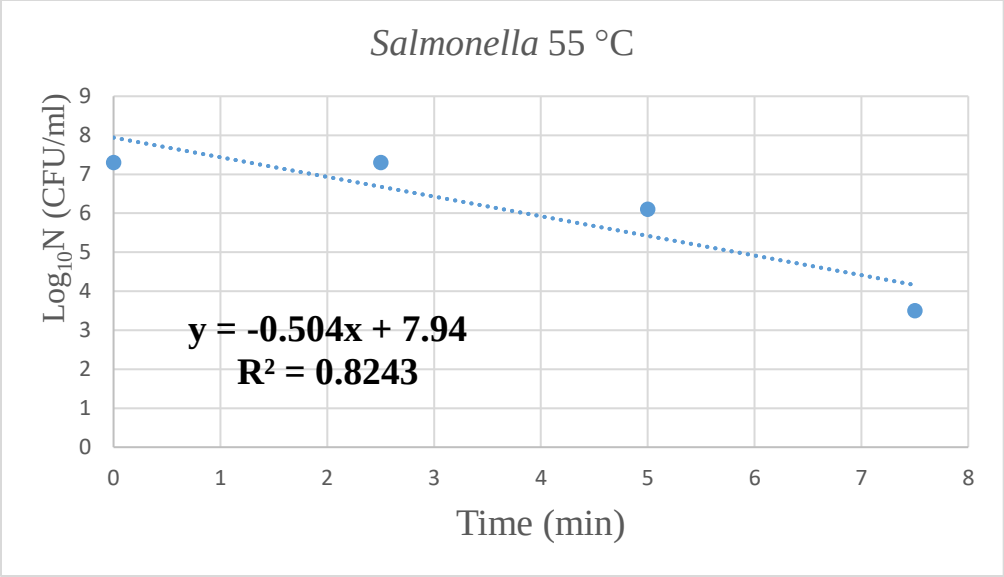

Figure 23. Survival curve (considering total cell count) of *S. enterica* at 55 °C heat treatment in smoothie

5.4.2 Survival of *Listeria monocytogenes* at 55 °C in smoothie

Listeria survival was monitored after heat treatment at 55 °C on Palcam *Listeria* Selective agar for the number of non-injured bacteria and on a TAL-Palcam *Listeria* Selective agar for the number of total bacteria. The difference between these values resulted in the number of injured cells.

After heat treatment of 2.5 minutes, the counts of non-injured and injured cells both decreased, with the injured cells having a higher standard deviation than the non-injured cells, suggesting a higher degree of variability in the injury process.

When the heat treatment time was increased to 5 minutes, the total cell count decreased to 6.85 log CFU/ml, and both non-injured and injured cells had lower counts compared to the control samples (Figure 24.). The injured cells had a similar count as the non-injured cells, indicating that a higher proportion of cells were injured.

At the highest heat treatment time of 7.5 minutes, the total cell count decreased significantly to 5.4 log CFU/ml, with both non-injured and injured cells having lower counts compared to the samples exposed to shorter heat treatment times. The injured cells had a much lower count than the non-injured cells.

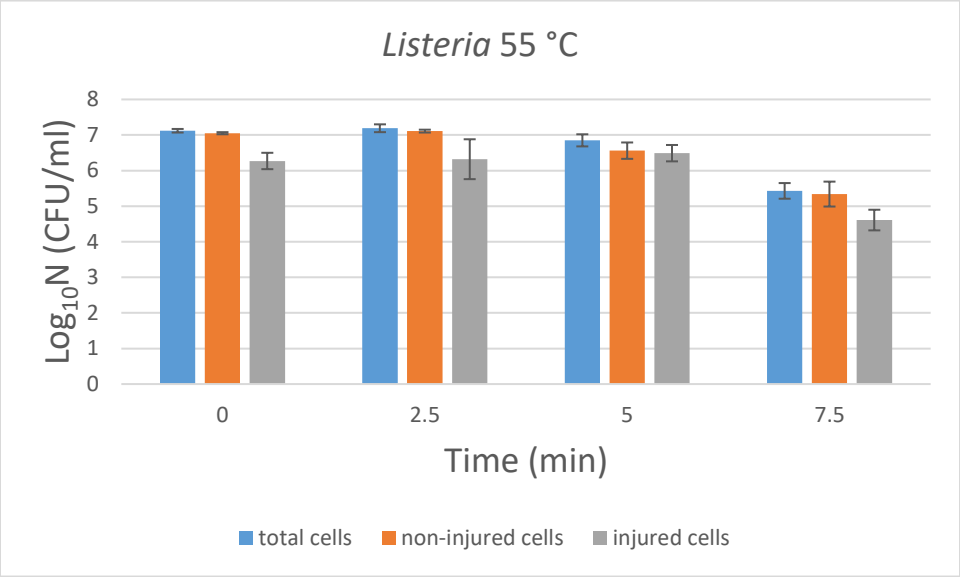

Figure 24. Number of survivals *of Listeria monocytogenes* at 55 °C in smoothie

When comparing these results with the previous experiment that examined the effect of temperature on *Listeria* in sterile distilled water at 55 °C, it appears that *Listeria* exhibits greater resistance to temperature when grown in smoothie, likely due to the differences in the inoculation medium. Therefore, the choice of inoculation medium can significantly impact the results of experiments investigating bacterial resistance to environmental stressors. The regression line (y=mx+b) was generated (Figure 25.), and the D_{55} value was determined to be 4.62 min. To compare the D-value obtained in this experiment, a study conducted by Sharma and co-workers (2005) found a D_{56} value of 7.7 minutes for *Listeria monocytogenes* (F8369) in watermelon juice as the test matrix. It is important to note that this difference in the results could be due to several factors, including the use of a slightly different temperature, a different strain of *Listeria*, and a different experimental medium. Other study connected by Dufort and co-workers (2017) found a D_{54} value of 3.49 minutes for *Listeria monocytogenes* in acidified tomato purée as the test matrix. It is important to note that this difference in the results can be explained by the slightly different temperature and different experimental medium.

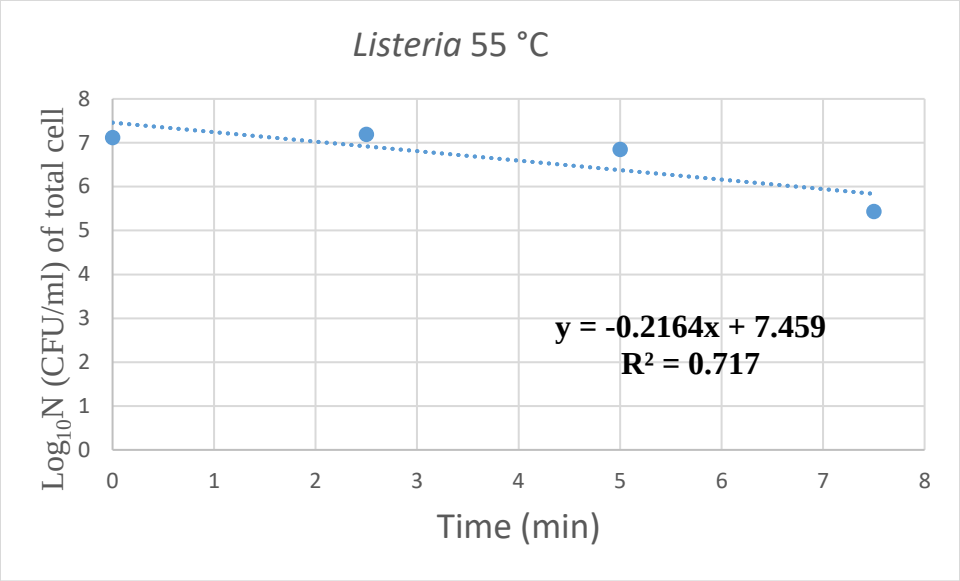

Figure 25. Survival curve (considering total cell count of *L. monocytogenes* at 55 °C heat treatment in smoothie

5.5 Heat treatment at 65 °C in smoothie

5.5.1 Survival of *Salmonella enterica* at 65 °C in smoothie

Salmonella survival was monitored after heat treatment at 65 °C on XLD Agar for the number of non-injured bacteria and on a TAL-XLD agar for the number of total bacteria. The number of injured cells was determined by the difference between these values.

The results of the experiment suggest that the heat treatment of *Salmonella* in Smoothie has a significant effect on the total number of cells (Figure 26.), as well as on the number of injured and non-injured cells. The $\log_{10}$N values of total cells decreased with increasing heat treatment time.

The non-injured cells showed a similar trend, with a decrease in their numbers with increasing heat treatment time. This suggests that the heat treatment was able to kill or inactivate some of the non-injured cells as well. The standard deviations for the $\log_{10}$N values were generally small, indicating that the data was relatively consistent and reliable. However, the standard deviation for the non-injured cells in the 2-minute heat treatment group was relatively large, indicating some variability in the data. Overall, the results suggest that even short exposures to high temperatures can significantly reduce the number of viable cells.

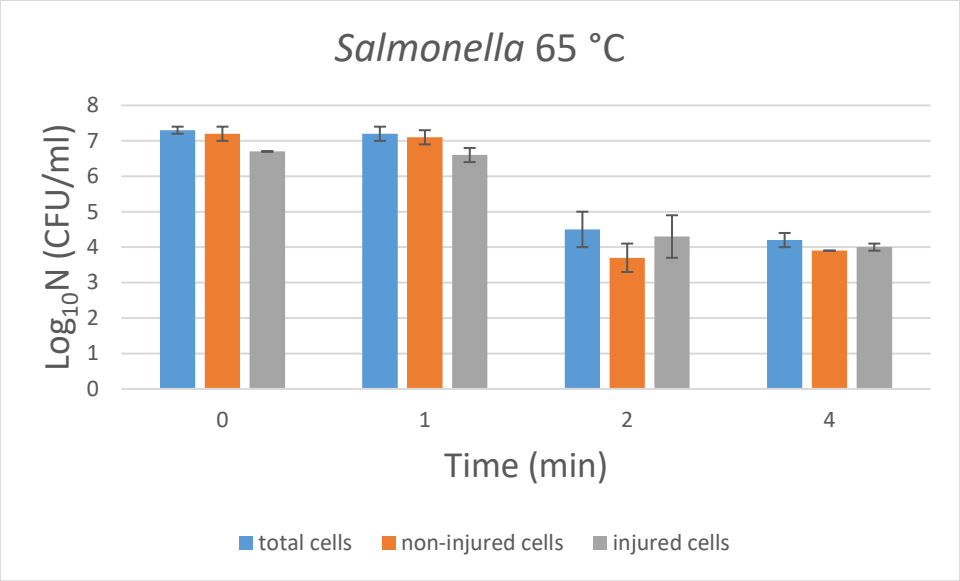

Figure 26. Number of survivals *of Salmonella enterica* after 65 °C in smoothie

The regression line (y=mx+b) was generated (Figure 27.), and the D_{65} value was determined to be 1.15 min. This value was comparable to a previous study conducted by (Hussien et al., 2022) where heat-tolerant *Salmonella sp*. bacteria were isolated from chicken and subjected to a thermal treatment at 73 degrees for 15 seconds. The surviving bacteria were further studied, and a subsequent heat treatment was conducted at 65 °C where the test matrix was TSB, with a resulting D_{65} value of 1.02. The observed small difference in the D values can be attributed to various factors, including the absence of a preliminary heat treatment of the bacteria in my experiment, differences in bacterial strains, variations in the test medium, and discrepancies in the experimental conditions and procedures as a whole.

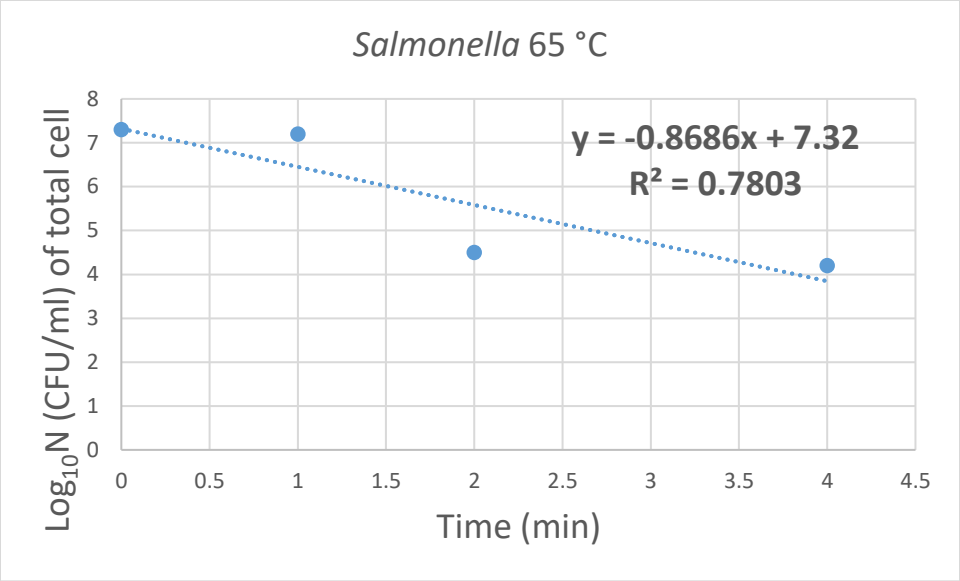

Figure 27. Survival curve (considering total cell count of *S. enterica* at 65 °C heat treatment in smoothie

5.5.2 Survival of *Listeria monocytogenes* at 65 °C in smoothie

Listeria survival was monitored after heat treatment at 65 °C on Palcam *Listeria* Selective agar for the number of non-injured bacteria and on a TAL-Palcam *Listeria* Selective agar for the number of total bacteria. The difference between these values resulted in the number of injured cells.

The results of the experiment showed that the heat tolerance of *Listeria* is influenced by the duration of exposure to high temperatures. The $\log_{10}N$ of total cells decreased as the duration of heat treatment increased (Figure 28.). In particular, the total number of cells decreased from 7.2 log CFU/ml in the control samples to 4.7 log CFU/ml after 4 minutes of heat treatment. This indicates that the bacteria were significantly affected by the high temperature and that the longer the exposure time, the greater the reduction in cell numbers.

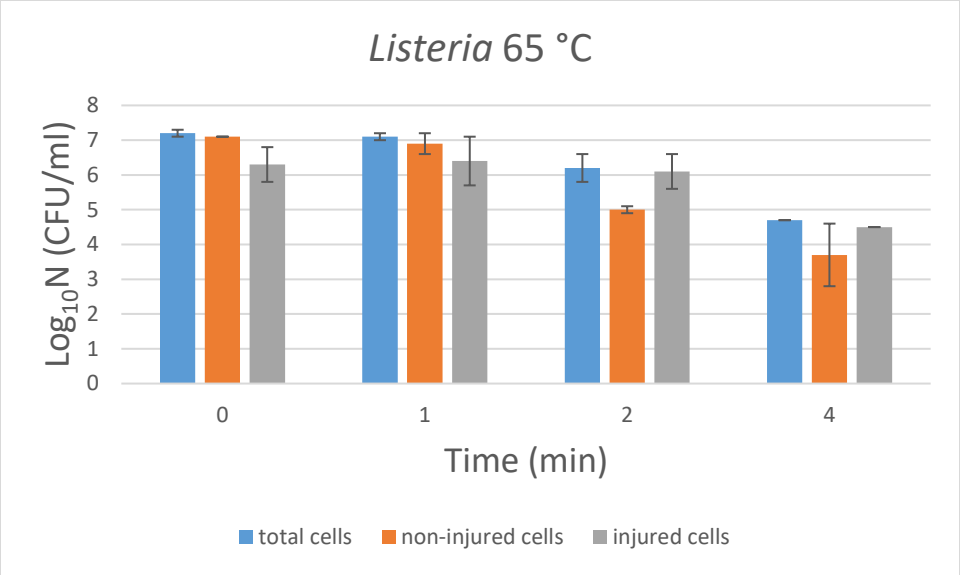

Figure 28. Number of survivals *of Listeria monocytogenes* at 65 °C in smoothie

In addition, the proportion of injured cells also increased with longer exposure times. This is particularly evident in the results obtained after 4 minutes of heat treatment, where the proportion of injured cells was higher than that of non-injured cells. This suggests that the longer the exposure time, the greater the damage to the bacterial cells.

It is important to note that the standard deviation values associated with the measurements of each sample suggest some variability in the results. This is expected given the complex nature of growth matrix.

When comparing these results with the previous experiment that examined the effect of temperature on *Listeria* in sterile distilled water at 65 °C, similarly to the heat treatments at 55 °C, it appears that *Listeria* exhibits greater resistance to temperature when grown in smoothie, likely due to the differences in the inoculation medium. Therefore, the choice of inoculation medium can significantly impact the results of experiments investigating bacterial resistance to environmental stressors.

The regression line (y=mx+b) was generated (Figure 29.), and the D_{65} value was determined to be 1.51 min. A study by Ágoston (2009) found D_{65} value of 1.29 minutes for *L. monocytogenes* (4 ab No 10) (a meat isolate) in case of TSB medium test matrix. This D value is slightly lower than the

D-value obtained in the current experiment. This may be due to the differences in the test matrix, bacterial strain, and heating conditions used in the two experiments.

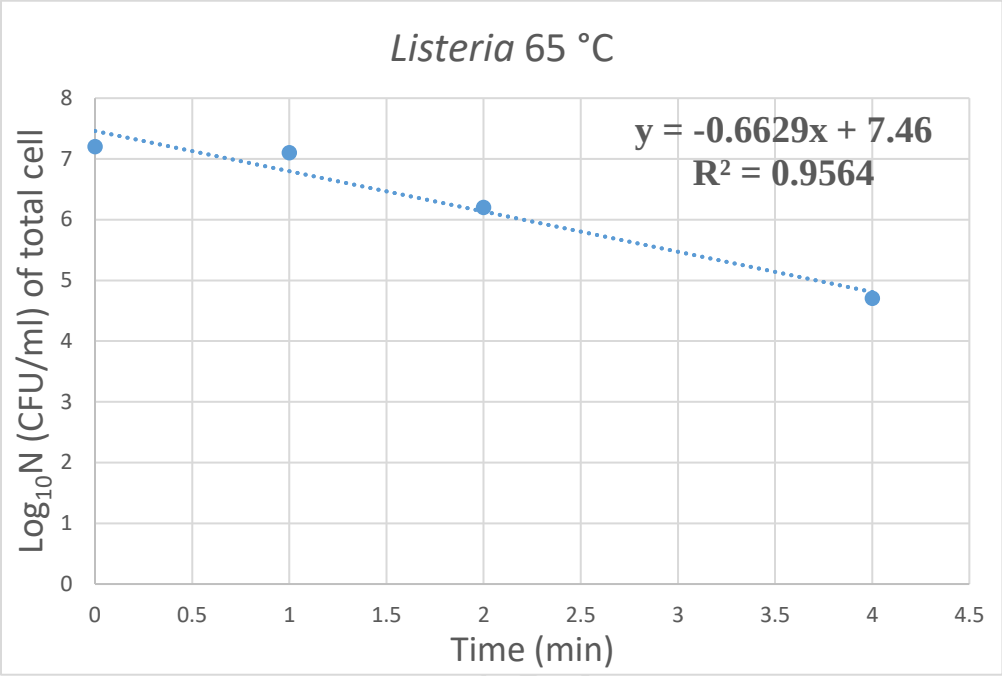

Figure 29. Survival curve (considering total cell count of *L. monocytogenes* at 65 °C heat treatment in smoothie

5.6 Heat treatment at 75 °C in smoothie

5.6.1 Survival of *Salmonella enterica* at 75 °C in smoothie

Salmonella survival was monitored after heat treatment at 75 °C on XLD agar (Figure 30.) for the number of non-injured bacteria and on a TAL-XLD agar (Figure 31.) for the number of total bacteria. The number of injured cells was determined by the difference between these values.

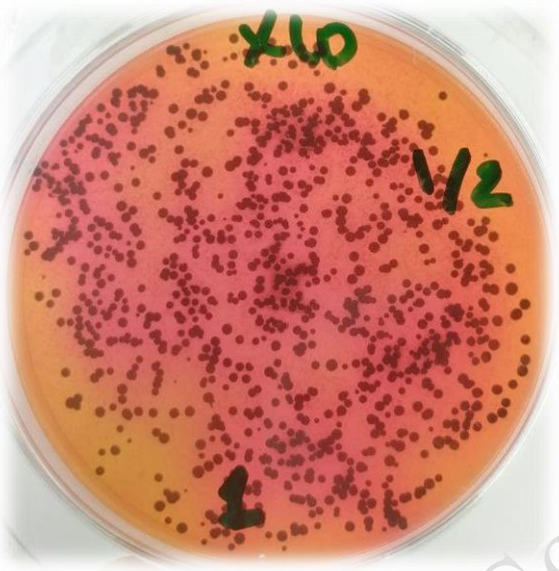

Figure 30. Growth of *S. enterica* on XLD agar after 1-min heat treatment at 75 °C

Figure 31. Growth of *S. enterica* on XLD-TAL agar after 1-min heat treatment at 75 °C

After exposing the *Salmonella* to 0.5 min of heat treatment, the $\log_{10}$N of total cell remained unchanged compared to the control samples (Figure 32.). However, the number of non-injured cells decreased slightly, while the number of injured cells increased. This suggests that some *Salmonella* cells were able to survive the heat treatment, but some were injured and unable to grow on selective

agar. When the *Salmonella* were exposed to 1 min of heat treatment, the $\log_{10}$N of total cell decreased significantly, indicating that the heat treatment had a significant effect on the bacterial growth. Both non-injured cells and injured cells decreased in number compared to the control and 0.5 min heat treatment conditions, with injured cells showing a greater reduction in numbers. This suggests that more cells were injured or killed by the 1 min heat treatment than the 0.5 min heat treatment.

At 1.5 min of heat treatment, no *Salmonella* cells were able to grow, indicating that the heat treatment was lethal to all cells. This result confirms that the heat tolerance of *Salmonella* is limited and that longer exposure to high temperatures can kill all cells.

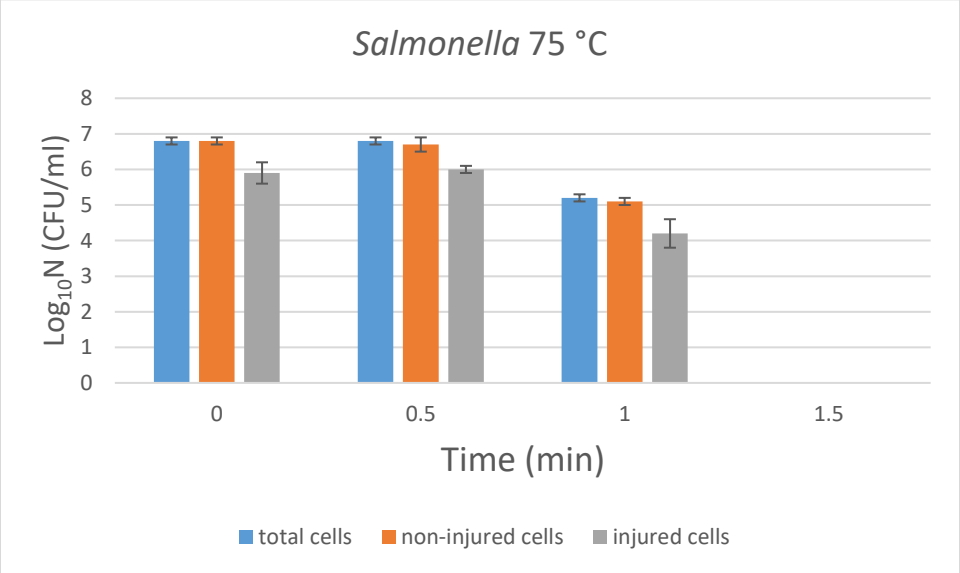

Figure 32. Number of survivals *of Salmonella enterica* at 75 °C in smoothie

The regression line (y=mx+b) was generated (Figure 33.), and the D_{75} value was determined to be 0.62 min. This value was comparable to a previous study conducted by Hussien and co-workers (2022) where heat-tolerant *Salmonella sp*. bacteria were isolated from sesame paste and subjected to a thermal treatment at 73 degrees for 15 seconds. The surviving bacteria were further studied, and a subsequent heat treatment was conducted at 75 °C where the test matrix was TSB, with a resulting D_{75} value of 0.38. The observed difference in the D values can be attributed to various factors, including the preliminary heat treatment of the bacteria in the recent experiment,

differences in bacterial strains, variations in the test medium characteristics, and discrepancies in the experimental conditions and procedures as a whole.

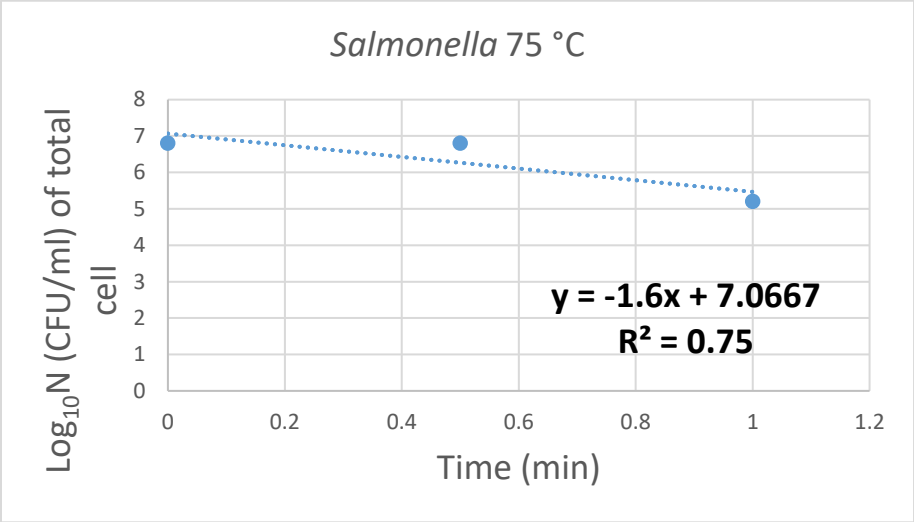

Figure 33. Survival curve (considering total cell count of *S. enterica* at 75 °C heat treatment in smoothie

A heat resistance curve was plotted based on the results of the three heat treatment experiments. Z value was calculated using the formula z = -1/m, which was derived from the regression line's negative slope over the $\log_{10}$ of D values at the appropriate temperatures.

The regression line (y=mx+b) was generated (Figure 34.), and the z value was determined to be 39.84 °C.

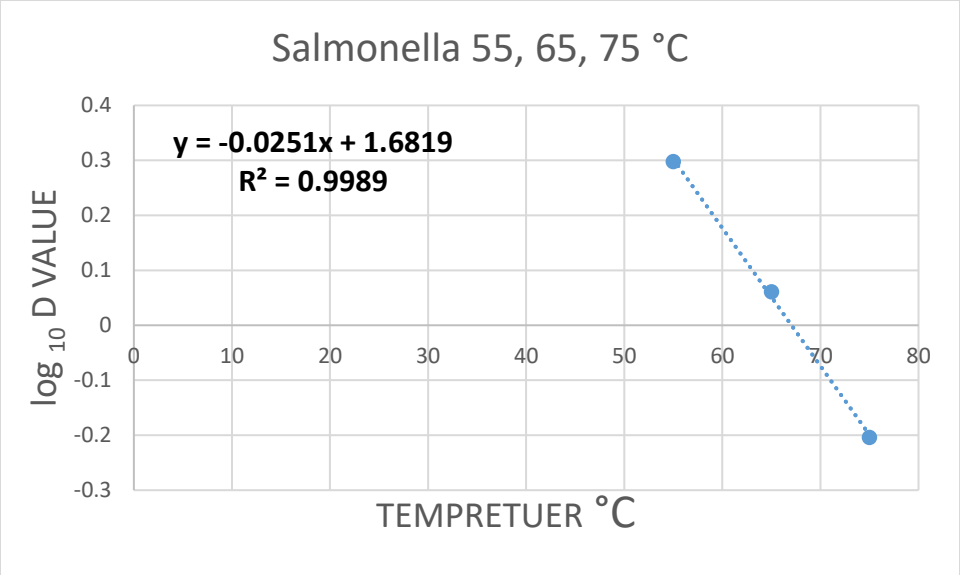

Figure 34. z value for *S. enterica* in Smoothie

5.6.2 Survival of *Listeria monocytogenes* at 75 °C in smoothie

Listeria survival was monitored after heat treatment at 75 °C on Palcam *Listeria* Selective agar (Figure 35.), for the number of non-injured bacteria and on a Palcam *Listeria* Selective-TAL agar for the number of total bacteria (Figure 36.). The difference between these values resulted in the number of injured cells.

Figure 35. Growth *L. monocytogenes* on Palcam agar after 0.5-min heat treatment at 75 °C

Figure 36. Growth of *L. monocytogenes* on Palcam-TAL agar after 0.5-min heat treatment at 75 °C

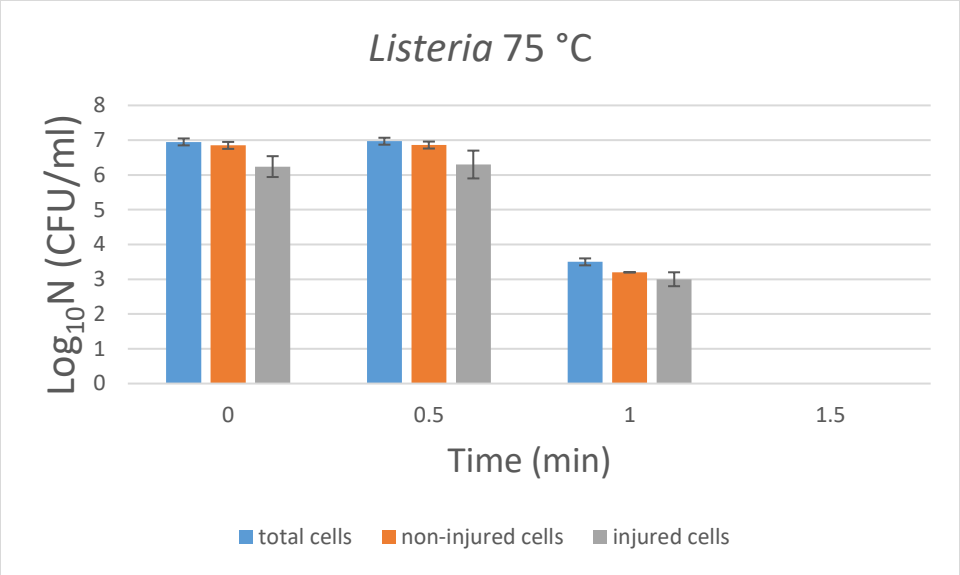

Figure 37. Number of survivals *of Listeria monocytogenes* at 75 °C in smoothie

From the graph we can see that when exposed to 75 degrees Celsius for 0.5 min, the concentration of *Listeria* cells did not change significantly, with a $\log_{10}$N of total cells, non-injured cells, and injured cells ranging from 6.3 to 6.97 log CFU/ml. However, the concentration of injured cells increased slightly. When exposed to 75 degrees Celsius for 1 min, the concentration of *Listeria* cells decreased significantly, with a $\log_{10}$N of total cells, non-injured cells, and injured cells ranging from 3 to 3.5 log CFU/ml. The concentration of injured cells decreased slightly compared to the non-injured cells. When exposed to 75 degrees Celsius for 1.5 min, there was no detectable growth of *Listeria* cells, whether non-injured or injured. This suggests that the heat treatment was severe enough to kill all *Listeria* cells.

Overall, the results demonstrate that exposure to high temperatures can significantly affect the survival of *Listeria* cells in smoothie medium. The longer the exposure time, the more significant is the effect on the concentration of *Listeria* cells.

The regression line (y=mx+b) was generated (Figure 38.), and the D_{75} value was determined to be 0.21 min. A comparison of this result with a study by Márquez-González and co-workers (2022) revealed that a D_{75} value of 0.3 minutes was obtained for *Listeria monocytogenes* in cheese curd packages as the test matrix. It is postulated that the observed discrepancy in the results may be attributed to differences in the experimental conditions, including the reaction medium and other experimental details. Notably, the result reported by Márquez-González and co-workers (2022) is

in agreement with the D_{75} value of 0.28 minutes obtained when using sterile distilled water as the test matrix.

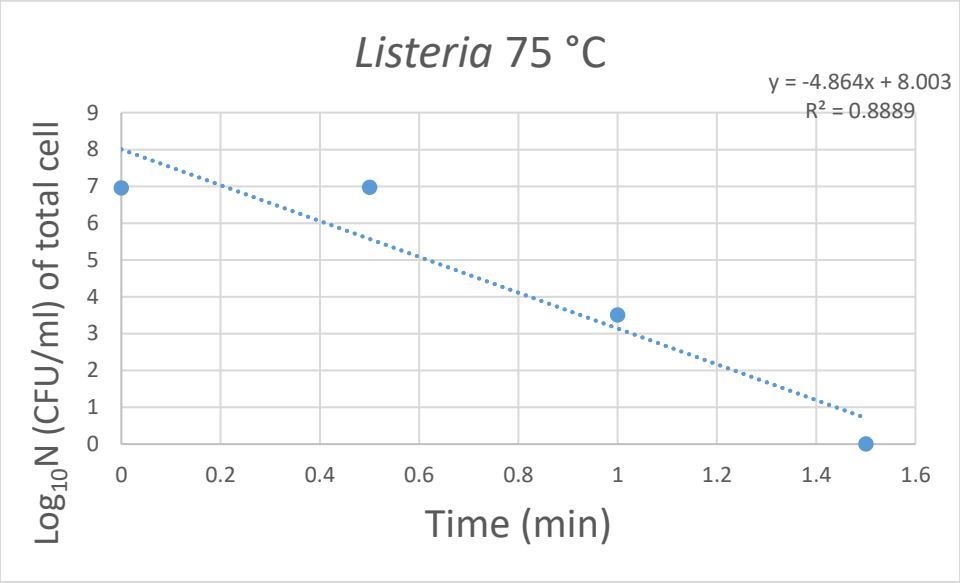

Figure 38. Survival curve (considering total cell count of *L. monocytogenes* at 75 °C heat treatment in smoothie

A heat resistance curve was plotted based on the results of the three heat treatment experiments. The regression line (y=mx+b) was generated (Figure 39.), and the z value was determined to be 14.79 °C.

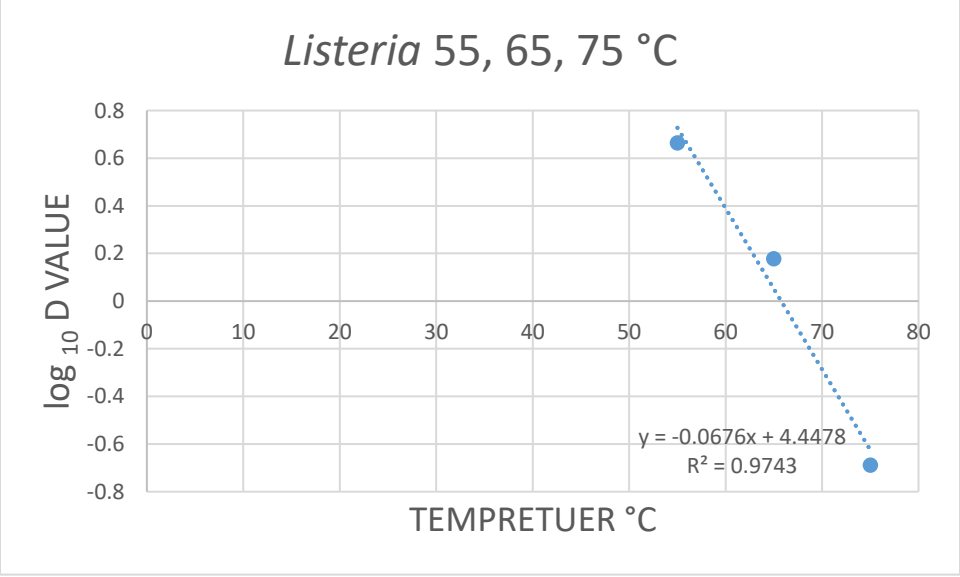

Figure 39. z value for *L. monocytogenes* in smoothie.

5.7 Heat treatment at 55 °C in strawberry puree

Salmonella survival was monitored after heat treatment at 55 °C on XLD agar for the number of non-injured bacteria. In this experiment, after the lowest heat treatment at 55 °C for 2.5 minutes there was no growth even on the TAL plates (neither for *Listeria* nor for *Salmonella*). According to Teribia and co-workers (2021) the strawberry puree pH varies between 2.5 and 3.5 and it is less than the the minimum pH (4.05) value for growth of *Salmonella* (Jay, 2000). The low pH value of the matrix can inhibit the test strain. This low pH value in combination with heat treatment explains these results. Similar observations were made for *Listeria monocytogenes*, of which the minimum pH value for growth is 4.0.

6. Summary

This thesis discusses food safety and the importance of ensuring that food is free from harmful microorganisms that can cause severe health consequences. Two common foodborne pathogens are *Listeria monocytogenes* and *Salmonella enterica*, which can survive and grow in fruit puree-based products. The ability of bacteria to survive and proliferate in food products is influenced by various factors such as temperature, pH, and water activity. Heating is frequently used to inactivate harmful bacteria in food products, and the heating temperature, heating period, and heat resistance of microorganisms all affect the effectiveness of heat treatment. The goal of this thesis was to study the thermal tolerance of *Listeria monocytogenes* and *Salmonella enterica* bacteria in different fruit-based media. The thesis aims to determine the effect of mild heat treatment on the number of surviving *Listeria monocytogenes* and *Salmonella enterica* in liquid foods, specifically in strawberry puree, smoothies, and distilled water. By analyzing the thermal destruction kinetics of the targeted bacteria in these matrices, the study aims to calculate the D-values. This research contributes to the advancement of knowledge on food safety measures to ensure the elimination of pathogenic microorganisms in food.

In the case of sterile distilled water as a test matrix experiments investigated the heat tolerance of *Salmonella enterica* and *Listeria monocytogenes* at temperatures of 55 °C, 65 °C, and 75 °C. The results demonstrated that longer durations of heat treatment were more effective in reducing the viability of both bacteria. However, the experiments also demonstrated that some cells were able to survive the heat treatment and represent a potential source of contamination. The D-values for *Salmonella enterica* were 4.39, 1.1, and 0.24 minutes at 55 °C, 65 °C, and 75°C, respectively, while for *Listeria monocytogenes*, the D-values were 2.58, 0.79, and 0.28 minutes at 55 °C, 65 °C, and 75 °C, respectively. These findings suggest that both bacteria are sensitive to heat treatment, and exposure to high temperatures can lead to a significant reduction in cell counts.

In the case of smoothie as a test matrix the experiments investigated the survival of *Salmonella enterica* and *Listeria monocytogenes* when exposed to different temperatures and time periods. For *Salmonella enterica*, longer heat treatment times were found to effectively reduce the survival of the bacteria, with D-values ranging from 1.94 to 0.62 minutes at temperatures of 55 °C to 75 °C, respectively. Similarly, for *Listeria monocytogenes*, longer exposure times resulted in greater

reductions in cell numbers and a higher proportion of injured cells. The D-values for *Listeria monocytogenes* ranged from 4.62 to 0.21 minutes at temperatures of 55 °C to 75 °C, respectively. These findings highlight the importance of considering the inoculation medium when investigating bacterial resistance to environmental stressors in smoothies.

In the case of strawberry puree as a test matrix, the study investigated the survival of *Salmonella* and *Listeria monocytogenes* after heat treatment at 55 °C. The results showed that there was no growth of either of the bacteria even on TAL plates after the lowest heat treatment of 2.5 minutes. This is attributed to the low pH value of the strawberry puree which inhibits the growth of both bacteria, in addition to the heat treatment. The pH value of the strawberry puree was found to be between 2.5 and 3.5, which is less than the minimum pH value required for the growth of *Salmonella* and *Listeria monocytogenes*.

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