EFFECT OF HIGH HYDROSTATIC PRESSURE PROCESSING (HHPP) ON *SALMONELLA ENTERICA* IN PEANUT BUTTER

By

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ABSTRACT OF THE THESIS

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American consumers eat more than 700 million pounds of peanut butter each year, accounting for approximately half the edible use of peanuts in the United States. *Salmonella* is a unique microorganism that can survive in peanut butter as demonstrated by two large outbreaks in 2007 and 2008, creating the need for methods to augment and improve the current peanut butter manufacturing processes to make them even safer. High Hydrostatic Pressure Processing (HHPP) is a popular processing method used to process foods such as guacamole, meats, oysters, jellies and juices to ensure microbiological safety while retaining quality and organoleptic properties. The application of HHPP as an alternative processing method to inactivate *Salmonella* in peanut butter was the focus of this research.

The objective of this research was to optimize the pressure and time conditions of HHPP for maximum inactivation of *Salmonella* inoculated in creamy peanut butter. It was found that at varying combinations of pressures between 400 and 600 MPa and hold times between 4 and 18 min, the reductions in *Salmonella* concentration in peanut butter,

from an initial level of 10^6- 10^7 CFU/g, were only between 1.6 and 1.9 log CFU/g. This led to further exploration of the effect of (i) pressure cycling during HHPP, (ii) varying water activity of peanut butter, and (iii) added nisin in combination with HHPP. The maximum log reduction achieved in all cases was 2 log CFU/g. *Salmonella* was inactivated to below detection limit only when the water activity of peanut butter was increased to an extreme value of 0.96, rendering it unrecognizable as peanut butter.

It can be concluded that HHPP is not a suitable processing method for significantly improving the microbiological safety of *Salmonella* contaminated peanut butter. However, the intriguing results from this research will sow the seeds for future research on the molecular mechanism associated with *Salmonella* survival in low water activity foods like peanut butter during HHPP.

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Tanya D’souza

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I. INTRODUCTION

I.1 Peanut

The peanut or groundnut (*Arachis hypogaea*), is a species of the legume family (Fabaceae). Peanuts are known by many other local names such as earthnuts, ground nuts, goober peas, monkey nuts, pygmy nuts and pig nuts. Despite its name and appearance, the peanut is not a nut, but rather a legume. The peanut plant is native to South America but is now cultivated widely in warm countries (Mayntz, 2012). Peanuts are high in protein but lack essential amino acids tryptophan, methionine and cysteine. Peanuts are primarily composed of unsaturated fatty acids (approximately 50%), with linoleic and oleic fatty acids being the major ones (Maguire, 2004). Peanuts also have high levels of squalene, α-tocopherol, stigmasterol, campesterol, and β-sitosterol that are believed to reduce chronic heart disease (Maguire, 2004).

I.2 History of Peanut butter and its consumption in the US

Peanut butter is thought to have begun with a medical doctor in the city of St. Louis in the 1890's. This doctor was looking for a high protein food for poor people with bad teeth who could not chew meat. This doctor originally used a meat grinder to grind the peanuts into peanut paste. The doctor took his idea to George A. Bayle, Jr., who owned a food products company, and the resulting peanut paste was packaged and sold in barrels. Around this time, Dr. John Kellogg, the staff physician at Battle Creek Sanitarium in Battle Creek, Michigan, also began making peanut paste for his patients as a source of protein that did not contain meat. He and his brother, W.K. Kellogg, patented a peanut butter process in 1895 (Filippone, 2011). The manufacturing process was

mechanized by George A. Bayle, Jr., and a patent for a peanut-butter machine was issued to Abrose W. Straub in 1903 (Filippone, 2011).

Peanut butter was available to the Australian public in 1899 (Holloway, 2011), and was produced by Edward Halsey at the Sanitarium Health Food Company. In 1908, Krema Products Company, located in Columbus, Ohio, started to sell peanut butter. This company is the oldest of the peanut butter producers still in business today. The next state to produce peanut butter was California. In 1922, peanut butter was mass produced when J. L. Rosefield of Rosefield Packing Company of Alameda, California perfected a process to keep the oil from separating in the peanut butter along with spoilage prevention methods. He marketed this commercial peanut butter under the name Skippy® in 1933 as churned peanut butter, which was a smoother, creamier version of the coarse-textured original. In 1958, Proctor & Gamble started producing a popular brand of peanut butter today, Jif (Holloway, 2011).

Americans consume on average over 1.5 billion pounds of peanut butter and other peanut products each year (Dvorak, 2011). Peanut butter is consumed in 90 percent of households within the U.S. An average American consumes more than six pounds of peanuts and peanut butter products each year. **Figure 1** depicts the total peanut butter consumption in the U.S. from 1990 – 2010.

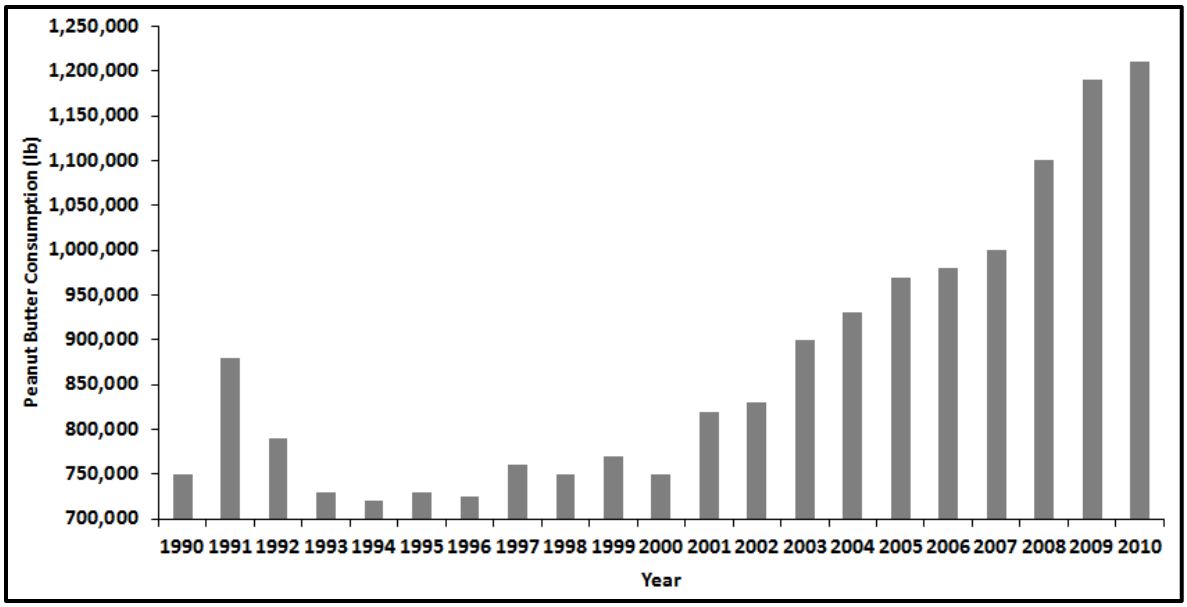


Figure 1: The total peanut butter consumption in the U.S. from 1990 – 2010 (National Agricultural Statistics Service)

I.3 Peanut Butter

Peanut butter, defined simply, is a food paste made primarily from ground dry roasted peanuts. According to 21 CFR 164.150 of the USFDA, peanut butter is the food prepared by grinding one of the shelled and roasted peanut ingredients – blanched or unbleached peanuts to which may be added safe and suitable seasoning and stabilizing ingredients that do not in the aggregate exceed 10 percent of the weight of the finished food. These seasoning and stabilizing ingredients may include salt, sugar, dextrose, honey and hydrogenated vegetable oils. Artificial flavorings, artificial sweeteners, chemical preservatives, and color additives are not suitable ingredients in peanut butter (21 CFR 164.150: Peanut Butter). Differences in manufactured peanut butter reflect variations in product formulations and processing conditions. A typical peanut butter consists of 90% peanut paste, 1-5% hydrogenated vegetable oil, 1-6% sugar, 1-1.5% salt

and 0.5-1.5% emulsifier (APV, 2008). The USFDA standard of identity for peanut butter requires no less than 90 percent peanuts and no more than 55 percent fat (FDA Food Standard Innovations: Peanut Butter's Sticky Standard). Peanut butter has a high level of monounsaturated fats. Peanut butter provides protein, vitamins B3 and E, magnesium, folate, dietary fiber, arginine and high levels of the antioxidant p-coumaric acid. **Table 1** shows the nutrient data for smooth style peanut butter containing salt.

Nutrient	**Amount per 100 g**
Water	1.81 g
Protein	25.09 g
Total fat	50.39 g
Carbohydrate	19.56 g
Fiber	6.0 g
Sugar	9.22 g
Calcium	43.0 mg
Iron	1.87 mg
Magnesium	154.0 mg
Phosphorus	358.0 mg
Potassium	649.0 mg
Sodium	459.0 mg
Zinc	2.91 mg
Thiamin	0.073 mg
Riboflavin	0.105 mg

Niacin	13.403 mg
Vitamin B6	0.543 mg
Folate, DFE	74 mcg_DFE
Vitamin E (alpha-tocopherol)	8.99 mg
Vitamin K	0.6 µg

Table 1: Nutrient data for peanut butter, smooth style with salt (Source: USDA Nutrient Data Laboratory)

I.4 Textures of peanut butter

Peanut butter is available in three textures (United States Standards for Grades of Peanut Butter §52.3062):

- Smooth texture means the peanut butter has a very fine, very even texture with no perceptible grainy peanut particles.
- Medium texture means the peanut butter has a definite grainy texture with perceptible peanut particles approximating not more than 1/16 inch in any dimension.
- Chunky or crunchy texture means peanut butter, which has a partially fine or partially grainy texture with substantial amount of peanut particles larger than 1/16 inch in any dimension.

I.5 Types of peanut butter

There are two types of peanut butter (United States Standards for Grades of Peanut Butter §52.3063):

- **Stabilized type:** Stabilized peanut butter is prepared by any special process and/or with any suitable added ingredient(s) designed to prevent oil separation.
- **Non-stabilized type:** Non-stabilized peanut butter is prepared without special process or added ingredient(s) to prevent oil separation. These are commonly termed as natural or organic peanut butters.

I.6 Grades of peanut butter

There are 3 U.S. grades of peanut butter (United States Standards for Grades of Peanut Butter §52.3065)

- **U.S. Grade A or U.S. Fancy** is the quality of peanut butter that has a good color, that has a good consistency, that is practically free from defects, that has a good flavor and good aroma, that has uniform dispersion of any added ingredient(s), and that scores not less than 90 points when scored in accordance with the scoring system (United States Standards for Grades of Peanut Butter §52.3065)
- **U.S. Grade B or U.S. Choice** is the quality of peanut butter that has a reasonably good color, that has a reasonably good consistency, that is reasonably free from defects, that has a reasonably good flavor and aroma, that has reasonably uniform dispersion of any added ingredient(s), and that scores not less than 80 points when scored in accordance with the scoring system.
- **Substandard** is the quality of peanut butter that fails to meet the requirements of U.S. Grade B.

I.7 Peanut Butter Manufacturing Process

Commercial peanut butter manufacture process involves several steps as shown in **Fig. 2**. First, the shelled peanuts, which may consist of a blend of different types, are dry roasted at about 200°C for 20 to 30 min. The roasting process removes moisture as well as imparts the desired color and flavor to the peanut. During roasting the peanut skins can soak up as much as 27% of the peanut oil. After roasting, the peanuts are quickly cooled to 100°C and then blanched by passing through warm air to loosen the skins and then through large rollers to remove the skins. After blanching, the peanuts are inspected and the scorched, rotten nuts and foreign material are removed (APV, 2008).

Next, the peanuts are ground into paste in a 2-step process at 70-75˚C for 20 min. During the first stage the peanuts are ground to a chunky paste. During the second stage, various ingredients, including sugar, salt and melted stabilizers are added to bring about the smooth creamy texture of peanut butter (APV, 2008 and Ma, 2009). To make chunky peanut butter, peanut pieces approximately the size of one-eighth of a kernel, are mixed with regular peanut butter or incomplete grinding is used by removing a rib from the grinder (APV, 2008). From this point on, the product is can be kept under a nitrogen atmosphere to prevent exposure to oxygen and therefore lipid oxidation. The peanut butter is packaged and stored at about 50°C and left undisturbed for about 48 hours, so that crystallization of the mass is complete. Improper cooling and storage can cause cracking or shrinking of the peanut butter (APV, 2008). Variations in peanut butter can be made by changing the temperature and the duration of roasting, fineness of grind, type of peanuts selected and the amount and kind of ingredients added (APV, 2008).

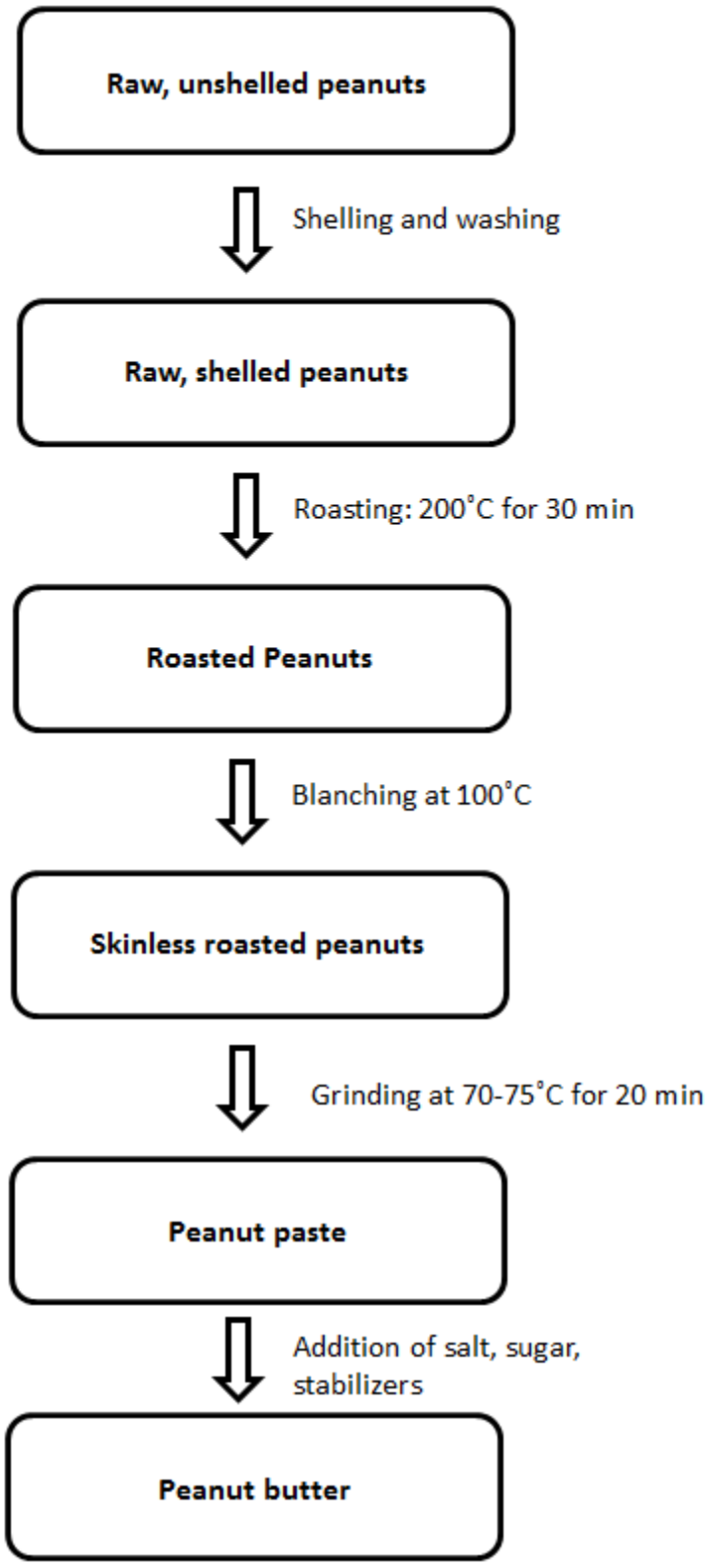


Figure 2: Peanut butter manufacture process

I.8 *Salmonella* spp.

Salmonella spp. is a genus of rod-shaped, gram-negative, non-spore-forming predominantly motile bacteria with flagella all over belonging to the family *Enterobacteriaceae*. *Salmonellae* are facultative anaerobes and contain two species *Salmonella enterica* and *Salmonella bongori* which currently include 2,443 and 20 serovars respectively (Montville and Matthews, 2005).

Salmonella spp. are associated with a number of foodborne and waterborne illnesses worldwide. Salmonellosis is the type of food poisoning that is caused by *Salmonella*. Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps within 12 to 72 hours after ingestion. The illness usually lasts 4 to 7 days, and most victims recover without treatment. However, in some people, the diarrhea may be so severe that the individual needs to be hospitalized. In that case the *Salmonella* infection may spread from the intestines to the blood stream, and then to other body sites and can cause death unless the person is treated with antibiotics. Every year, approximately 40,000 cases of salmonellosis are reported in the United States (CDC). Because many milder cases are not diagnosed or reported, the actual number of infections may be thirty or more times greater.

Salmonella serotype Typhimurium and *Salmonella* serotype Enteritidis are the most common in United States (CDC). Sources of infection include infected food, poor kitchen hygiene, fluids from sick or infected people or animals, polluted surface or standing water and unhygienically thawed meat. *Salmonella* is unique as it can survive several weeks in a dry environment as well as several weeks in water (CDC).

There have been several outbreaks of *Salmonella* in the recent past in various foods such as cantaloupe, turkey burgers, sprouts, ground beef, pine nuts, tomatoes, peppers, shell eggs, pistachios, frozen entrée meals (CDC) etc., which has led to growing awareness and extensive research on *Salmonella* survival and growth in foods as well as technologies to eliminate their survival in foods during manufacture. Peanut butter is one such food.

I.9 Outbreaks of *Salmonella* in peanut butter

Large outbreaks of foodborne illness caused by *Salmonella enterica* serovars have been associated with the consumption of foods with a high fat content and reduced water activity including peanut butter. **Table 2** depicts the *Salmonella* outbreaks in peanut butter, peanut and peanut butter snacks.

Table 2: *Salmonella* outbreaks in peanut butter, peanut and peanut butter snacks (Killelea et al., 1996; Shohat et al., 1996; Scheil et al., 1998; Kirk et al., 2004; CDC 2007; CDC 2009)

Product	Pathogen	Year	Outbreak Location	No. of confirmed cases
Peanut butter coated snack	*Salmonella* Agona PT15	1994 – 1995	Israel, United Kingdom, USA	2200
Peanut Butter	*Salmonella* Mbandaka	1996	Australia	15
Peanuts	*Salmonella* Stanley	2001	Australia, United Kingdom, Canada	93
	Salmonella Newport			12
Peanut Butter	*Salmonella* Tennessee	2006 - 2007	USA	628
Peanut Butter	*Salmonella* Typhimurium	2008 - 2009	USA, Canada	529

The first recorded outbreak of salmonellosis resulting from the consumption of peanut butter occurred in 1996, when 15 persons were infected with *S. enterica* serovar Mbandaka due to contaminated roasted peanuts that were processed into peanut butter and sold in South Australia (Scheil, 1998). During 1994 to 1995, an outbreak of *S. enterica* serovar Agona infection in Israel, England, Wales, and the United States infected 2,200 people and was associated with a peanut butter–coated snack produced in Israel (Killalea, 1996 and Shohat, 1996).

An international outbreak of *Salmonella* Stanley and *S.* Newport infection associated with consumption of 'Farmer' brand peanuts occurred in 2001. Three countries (Australia, Canada, and the United Kingdom reported isolating *S.* Stanley and/or *S.* Newport from unopened packets of these peanuts, which originated in China, and were distributed via Singapore. Both strains had distinctive pulsed field gel electrophoresis (PFGE) patterns. Ninety three cases of *Salmonella* Stanley were reported in Australia, Canada and UK and 12 reported cases of *Salmonella* Newport in these 3 countries (Kirk, 2004).

In the United States, from 2006 to 2007, 628 persons infected with *Salmonella enterica* serovar Tennessee were reported in 47 states as a result of consuming contaminated peanut butter later found to have been processed in a single facility in Georgia. In November 2006, public health officials at CDC and state health departments detected a substantial increase in the reported incidence of isolates of *Salmonella* serotype Tennessee. In a multistate case-control study conducted during February 2007, illness was strongly associated with consumption of either of two brands (Peter Pan or Great Value) of peanut butter produced at the same plant. Based on these

findings, the plant ceased production and recalled both products on February 14, 2007. The outbreak strain of *Salmonella* Tennessee subsequently was isolated from several opened and unopened jars of Peter Pan and Great Value peanut butter and from two environmental samples obtained from the plant. The source of the contamination was unknown, but raw peanuts were ruled out as the source since none of the other plants using the same raw peanuts tested positive for *Salmonella* (CDC 2007).

Another major outbreak of *Salmonella* in peanut butter in the US occurred in 2008 – 2009. On November 25, 2008, an epidemiologic assessment began of a growing cluster of *Salmonella* serotype Typhimurium isolates that shared the same pulsed-field gel electrophoresis (PFGE) pattern in PulseNet. As of January 28, 2009, 529 persons from 43 states and one person from Canada had been reported infected with the outbreak strain. Confirmed, reported onset of illness dates ranged from September 1, 2008, to January 16, 2009. A total of 116 patients were reported hospitalized, and the infection might have contributed to eight deaths. *Salmonella* Typhimurium–contaminated King Nut peanut butter and peanut paste produced by the Peanut Corporation of America (PCA) at a single facility in Blakely, GA, were determined to be the source of this outbreak and the reason for the largest single recall of over 400 peanut butter containing products from 54 companies in U.S. history. King Nut peanut butter was distributed in bulk packaging to institutions, food service industries, and private label food companies. King Nut peanut butter was not sold directly to consumers or distributed for retail sale in grocery stores (CDC 2009).

USFDA inspections showed that the PCA facility in Georgia had evidence of *Salmonella* contaminated product and 12 samples had tested positive for *Salmonella* on

initial testing. When re-tested, negative results were obtained and the company shipped out the product. Further inspection by the USFDA showed that the plant failed to properly clean peanut paste production lines and areas as close as three feet away from production lines, tested positive for *Salmonella* Seftenberg and Mbandaka. Mold growth was found within coolers and water drips were observed from overhead cooling fans (DeVault and McKilligin, 2009).

I.10 *Salmonella* survival in peanut butter

A study was conducted by Burnett et al. (2000), to understand the survival characteristics of *Salmonella* in peanut butter and peanut butter spreads. Five commercial peanut butters and two commercial peanut butter spreads were inoculated with a 5 strain cocktail of *Salmonella* and the samples stored at 21˚C and 5˚C for 24 weeks. There was greater survivability of *Salmonella* at lower temperatures 5˚C than at 21˚C but survival was seen at both temperatures. The degree of viability was peanut butter spreads > regular or low sugar low sodium peanut butters > natural peanut butter. It was speculated that cells of *Salmonella* clump or aggregate near the water phase and differences in the rate of inactivation was attributed to the differences in the size of the water and lipid droplets dispersed in the peanut meal (Burnett, 2000).

In another study by Park et al. (2008), a 3 strain cocktail of *Salmonella* Tennessee was inoculated in 5 commercial brands of peanut butter and was stored at 4˚C and 22˚C for 14 days. *Salmonella* was able to survive in the peanut butter at both temperatures and there were no significant differences in the levels of *Salmonella* at 4˚C and 22˚C after 14 days. From this study it was speculated that the small water droplet size

in peanut butter provides a limited environment and less nutrients for microbial growth. The procedure for inoculating and dispersing the inoculum into peanut butter breaks up its colloidal structure and in combination with the high fat content and unfavorable temperature, *Salmonella* Tennessee did not grow in peanut butter (Park et al., 2008).

Salmonella has been associated with survival in other low water activity foods like chocolate, margarine, butter, almonds and other nuts. In the last few decades, there have been a number of outbreaks of *Salmonella* in chocolate and other cocoa products. Chocolate has very low moisture content (0.5% – 1.0%). It has been suggested that the high fat content of chocolates apparently protects *Salmonella* cells against the action of gastric of acid in the stomach, which allows the cells to colonize the lower gastrointestinal tract and produce clinical symptoms even when a very small number of the cells is present in the product (Bell, 2002).

I.11 Heat resistance of *Salmonella* in peanut butter

Recent large foodborne outbreaks caused by *Salmonella enterica* serovars have been associated with consumption of foods with high fat content and reduced water activity, even though their ingredients usually undergo pasteurization. In a study by Shachar and Yaron (2006) focused on the heat tolerance of *Salmonella enterica* serovars Agona, Enteritidis, and Typhimurium in peanut butter, the *Salmonella* serovars in the peanut butter were resistant to heat and even at temperatures as high as 90˚C, only 3.2-log reduction in CFU/g was observed. The obtained thermal inactivation curves were upwardly concave, indicating rapid death at the beginning (10 min) followed by lower death rates and an asymptotic tail. The curves fitted the nonlinear Weibull model

(Shachar and Yaron, 2006), indicating that the remaining cells have a lower probability of dying. Very little decrease in the viable population (less than 2 log CFU/g) was noted in cultures that were exposed to a second thermal treatment. Peanut butter is a highly concentrated colloidal suspension of lipid and water in a peanut meal phase and it was hypothesized that differences in the local environments of the bacteria, with respect to fat content or water activity, explained the observed distribution and high number of surviving cells (0.1%, independent of the initial cell number). These results demonstrated that thermal treatments are inadequate to consistently destroy *Salmonella* in highly contaminated peanut butter and that the pasteurization process could not be improved significantly by longer treatment or higher temperatures.

Ma et al. (2009) conducted a study to determine the rates of thermal inactivation of three *Salmonella* Tennessee strains in peanut butter associated with an outbreak and to compare them to the rates of inactivation of *Salmonella* strains of other serotypes (Enteritidis, Typhimurium, and Heidelberg) and of clinical isolates of *Salmonella* Tennessee from sporadic cases. Commercial peanut butter was inoculated with *Salmonella* isolates and heated at 71, 77, 83, and 90˚C. The thermal inactivation curves were upwardly concave, indicating rapid death at the beginning (20 min) of heating followed by lower death rates thereafter. The first-order kinetics approach and nonlinear Weibull model were used to fit the inactivation curves and describe the rates of thermal inactivation of *Salmonella* in peanut butter. The calculated minimum times needed to obtain a 7-log reduction at 90˚C for the composited three outbreak-associated strains were significantly greater than those of the other strains. Approximately 120 min were needed to reduce the outbreak strains of *Salmonella* Tennessee by 7 log CFU/g. These

results indicated that the outbreak-associated *Salmonella* strains were more heat resistant than the other *Salmonella* strains tested, and this greater thermal resistance was not serotype specific. Thermal treatments of peanut butter at 90˚C for less than 30 min were not sufficient to kill large populations (5 log CFU/g) of *Salmonella* in highly contaminated peanut butter (Ma et al., 2009).

Mattick et al. (2001) studied the death of *Salmonella enterica* Serovar strains exposed to 54 combinations of temperature (55 to 80°C) and water activity (0.65 to 0.90). All *Salmonella* strains tested demonstrated that low water activity of 0.65 compared with 0.90 was detrimental to survival at 55˚C or 60°C, whereas at ≥70°C the lower water activity was always protective. The most heat resistant serovars over the range of conditions tested were serovar Typhimurium DT104 and serovar Enteritidis. Strains isolated from outbreaks associated with low water activity foods did not appear to be more heat tolerant at low water activity than did other strains. This indicates that *Salmonella* strains from outbreaks associated with low water activity foods may not have particular characteristics promoting their survival during heat processing and subsequent storage in low water activity foods but that their characteristics may instead relate to the contamination source (Mattick et al., 2001).

II. EFFECT OF HIGH HYDROSTATIC PRESSURE AND PRESSURE CYCLING ON A PATHOGENIC *SALMONELLA ENTERICA* SEROVAR COCKTAIL INOCULATED INTO CREAMY PEANUT BUTTER

II. 1. BACKGROUND

II.1.1 High Hydrostatic Pressure Processing (HHPP)

High Hydrostatic Pressure Processing (HHPP) is a method of food processing where food is subjected to elevated pressures to achieve microbial inactivation or to alter the food attributes in order to achieve consumer-desired qualities (Juneja and Sofos, 2002). During HHPP, foods (solids or liquids) may be subjected to pressures up to 1000 MPa (145,000 psi). To get an idea of how high is this high pressure, one has to imagine pressure on a dime if three big elephants (weighing 4-5 tons each) are made to stand on it. The main commercial advantage of high pressure processing is that it can be applied to a packaged product and, hence, any point of contamination (raw material or processing) is rendered insignificant. High pressure acts instantaneously and uniformly on a food product independent of size, shape, and food composition, and with minimum loss of food quality. There are several advantages of high pressure processing because of which it is gaining popularity in the recent past such as it retains the freshness and quality of foods, retains the flavor and color of foods, retains nutritional properties, denatures enzymes, extends shelf life, inactivates/kills microbes, reduces need for preservatives and eliminates post-process contamination.

HHPP is a batch or semi-continuous process. During HHHP, the food product to be processed is placed in a pressure vessel capable of withstanding high pressures. The food product is submerged in a pressure-transmitting medium which in most cases is water. Other liquids such as castor oil, silicon oil, sodium benzoate (aqueous), ethanol or glycol have also been used. The pressure in the vessel is increased by pumping more medium into the vessel or by using a piston to compress the medium under external force. Due to adiabatic compression, water temperature rises by approximately 3°C per 100 MPa. For foods high in fat, temperature increases can be larger (9°C per 100 MPa). Once the desired pressure is attained, the pump is turned off and the food product is held at that pressure for a desired period of time. After the required hold time has elapsed, the vessel is depressurized and the product removed. There are thus three stages of a HHPP cycle as shown in **Fig. 3**:

1. Pressurization: Pressure increases to desired pressure and temperature increases due to adiabatic compression heating
2. Hold Time: Pressure is held at desired value and temperature decreases due to heat loss to thick wall of vessel
3. Depressurization: Pressure rapidly decreases to ambient pressure and temperature decreases and ends up lower than initial temperature.

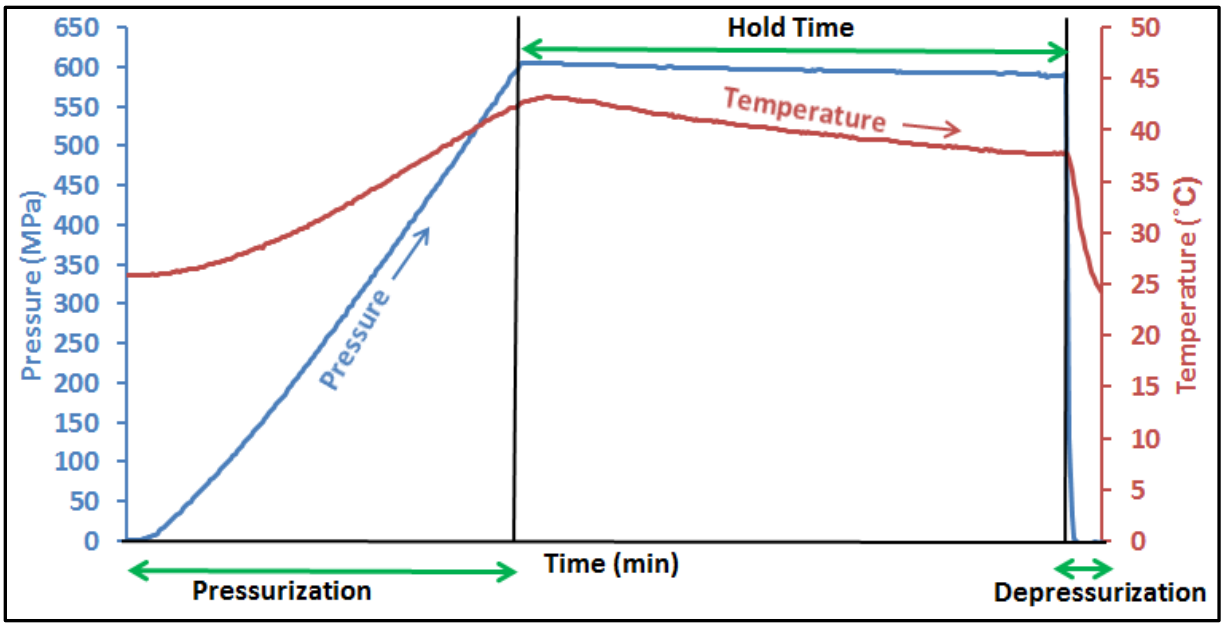


Figure 3: Variation of pressure and temperature with time during a HHPP cycle showing the three stages (600 MPa for 18 min).

II.1.2 Commercial Applications of HHPP

Strawberry, apple, and kiwi jams represented the first wave of pressure-treated commercial products introduced into the Japanese market in 1990. Avocado based products, especially guacamole, were subsequently commercialized in the United States. Fresherized Foods (formerly Avomex) began the first industrial production of guacamole in North America in 1997. By 2007, approximately 120 industrial HHPP installations were in use worldwide for commercial scale food production. Hormel Foods, Kraft Foods, Perdue, Foster Farms, and Wellshire Farms are examples of meat processors that have successfully utilized the technology for a variety of minimally processed meat products. Several seafood processors such as Motivatit Seafoods in Louisiana, have also employed HHPP to improve food safety and shelf life of shellfish with the added benefit of facilitating the removal of flesh from the shell (Balasubramaniam et al., 2008). Other

foods that use HHPP in their processing include meats and ready-to-eat meats, fruits, vegetables, juices, smoothies, jams, jellies and seafood (http://www.hiperbaric.com).

II.1.3 Microbial inactivation by HHPP

Hite in 1899 demonstrated that high pressure treatment can prevent souring of milk, showing that microorganisms can be inactivated by pressure (Hendrickx and Knorr, 2002). The mechanism of microbial inactivation by HHPP is still not well understood. Compression during high hydrostatic pressure processing (HHPP) increases the temperature of foods and inactivates microbial cells by inducing morphological changes, cell membrane perturbation, biochemical changes, and genetic changes (Hendrickx and Knorr, 2002). The increased permeability of the cell membrane due to high pressure is one of the factors responsible for inactivation. This increase in permeability is due to the denaturation of proteins in the cell membrane at high pressures. Evidence of membrane damage has been demonstrated by the leakage of ATP and metallic ions such as Na+, K+ and Ca2+, or increased uptake of fluorescent dyes such as propidium iodide that do not normally penetrate membranes of intact cells (Smelt, 1998; Kato, 1999).

Yersinia enterocolitica is the most sensitive to HHPP whereas cells of *Salmonella, Listeria monocytogenes, E.coli* O157:H7 and *Staphylococcus aureus* need higher pressures to be inactivated by HHPP. Yeasts and molds too are very sensitive to HHPP (Patterson et. al, 1995). **Table 3** depicts recent work on inactivation of vegetative bacteria by HHPP.

Table 3: Brief summary of recent work in inactivation of vegetative bacteria using HHPP (Patterson, 2005).

Vegetative Bacteria	Substrate	Treatment	Log Unit Reduction	Comments
Campylobacter jejuni	Pork slurry	300MPa/10min/ 25°C	6	-
Salmonella Seftenberg 775W	Strained baby food	340MPa/10min/ 23°C	<2	-
E.coli O157:H7 NCTC 12079	UHT milk Poultry meat	600MPa/15min/20°C	<2 3	Pressure-resistant strain
S.aureus	UHT milk Poultry meat	600MPa/15min/20°C	2 3	-
L.monocytogenes	UHT milk Poultry meat	375MPa/15min/20°C	<1 2	Most resistant of the three strains studied
Vibrio parahaemolyticus O3:K6	Oysters	300MPa/3min/10°C	5	Most resistant of ten strains studied
Pseudomonas Fluorescens	Ewe's milk	450MPa/10min/10°C	4	-

II.1.4 Studies conducted on *Salmonella* and HHPP in buffer and foods

HHPP has been reported to inactivate *Salmonella* in buffer solutions. Ritz et al. (2005) found that S*almonella* Typhimurium in buffer solution (pH 7) exposed to pressure of 400 MPa for 10 min were inactivated to below the limit of detection. However, they observed resuscitation of the pathogen after storage at 4ºC and 20ºC for 24 weeks (direct viable count). When *Salmonella* was exposed to 600 MPa for 10 min there was total destruction of viable non-culturable cells even after storage at the same conditions.

There are limited studies looking at inactivation kinetics of *Salmonella* by high pressure in a food matrix. High pressure processing inactivates both Gram positive and Gram negative organisms and retains the freshness, quality and nutrient value of foods. Due to several advantages of high pressure processing the potential of HHPP to inactivate *Salmonella* in peanut butter is explored in this research.

II.1.5 Hypothesis

The hypothesis for this research was that HHPP can inactivate *Salmonella enterica* serovars in inoculated creamy peanut butter to ensure its microbiological safety.

II.1.6 Rationale

Thermal processing has been shown not to eliminate *Salmonella* in high fat low water activity foods such as peanut butter (Shachar et al., 2006). Research has shown that *Salmonella enterica* serovars Agona, Enteritidis and Typhimurium are resistant to heat – even as high as 90°C and thermal treatments of highly contaminated peanut butter at 90˚C for less than 30 min were not sufficient to kill *Salmonella* even at higher temperatures and for longer duration (Ma et. al, 2009 and Shachar et al., 2006). It is thought that the high fat and low water activity environment protect *Salmonella* against thermal inactivation.

High pressure processing has been demonstrated to inactivate gram positive and gram negative micro-organisms in liquid and semi-solid foods as well as in buffer solutions. It also retains food quality and maintains the natural freshness of foods. It is an emerging non-thermal food processing technology with several advantages and hence its

potential to ensure the microbiological safety of *Salmonella* contaminated peanut butter was explored in this research.

II.1.7 Overall Objective

The overall objective of this research was to optimize conditions of HHPP (pressure, time, temperature and water activity) for maximum inactivation of *Salmonella* in peanut butter to ensure the microbiological safety of *Salmonella* contaminated peanut butter.

II.2. MATERIALS AND METHODS

II.2.1 Materials

II.2.1.1 Peanut Butter

16.3 oz. jars of Skippy® Creamy Peanut Butter were purchased from local supermarkets. The ingredients listed were roasted peanuts, sugar, hydrogenated vegetable oils (cottonseed, soybean, and rapeseed), and salt. The nutrition facts label indicated 16 g of fat and 7 g of protein per each 32-g serving, and each jar contained 462 g of peanut butter. Unopened jars of processed peanut butter were stored at room temperature. Once opened, the jars were stored at refrigeration to prevent rapid spoilage.

II.2.1.2 *Salmonella* strains

Six pathogenic strains of *Salmonella* were obtained from Dr. Linda Harris from the Department of Food Science and Technology in University of California, Davis, in glycerol stocks and were stored at -85˚C in a freezer. These strains were obtained from peanut butter and other nut related outbreaks. The strains were:

- *Salmonella* Enteritidis PT30 obtained from raw almonds in USA and Canada in a 2000 – 2001 outbreak (Isaacs et al., 2005)
- *Salmonella* Tennessee obtained from peanut butter in USA in a 2006 – 2007 outbreak (CDC 2007)
- *Salmonella* Oranienburg obtained from chocolate in Germany in a 2001- 2002 outbreak
- *Salmonella* Anatum obtained from peanut butter, peanut meal and peanut granules at the PCA facility in Blakely, Georgia, USA in 2008 (CDC 2009)

- *Salmonella* Enteritidis PT 9c obtained from raw almonds in USA in a 2003 – 2004 outbreak
- *Salmonella* Montevideo obtained from pistachio nuts and pistachio containing products in USA in a 2009 outbreak (CDC 2009).

II.2.1.3 Media for culturing and enumeration of *Salmonella*

1) 0.1% Peptone Water: This media was prepared by dissolving 1.5 grams of peptone powder (Difco™, Benkitson and Dickson, MD, USA) in 1 liter of distilled water. This solution was dispensed as 9 ml aliquots into boiling tubes and 225 ml portions in 500 ml glass bottles. This media was sterilized in the autoclave at 121˚C (250˚F) for 15 minutes.
2) Tryptic Soy Broth (TSB): This media was prepared by suspending 30.0 grams of the tryptic soy broth (Soybean – Casein Digest Medium) powder (Difco™, Benkitson and Dickson, MD, USA) in 1 liter of distilled water. This media was sterilized in the autoclave at 121˚C (250˚F) for 15 minutes.
3) Tryptic Soy Agar (TSA): This media was prepared by suspending 40.0 grams of the tryptic soy agar (Soybean – Casein Digest Agar) powder (Difco™, Benkitson and Dickson, MD, USA) in 1 liter of distilled water. This media was sterilized in the autoclave at 121˚C (250˚F) for 15 minutes.
4) Xylose Lysine Tergitol 4 Agar (XLT4): This media was prepared by suspending 59.0 grams of the XLT4 Agar Base (Difco™, Benkitson and Dickson, MD, USA) in 1 liter of distilled water. 4.6 ml of XLT4 Agar Supplement was added. A stir-bar was added and the bottle was heated on a hot-plate and media boiled.

5) XLT4 Agar Supplement: 4.6 ml of this supplement (Difco™, Benkitson and Dickson, MD, USA) was added during the preparation of the XLT4 Agar.

II.2.1.4 Blender

A 2000 ml blender jar (Sunbeam Products, Inc., Boca Raton, FL) was used to equally distribute the *Salmonella* inoculum into the peanut butter during preparation of inoculated peanut butter samples for high pressure processing.

II.2.1.5 High Pressure Processing Equipment

The high hydrostatic pressure processing unit at the Department of Food Science in Rutgers University was manufactured by Elmhurst, Inc., Albany, NY and is depicted in **Fig. 4**. The unit comprises a 10 liter stainless steel high pressure vessel with an external heating tank and a 20 HP intensifier pump to build a maximum pressure of 690 MPa (100,000 psi) in 3 min or less. The maximum depressurization time is 10 seconds. The equipment is rated for a temperature range of 5°C to 90°C and is capable of pressure hold times of up to 60 min. It also has a pressure cycling capability. The setup is shown in **Figure 5.** The length of the stainless steel cylinder is 1090 mm and the external diameter 445 mm. The internal bore diameter of the pressure cavity in the stainless steel vessel is 127 mm, its length is 823 mm, and the wall thickness is 142 mm. The high pressure vessel remains in horizontal position when not in use. During experiments, peanut butter samples (jars and/or pouches) were loaded into the pressure cavity in this position, top closure inserted and the vessel made vertical and filled with water at room temperature (22˚C), the pressurizing medium. Using the PLC control panel, the desired

pressure in kpsi and hold time in minutes, were set. The increase in water temperature due to adiabatic heating during the cycle was measured using three thermocouples (type K) located at the top, at the center and the bottom of the vessel. Data on pressure, temperature and time were logged using LabVIEW 7 ® (National Instruments, Austin, TX) software on a computer.

Figure 4: Rutgers 10 liter High Hydrostatic Pressure Processing Unit

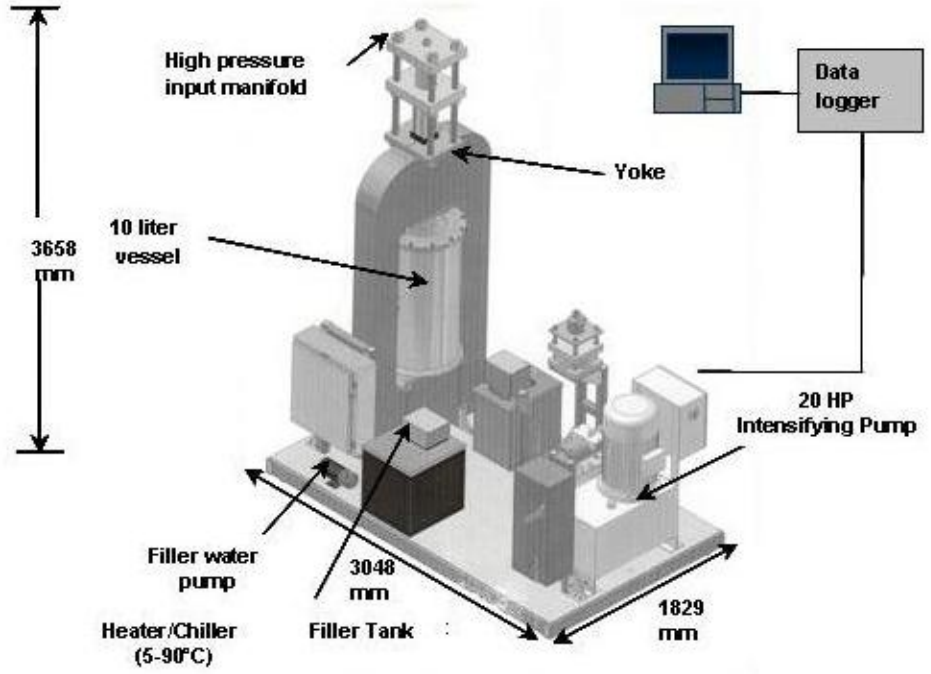


Figure 5: Detailed setup of the HHPP unit at Rutgers University

II.2.1.6 Nature's Promise Organic Peanut Butter

Four 16 oz. (453 g) jars of Nature's Promise organic peanut butter was purchased from a local supermarket. It was creamy and had no salt added. Its ingredient was only peanuts. Each 32 g (2 Tbsp.) serving contained 18 g of fat and 8 g of protein. Unopened jars of processed peanut butter were stored at room temperature. Once opened, the jars were stored at refrigeration to reduce oil separation.

II.2.2 Methods

II.2.2.1 Bacterial cultures and inoculum preparation

Pathogenic strains of *Salmonella* Enteritidis PT30, *Salmonella* Tennessee, *Salmonella* Oranienburg, *Salmonella* Anatum, *Salmonella* Enteritidis PT 9c, and *Salmonella* Montevideo were stored at - 85˚C in a freezer. Each culture was inoculated into 10 ml of tryptic soy broth (BD, Sparks, MD) in a 15-ml conical centrifuge tube (Fisher Scientific, Pittsburgh, PA), vortexed, and incubated at 37˚C for 18 to 24 h. After incubation, 5 ml of overnight culture of each strain was transferred to a single 50-ml conical centrifuge tube (Fisher Scientific), and the mixture was vortexed to produce a cocktail of six *Salmonella* strains. The *Salmonella* cocktail was serially diluted in 0.1% peptone water, and 100 ml was spread plated on Xylose Lysine Tergitol 4 (XLT4) agar (BD). Plates were incubated for 24 h at 37˚C, and colonies counted to determine the level of *Salmonella* in the overnight culture to be approximately 1 X 10^9 CFU/ml. This method of preparation of overnight culture of each strain and subsequent preparation and plating of the cocktail was followed for the control experiment in peptone buffer, single-cycle high-pressure experiments, and pressure-cycling experiments.

II.2.2.2 HHPP of inoculated peptone water in plastic bottles

Twenty-fluid-ounce (592 ml) polyethylene terephthalate water bottles (Poland Spring, Poland, ME) were purchased and emptied. Six bottles were filled with sterile 0.1% peptone water (BD), and 1% of the volume of the overnight culture of the pathogenic *S. enterica* serovar cocktail was added to each bottle. Serial dilutions of the *Salmonella*-inoculated peptone buffer were made in 0.1% peptone buffer, and 100-ml

amounts were spread plated on XLT4 agar to determine the initial level of *Salmonella* in the buffer. The bottles were sealed with paraffin film. Three bottles were maintained at room temperature, and three bottles were high pressure processed at 600 MPa for 18 min. After the high pressure processing, serial dilutions in 0.1% peptone buffer were made for both the processed and unprocessed samples and 100-ml aliquots were spread plated. The plates were then incubated for 24 h at 37˚C, and colonies were counted to determine the log reduction of *Salmonella* by HHPP in peptone buffer.

II.2.2.3 Adiabatic heating value of peanut butter

The adiabatic heating value of peanut butter was determined in order to understand the maximum temperature attained by the peanut butter at various pressures during HHPP, starting at the initial room temperature. In order to determine this value, insulated 50-ml falcon tubes (**Fig. 6**) filled with peanut butter were fitted over two thermocouples of the HHPP equipment and high pressure processed at 600 MPa for 1 min. Based on the change in temperature with pressure data during this HHPP cycle, the dT/dP value (adiabatic heating value, where T is temperature and P is pressure) of peanut butter was calculated from the slope of a plot of dT versus dP.

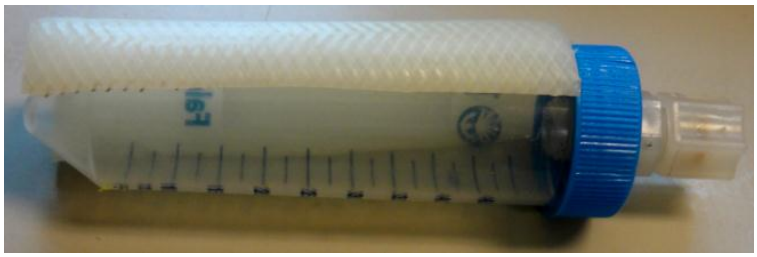


Figure 6: Setup of insulated falcon tube which was filled with peanut butter and fitted over two thermocouples of HHPP unit

II.2.2.4 Preparation of inoculated creamy peanut butter

Jars of Skippy® Creamy Peanut Butter in low density polyethylene jars was purchased. Water activity was measured using a digital aw meter (Rotronic Instrument Corp., Hauppauge, NY) and was found to be 0.17 ± 0.02 at 22˚C, based on an average of 10 measurements. For inoculated peanut butter, the purchased peanut butter was removed from the original containers and added to a 2,000-ml blender jar (Sunbeam Products, Inc., Boca Raton, FL), and a 1% inoculum by weight was added. The inoculated peanut butter was blended at high speed (speed setting 10), and during blending, the open top of the blender jar was covered with an aluminum foil cover to contain any aerosols generated. Part of the inoculated peanut butter was repacked, in a sterile environment under the laminar air flow hood, into the original plastic peanut butter jars (462 g each), and part was packed into two heat-sealable pouches that were cut out, filled with inoculated peanut butter (50 g each) and vacuum packed using a FoodSaver® vacuum sealer (Sunbeam Products, Inc., Boca Raton, FL) to prevent the pouches from bursting due to air pockets during high pressure processing. This process was repeated for each HHPP pressure-time condition under consideration.

II.2.2.5 HHPP of inoculated peanut butter samples in jars and pouches

The experimental design consisted of five pressure-time conditions which are shown in **Table 4**. Three pressure-time conditions for HHPP were 400 MPa for 18 min, 500 MPa for 9 min, and 600 MPa for 4 min along with an additional two extreme conditions of 400 MPa for 4 min and 600 MPa for 18 min. The initial temperature of the pressurizing medium (water) was room temperature (21 to 24˚C) for all conditions.

Inoculated peanut butter samples (two jars and two pouches) for each experimental condition were high pressure processed under the five conditions in the 10-liter HHPP vessel (Elmhurst Research, Inc., Albany, NY). During an HHPP run, the sample underwent pressurization, hold time at the desired pressure, and depressurization. Pressurization varied between 2 to 3 min based on the desired pressure. Depressurization occurred in less than 10 s. The temperature of the water inside the vessel was initially at 20 to 25˚C, would go up to a maximum of 37˚C during pressurization, drop by a few degrees due to heat loss to the vessel wall, and then drop rapidly to a few degrees below the initial temperature after depressurization. A sealed jar of uninoculated creamy peanut butter with its tamper-proof seal intact was also high pressure processed at 600 MPa for 18 min.

Table 4: Experimental conditions of pressure and time used for high pressure processing of inoculated peanut butter

	Pressure (MPa)		
Time (min)	**400**	**500**	**600**
4	X		X
9		X	
18	X		X

II.2.2.6 Enumeration of *Salmonella* counts in peanut butter before and after HHPP

25 g of an inoculated but unprocessed peanut butter (control) was weighed in a two chamber filter bag (Fisher Scientific, Pittsburgh, PA) and 225 ml of 0.1% peptone

water was added. The filter bag was stomached for 3 min, and ten-fold serial dilutions with 0.1% peptone water were made. 100 µl aliquots of the sample were spread plated in duplicate on XLT4 agar. The double bagged vacuum packed high pressure processed samples were removed from the bags and 25 g of each sample from the jars and pouches was weighed in two chamber filter bags. To each filter bag 225 ml of 0.1% peptone water was added. Each filter bag was put in the stomacher for 3 min and ten-fold serial dilutions with 0.1% peptone water were made. 100 µl aliquots of each sample were spread plated in duplicate on XLT4 agar. Plates for both the control and high pressure processed samples were incubated for 24 h at 37˚C. Black colonies of *Salmonella* were counted after incubation. Experiments for each pressure-time HHPP condition were conducted individually, and the data on HHPP of *Salmonella*-inoculated peanut butter were collected in duplicate.

II.2.2.7 Pressure cycling of inoculated creamy peanut butter samples during HHPP

It was reported that repeated cycles of pressurization hold time and depressurization may help to induce more pressure related changes, resulting in more extensive microbial inactivation in discontinuous HHPP (Goodridge, 2006). Three sets of conditions were selected for pressure-cycling experiments: 400 MPa for 3 cycles of 6 min each, 600 MPa for 3 cycles of 6 min each, and an extreme condition of 600 MPa for 10 cycles of 6 min each. The preparation of the inoculum, inoculation of creamy peanut butter, preparation of controls, and samples for high pressure processing, were carried out in the same manner as described for non-cycling HHPP experiments previously. Samples were high pressure processed at 400 MPa for 3 cycles of 6 min each, 600 MPa for 3

cycles of 6 min each, and 600 MPa for 10 cycles of 6 min each. Enumeration of *Salmonella* counts in peanut butter before and after pressure cycling was carried out in same manner as described non-cycling HHPP experiments. Experiments for each pressure-cycling condition were conducted individually two times, and the data on HHPP of *Salmonella*-inoculated peanut butter for each experiment were collected in duplicate.

II.2.2.8 Contribution of temperature during HHPP to inactivation of *Salmonella* in peanut butter

An experiment was designed to mimic the temperature profile of peanut butter during HHPP at 600 MPa for 4 min in the HHPP equipment to understand the contribution of temperature alone during HHPP to inactivation of *Salmonella* in peanut butter. The maximum temperature that the peanut butter reached due to adiabatic heating during a 600 MPa HHPP run as determined from temperature recorded data, was 52˚C. Hence, the desired temperature of peanut butter for this experiment was 52°C and the hold time was 4 min. Overnight culture of the *Salmonella enterica* serovars strains at 37˚C was prepared and cocktail prepared after incubation as described earlier in this chapter. 462 g of peanut butter from a 16.3 oz (462 g) Skippy® jar of peanut butter was blended with 4.6 ml of overnight culture (1% of the weight of peanut butter). 225 ml of peptone water was added to 25 g of inoculated peanut butter and put in the stomacher for 2 min. Ten-fold dilutions were made and the peanut butter sample plated on XLT4 agar plates in 100 µl aliquots to determine initial recovery of *Salmonella* in peanut butter (control). A small pouch with 10 g inoculated peanut butter was prepared and heat sealed with a thermocouple wire inserted. The pouch with thermocouple was connected to a data

acquisition system. The pouch was inserted into a water bath preheated to 52 ˚C and held for 4 min and then immediately put into a tub of ice to rapidly cool. 10-fold dilutions of this thermally processed sample was made and plated in 100 µl aliquots plated on XLT4 agar.

II.2.2.9 Effect of high temperature (50˚C) high pressure processing on *Salmonella* inoculated peanut butter

Overnight culture of the *Salmonella enterica* serovars strains at 37˚C was prepared and cocktail prepared after incubation as described earlier in this chapter. The vessel was filled with water and pre-heated to 50˚C overnight. 1% of the inoculum was inoculated into a large amount of peanut butter taken in the blender jar and blended until the inoculum was evenly distributed into the peanut butter. Two jars and two pouches of inoculated peanut butter were prepared and were loaded into the high pressure vessel where both the vessel and the pressurizing medium (water) were preheated to 50˚C using a hot iron rod connected to an external temperature controlled 13 l water tank. HHPP was carried out at 50˚C initial temperature, at 600 MPa for 18 min. 25 g of the unprocessed inoculated peanut butter samples (control) and the high pressure processed jar and pouch samples were weighed out, diluted with 225 ml peptone buffer and put in the stomacher for 2 min. Ten-fold dilutions were made and 100 µl aliquots of the control and processed samples plated on XLT4 agar.

II.2.2.10 Effect of low temperature (7˚C) high pressure processing on *Salmonella* inoculated peanut butter

Overnight culture of the *Salmonella enterica* serovars strains at 37˚C was prepared and cocktail prepared after incubation as described earlier in this chapter. The vessel pre-chilled to 7˚C by circulating cold water through the vessel overnight. 1% of the inoculum was inoculated into a large amount of peanut butter taken in the blender jar and blended until the inoculum was evenly distributed into the peanut butter. Two jars and two pouches of inoculated peanut butter were prepared and placed in a water bath at 7˚C for 5 min and were loaded into the high pressure vessel where both the vessel as well as the pressurizing medium (water) were pre-chilled to 7˚C. HHPP was carried out at 7˚C, at 400 MPa for 18 min. 25 g of the unprocessed inoculated peanut butter samples (control) and the high pressure processed jar and pouch samples were weighed out, diluted with 225 ml peptone buffer and put in the stomacher for 2 min. Ten-fold dilutions were made and 100 µl aliquots of the control and processed samples plated on XLT4 agar.

II.2.2.11 Effect of HHPP on *Salmonella* inoculated organic creamy peanut butter

Nature's Promise organic creamy peanut butter does not contain any hydrogenated vegetable oils nor any preservatives like salt. It only contains peanuts. To understand whether these components of Skippy® creamy peanut butter play any role in the 2 log reduction of *Salmonella* in peanut butter, an experiment was conducted using Nature's Promise organic peanut butter.

Overnight culture of the *Salmonella enterica* serovars strains at 37˚C was prepared and cocktail prepared after incubation as described earlier in this chapter. 1% of the inoculum was inoculated into a large amount of organic peanut butter taken in the blender jar and blended until the inoculum was evenly distributed into the peanut butter. Two jars and two pouches of inoculated peanut butter were prepared and loaded into the HHPP vessel. HHPP was carried out at 600 MPa for 18 min. 25 g of the unprocessed inoculated peanut butter samples (control) and the high pressure processed jar and pouch samples were weighed out, diluted with 225 ml peptone buffer and put in the stomacher for 2 min. Ten-fold dilutions were made and 100 µl aliquots of the control and processed samples plated on XLT4 agar.

II.3. RESULTS AND DISCUSSION

II.3.1. Effect of HHPP on inoculated peptone water in plastic bottles

Figure 7 shows the effect of HHPP at 600 MPa for 18 min on *Salmonella*-cocktail inoculated peptone water in plastic PET bottles. This experiment serves as a control.

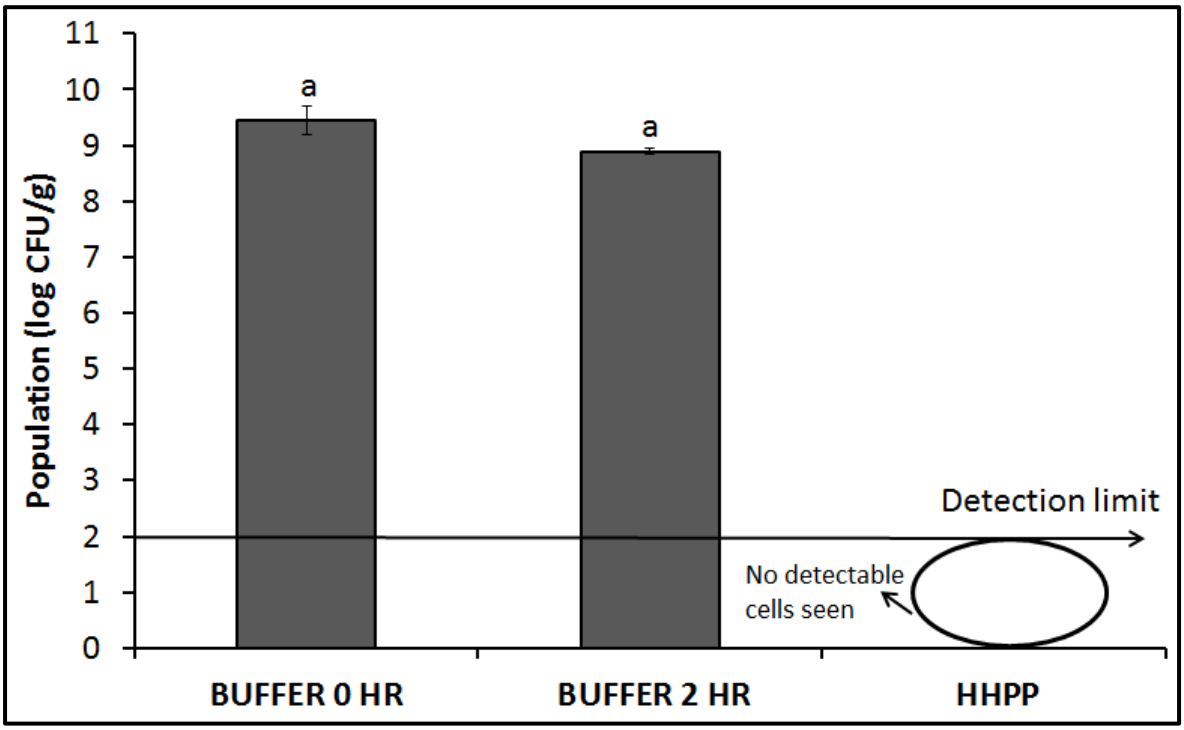


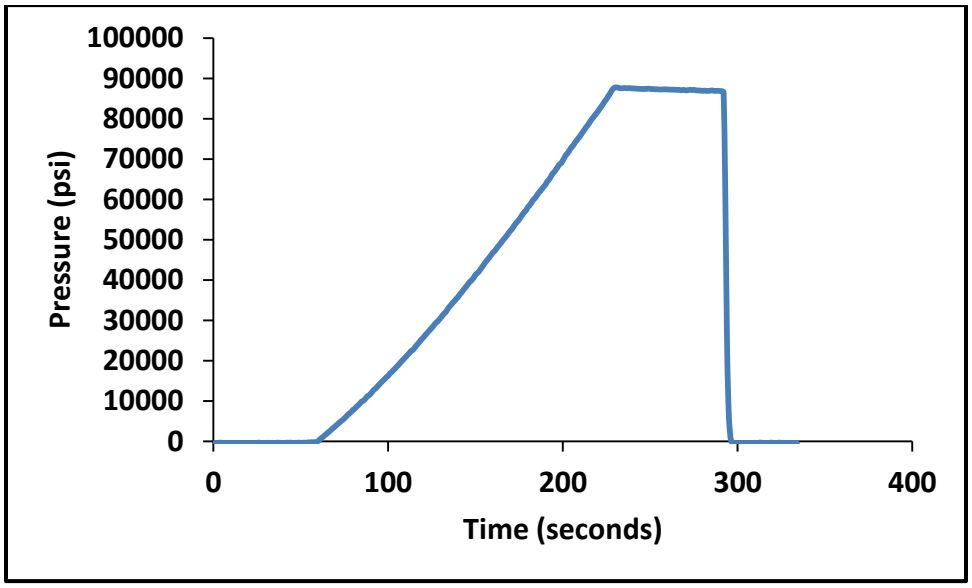
Figure 7: Effect of HHPP on *Salmonella* in peptone water. Same lowercase letters indicate results imply no significant difference ($p<0.05$)

The *Salmonella* cocktail–inoculated polyethylene terephthalate (PET) bottles containing 0.1% peptone water originally contained 9.45 ± 0.45 log CFU/ml. For the unprocessed samples spread plated on XLT4 2 h after inoculation, the level of *Salmonella* was 8.88 ± 0.09 log CFU/ml. No detectable cells were observed in the high pressure processed samples, i.e., the *Salmonella* cocktail was inactivated to below the detection

limit of 100 CFU/ml (D'souza, et. al., 2012). This is similar to the results obtained by Ritz et al. (2006), where *Salmonella* Typhimurium ATCC 13311 in phosphate and citrate buffers was inactivated to levels below the detection limit after HHPP at 600 MPa for 10 min. This showed that HHPP is very effective at reducing the microbial load in foods with higher water activities (Goodridge, 2006).

II.3.2. Adiabatic heating value of peanut butter

The temperature (˚C) and pressure (psi) data as recorded by LabVIEW 7 ® software as a function of time in the HHPP unit from the two thermocouples, was plotted in a graph format and the slope and coefficient of determination (R^2) of the trend line were calculated. **Figure 8** shows the pressure vs time data and temperature vs time data for peanut butter during HHPP at 600 MPa for 1 min.



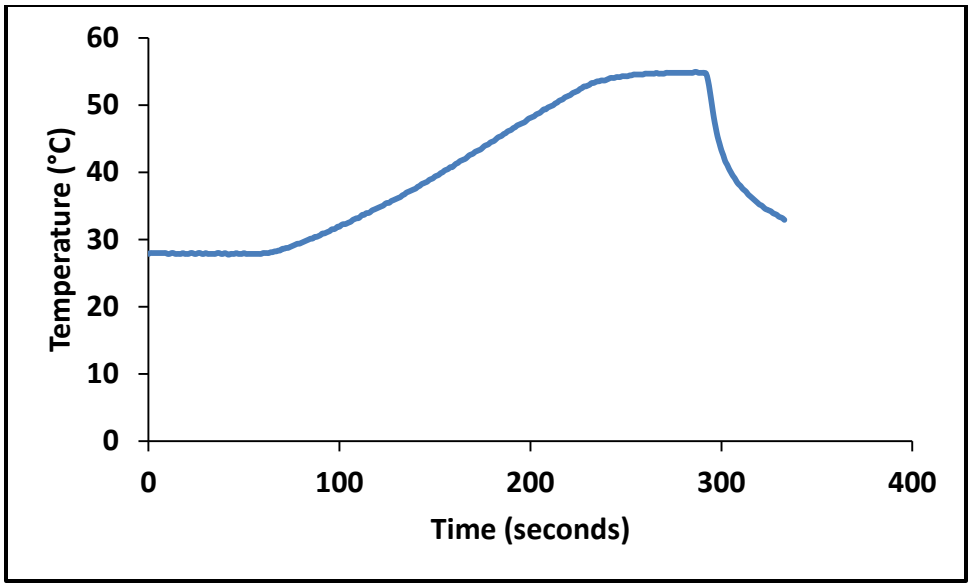
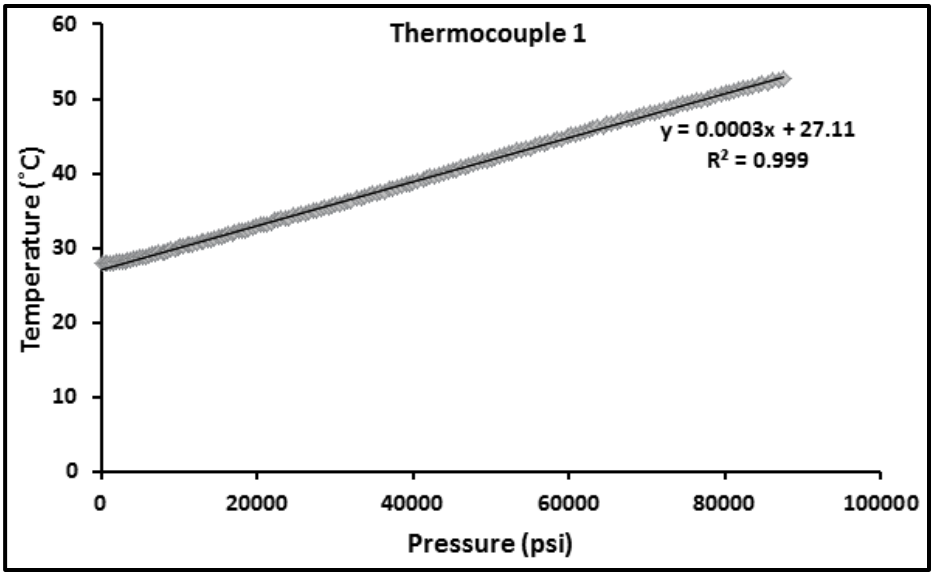


Figure 8: Pressure vs. time and temperature vs. time data for peanut butter during HHPP at 600 MPa for 1 min

Figure 9 below shows the temperature vs. pressure data for peanut butter during HHPP at 600 MPa for 1 min obtained from both thermocouples.



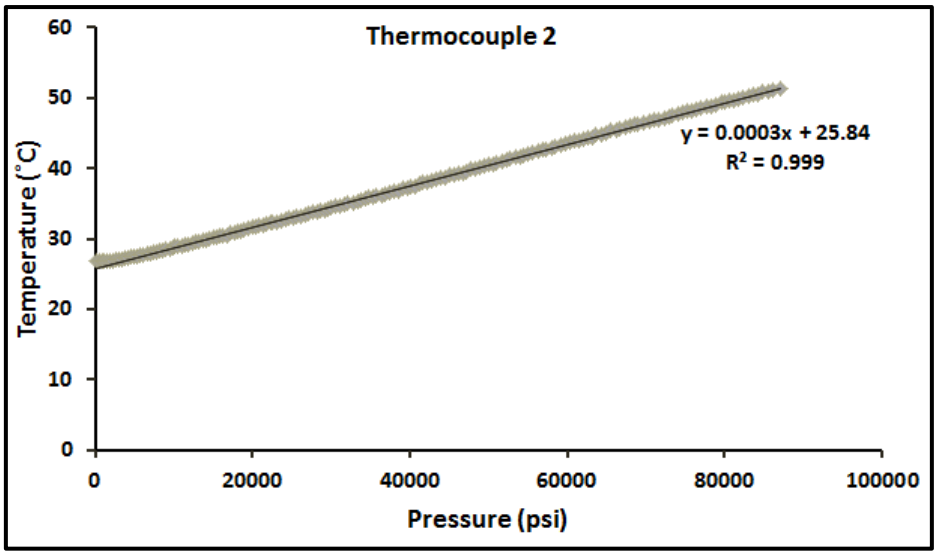


Figure 9: Temperature vs. Pressure data for peanut butter at 600 MPa for 1 min from 2 thermocouples of the HHPP unit

Thermocouple 1: 203 mm from top of pressure cavity

Thermocouple 2: 318 mm from top of pressure cavity

The slope dT/dP represents the adiabatic heating value of peanut butter and was calculated in the unit ˚C/100MPa as follows:

For thermocouples 1 and 2:

Slope: 0.0003 ℃/psi

$$\frac{\mathrm{d}T}{\mathrm{d}P} = \frac{0.0003 * 14.6}{0.1}$$

$$\frac{\mathrm{d}T}{\mathrm{d}P} = 0.044\ ^\circ C/MPa$$

$$\frac{\mathrm{d}T}{\mathrm{d}P} = 4.4\ ^\circ C/100MPa$$

Adiabatic heating value of peanut butter: 4.4℃ per 100 MPa

This value helped understand the temperature to which peanut butter rises during a HHPP run. For example, if peanut butter was initially at 25˚C after HHPP at 600 MPa, due to adiabatic heating, its temperature would go up to a maximum of 25 + (4.4 X 6) = 51˚C approximately.

II.3.3. Effect of HHPP on uninoculated and inoculated peanut butter

High pressure processing of the uninoculated, sealed peanut butter jar at 600 MPa for 18 min did not affect the structural integrity of the jar or the tamper-proof seal. There were no visual adverse effects of HHPP on the peanut butter either. This indicated that post-packaging processing of peanut butter with HHPP is compatible with the currently used peanut butter packaging materials. **Figure 10** shows a jar and pouch of inoculated peanut butter before HHPP.

Figure 10: Jar and pouch of inoculated unprocessed peanut butter

The change in pressure and temperature with time during HHPP of inoculated peanut butter at 600 MPa for 18 min is shown in **Fig. 3**.

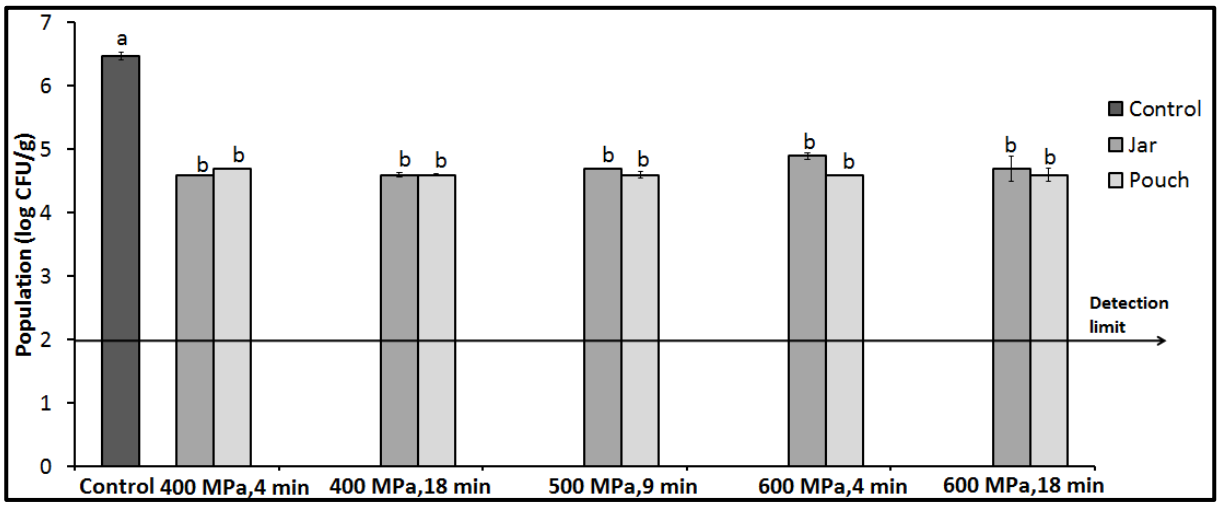


Figure 11: Populations of the pathogenic cocktail of *Salmonella* enterica serovars in the control (recovered in peanut butter) and jars and pouches of creamy peanut butter under the five sets of HHPP conditions. Same lowercase letters indicate results are not significantly different ($p<0.05$).

The mean initial level of *Salmonella* recovered in the peanut butter after blending was 6.48 ± 0.06 log CFU/g. As seen in **Fig. 11**, the log reductions of *Salmonella* in peanut butter after HHPP for both the jars and the pouches under all five sets of conditions varied from 1.6 to 1.9 log CFU/g. These results were in contrast to the results of the control experiment, where *Salmonella* was inactivated to below the detection limit in peptone buffer (**Fig. 7**).

Adiabatic heating of the peanut butter during HHPP resulted in the temperature of the peanut butter rising to 45, 49, and 53˚C at 400, 500, and 600 MPa, respectively, from an initial temperature of 29˚C. Based on research where *Salmonella* in peanut butter was subjected to heat alone, it can be estimated that heating in the range of 45 to 53˚C for 4 to 18 min would be expected to result in no more than a 0.3 log reduction, and in many

cases much less. The effect of adiabatic heating in addition to pressure can help to explain the difference between these results and those of Grasso et al. (2010), where pre-chilled samples of peanut butter were high pressure processed such that the final peanut butter temperature did not exceed 45˚C, and it was seen that there were no significant reductions ($p < 0.05$) between the unprocessed positive control and each of the inoculated peanut butter samples that were high pressure processed at 600 MPa for 5 min at 45˚C. The research done by Grasso et al. (2010) also used a single avirulent strain of *S. enterica* serovar Typhimurium ATCC 53647, which differed from the cocktail of pathogenic *S. enterica* serovar strains obtained from peanut butter and other nut outbreaks used in this research (D'souza et al., 2012).

II.3.4. Effect of pressure cycling during HHPP on inoculated creamy peanut butter samples

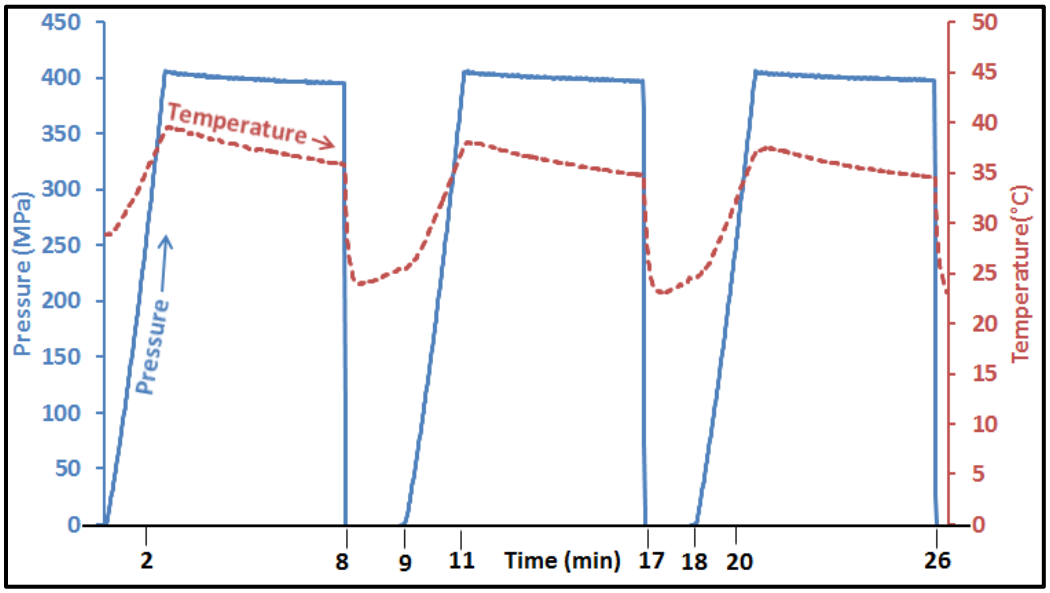


Figure 12: Pressure and temperature variation with time at 400 MPa, 3 cycles, 6 min each

Figure 12 shows the variation in pressure and temperature with time at 400 MPa, 3 cycles, 6 min each. Three cycles of pressurization, hold time and depressurization are clearly depicted.

The mean initial level of *Salmonella* inoculum recovered in the peanut butter after blending and before pressure cycling was 6.53 ± 0.21 log CFU/g. **Figure 13** shows the effect of pressure cycling on *Salmonella* in peanut butter in both jars and pouches.

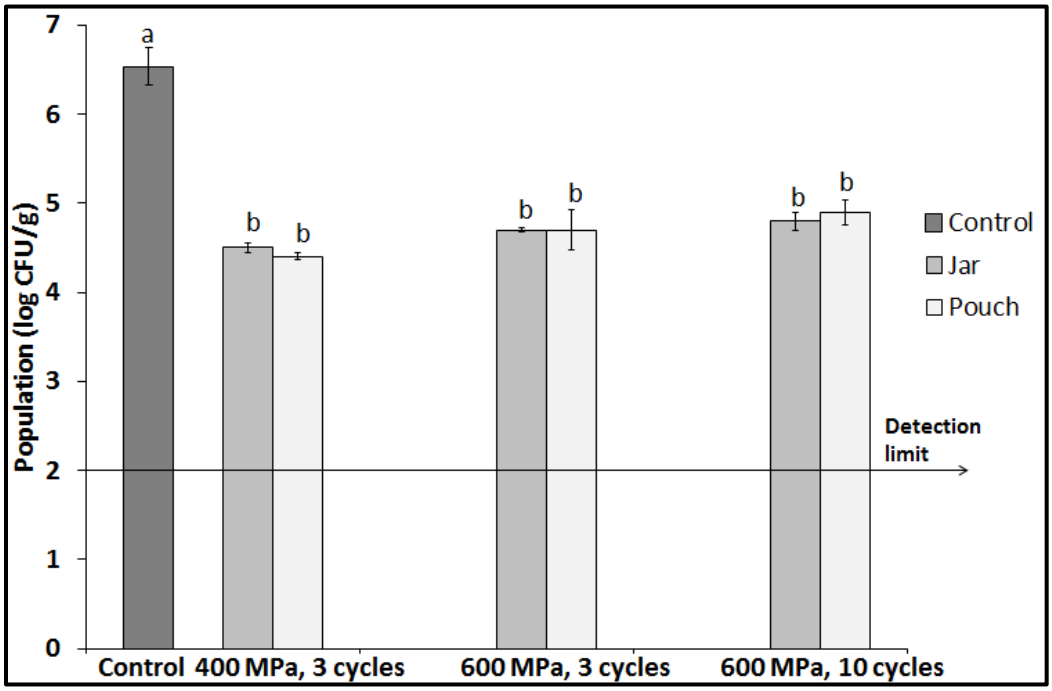


Figure 13: Populations of the pathogenic cocktail of *Salmonella* enterica serovars in the control (recovered in peanut butter) and jars and pouches of creamy peanut butter pressure cycled under the three sets of HHPP conditions. Same lowercase letters indicate results are not significantly different ($p<0.05$).

The log reduction achieved by all the pressure-cycling experiments varied from 1.8 to 1.9 log CFU/g. Pressure cycling does not cause greater inactivation of *Salmonella* in peanut butter than a single cycle of HHPP (D'souza et al., 2012). This disagrees with

the results obtained by Goodridge et al. (2006), where a greater log reduction of *Salmonella* Enteritidis inoculated on the surface of raw almonds, a low-water-activity food, occurred by pressure cycling than by steady pressure. It is possible that the raw almond surface represents a different environment than that seen in the peanut butter matrix, such that pressure cycling is more effective.

II.3.5 Contribution of temperature during HHPP to inactivation of *Salmonella in* peanut butter

Figure 14 shows the temperature-time data for inoculated peanut butter when the temperature profile of peanut butter during a 600 MPa 4 min HHPP cycle is mimicked in a water bath.

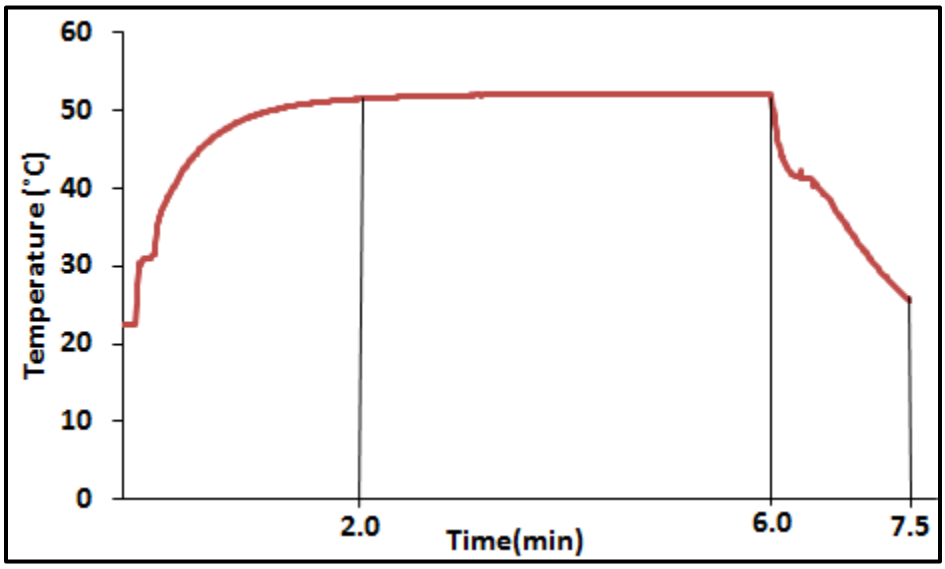


Figure 14: Variation of temperature with time during thermal processing of peanut butter in a water bath

Figure 15 compares the level of *Salmonella* in peanut butter after thermal processing and high pressure processing with the initial level of *Salmonella* in peanut butter.

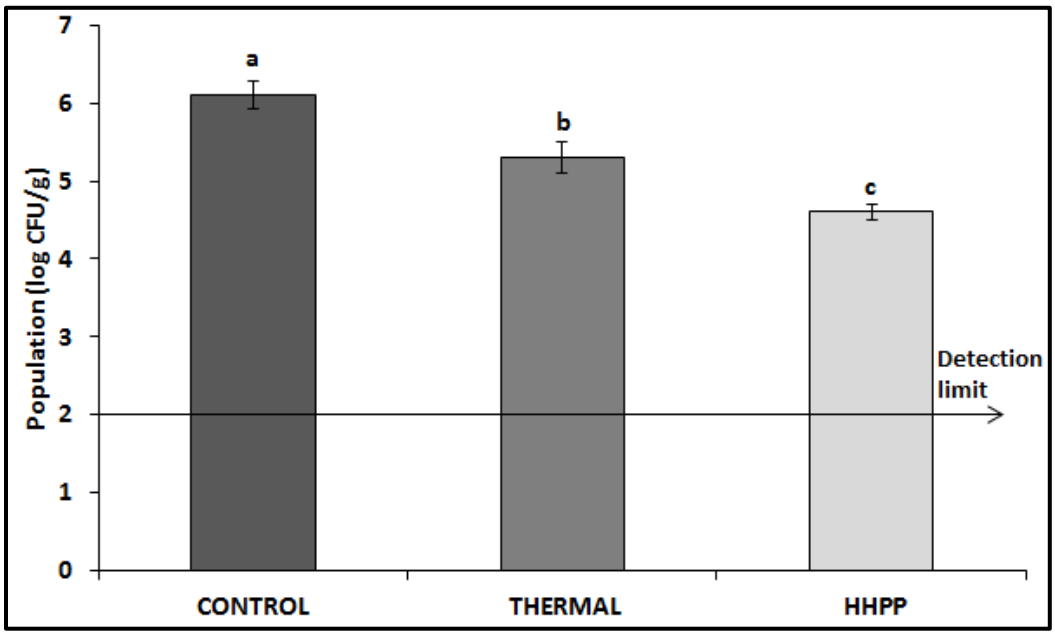


Figure 15: Populations of the pathogenic cocktail of *Salmonella* enterica serovars in the control (recovered in peanut butter), in the thermally processed samples and in the high pressure processed samples (600 MPa for 4 min). Same lowercase letters indicate results are not significantly different ($p<0.05$).

The thermal processing experiment was carried out as close as possible to the thermal changes peanut butter undergoes during a HHPP cycle of 600 MPa for 4 min. The pressurization time for a 600 MPa cycle is approximately 3 min and in this experiment, it took 2 min in the water bath for the temperature of peanut butter in the pouch to come up to 52˚C. The hold time was maintained at 4 min. Depressurization takes place in less than 10 seconds and the temperature rapidly drops to 24˚C. However during thermal processing of peanut butter, when the peanut butter pouch was transferred from the water bath into a tub of ice it took about 1.5 min to come down to 24˚C. From

Fig. 15, it is seen that temperature alone contributes to almost half of the log reduction achieved by HHPP, which is a combination of pressure and temperature factors acting on inoculated peanut butter. Hence, it can be estimated that during HHPP, temperature plays almost an equal role in combination with pressure to achieve the 1.6 – 1.9 log reduction of *Salmonella* in peanut butter achieved so far at the various pressure-time combinations of HHPP used.

II.3.6 Effect of high temperature (50˚C) high pressure processing on *Salmonella* inoculated peanut butter

Figure 16 shows the inactivation of *Salmonella* in both the jar and pouch after HHPP at 50˚C compared to unprocessed inoculated peanut butter.

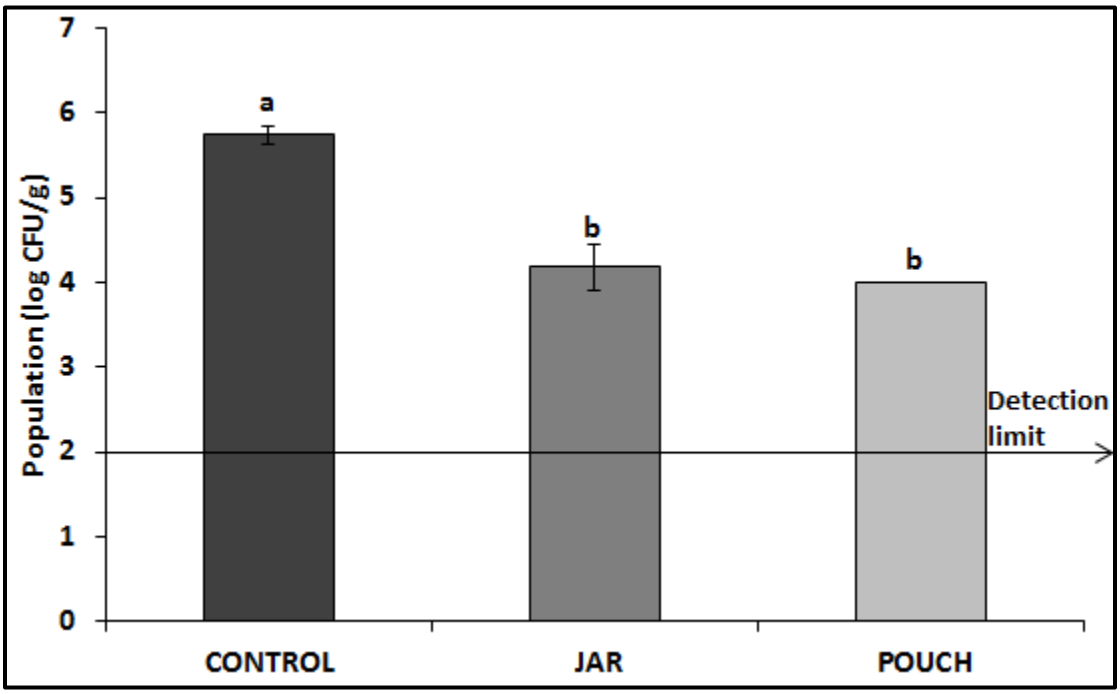


Figure 16: Populations of *Salmonella* in the inoculated unprocessed peanut butter (control) and the jar and pouch after HHPP at 50˚C. Same lowercase letters indicate results are not significantly different ($p<0.05$).

Based on the adiabatic heating value of peanut butter the temperature of peanut butter would have at least gone up to 70˚C, which differs slightly for the jar and pouch due to differing surface areas. The mean initial recovery of *Salmonella* in the peanut butter after blending was 5.73 ± 0.14 log CFU/g. As seen in **Fig. 16**, the mean log reductions achieved in the jar was 1.6 log CFU/g and in the pouch 1.7 log CFU/g which was comparable to the log reductions achieved at the various pressure-time combinations of HHPP at room temperature. Hence, it can be estimated that HHPP at higher temperatures does not enhance inactivation of *Salmonella* in peanut butter. It can also be understood that there is an almost equal contribution of temperature to inactivation achieved during this HHPP cycle as shown in section II.3.5 (**Fig. 15**).

II.3.7 Effect of low temperature (7˚C) high pressure processing on *Salmonella* inoculated peanut butter

Fig. 17 shows the inactivation of *Salmonella* in both the jar and pouch after HHPP at 7˚C compared to unprocessed inoculated peanut butter.

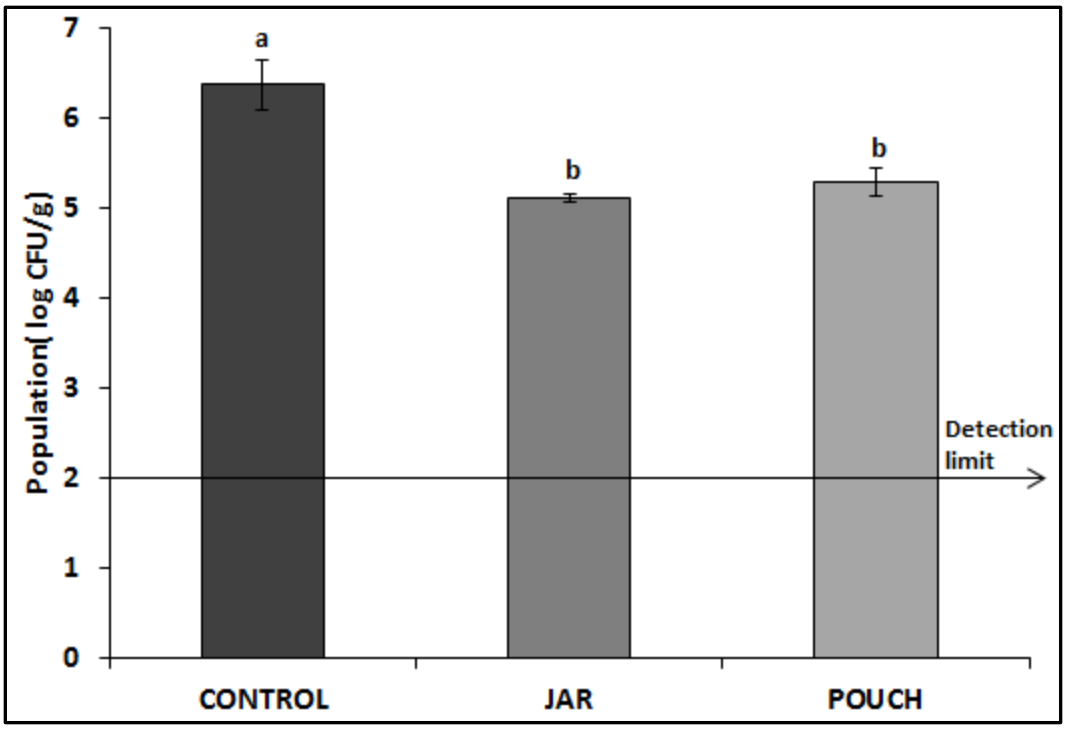


Figure 17: Populations of *Salmonella* in the inoculated unprocessed peanut butter (control) and the jar and pouch after HHPP at 7˚C. Same lowercase letters indicate results are not significantly different ($p<0.05$).

Based on the adiabatic heating value of peanut butter the temperature of peanut butter would have gone up to room temperature 23˚C. The mean initial recovery of *Salmonella* in the peanut butter after blending was 6.37 ± 0.39 log CFU/g. The mean log reductions achieved in the jar was 1.2 log CFU/g and in the pouch 1.1 log CFU/g which was significantly slightly less comparable to the log reductions achieved at the various pressure-time combinations of HHPP at room temperature. This can be explained by the minimal contribution of temperature during HHPP to the log reduction, due to a low initial temperature. Hence, it can be estimated that HHPP at lower temperatures does not enhance inactivation of *Salmonella* in peanut butter.

II.3.8 Effect of HHPP on *Salmonella* inoculated organic creamy peanut butter

The level of *Salmonella* initially recovered in organic peanut butter was 5.91 ± 0.01 log CFU/g and after high pressure processing the log reductions achieved in the jar and pouch was approximately 2 log CFU/g as seen in **Fig. 18**. This is statistically comparable to the results obtained with Skippy® creamy peanut butter, suggesting that the ingredients other than peanuts present in it such as salt and hydrogenated vegetable oils, do not contribute to reducing or enhancing *Salmonella* inactivation by HHPP in peanut butter.

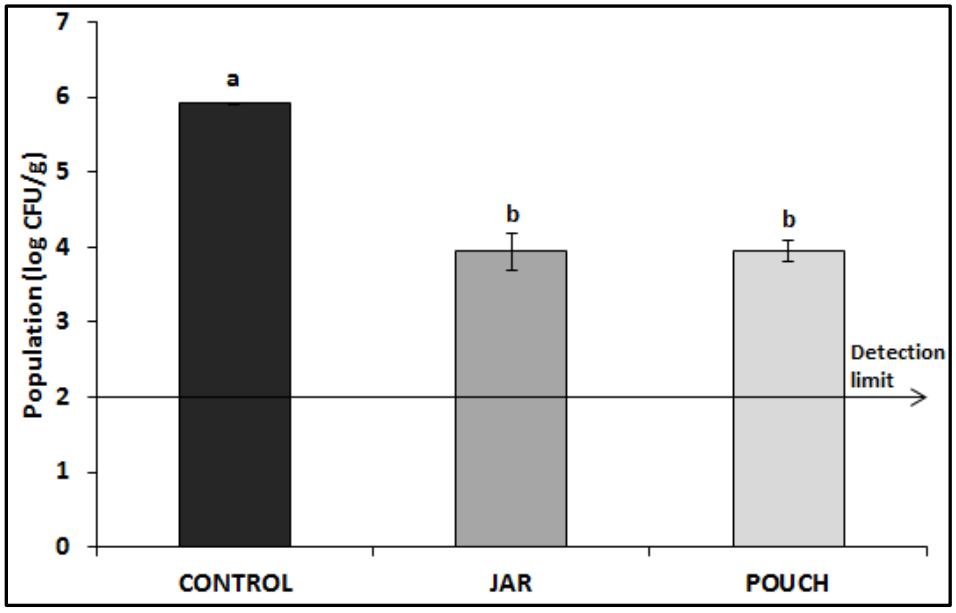


Figure 18: Populations of *Salmonella* in the inoculated unprocessed organic peanut butter (control) and the jar and pouch after HHPP at 600 MPa for 18 min. Same lowercase letters indicate results are not significantly different ($p<0.05$).

Thus, in this chapter, it was shown that various combinations of pressure and time during HHPP as well as pressure cycling could only achieve a 1.6-1.9 log reduction of *Salmonella* in peanut butter. It was also established that temperature contributed almost equally to pressure during HHPP to this inactivation of *Salmonella* achieved.

III. EFFECT OF HIGH HYDROSTATIC PRESSURE ON THE PATHOGENIC *SALMONELLA ENTERICA* INOCULATED INTO CREAMY PEANUT BUTTER WITH MODIFIED COMPOSITION

III. 1. BACKGROUND

III.1.1 Basis for experiments with peanut butter of a modified composition

Results presented in part II of this thesis showed that pressure, time and temperature did not achieve a commercially significant log reduction of *Salmonella* in peanut butter. To enhance the comprehensiveness of this study, the next closest options to inflict inactivation of *Salmonella* in peanut butter by HHPP was to modify its composition. Starting with understanding *Salmonella* behavior in individual components of peanut butter, this study involved studying the effect of HHPP on peanut butter by modifying its water activity with addition of distilled water and 100% peanut oil in different calculated proportions as well as peanut butter with addition of calculated amounts of nisin, scientifically proven to be effective in combination with HHPP on *Salmonella* and other Gram negative bacteria in certain foods. A survival study was also conducted in both inoculated, unprocessed peanut butter and inoculated, high-pressure processed over 10 weeks to study the survival pattern of *Salmonella* in peanut butter over time.

III.1.2 *Salmonella* behavior in individual components of peanut butter

Early results presented in this thesis showed only modest (1.6 – 1.9 log CFU) reduction in *Salmonella* concentration. In an effort to understand inactivation

mechanisms and to improve inactivation, efforts were to study *Salmonella* survival in peanut oil and peanut meal (the two major components of peanut butter matrix) separately.

III.1.3 Modifying the water activity of peanut butter

It was essential to change (both increase and decrease) the water activity of peanut butter and study its effect on inactivation of *Salmonella* by HHPP. The simplest way to increase the water activity of peanut butter is to add distilled water and the simplest way to decrease the water activity of peanut butter is to add peanut oil. These simple additions also minimize the influence of additional factors on the *Salmonella* inactivation by HHPP.

III.1.4 Effect of Nisin in combination with HHPP

Nisin is an antimicrobial peptide or bacteriocin produced by *Lactococcus lactis* subsp. *lactis* that is highly effective against gram-positive bacteria and spores (Delves-Broughton, 2005). It is a natural, toxicologically safe food preservative. Nisin was approved by FDA in 2001 with GRAS status for usage at levels ranging from ~1-25 ppm in dairy products, meat products and canned foods as a preservative (Delves-Broughton, 2005). It shows little or no activity against Gram – negative bacteria, yeasts and molds (Delves-Broughton, 2005). Since 1953, it has been sold by Danisco (KS) under the trade name Nisaplin®, which contains approximately 2.5% pure nisin, the rest being milk and milk solids derived from the fermentation of a modified milk medium by nisin producing strains of *L. lactis*. It is most stable in the pH range of 3.0-3.5 (Delves-Broughton, 2005).

Although nisin has not been commonly known to be effective against Gram-negative bacteria like *Salmonella*, recent studies have shown that nisin, in combination with HHPP may inactivate Gram-negative organisms (Lee and Kaletunc, 2010). The effects of HHPP and nisin treatment alone and in combination on cellular components and viability of two *Salmonella enterica s*erovar Enteritidis strains in buffer were evaluated (Lee and Kaletunc, 2010). Inactivation from a concentration of 9.2 log CFU/ml to below detection limit (1.0 log CFU/ml) was observed after a pressure treatment at 500 MPa for one strain and 450 MPa for the other strain. When nisin was added, a similar reduction was obtained at 400 MPa for one strain and 350 MPa for the other strain. These researchers hypothesized that HHPP caused alterations in the outer cytoplasmic membrane of Gram negative bacteria thus facilitating penetration of nisin into the cell thus causing cell death (Lee and Kaletunc, 2010). In another study, pressurization in the presence of nisin increased the inactivation of generic *E. coli* (also Gram-negative) by an additional 3 log units in skim milk at 550 MPa (Garcia-graells et al., 1999). Based on these studies, the effect of HHPP in combination with nisin was explored for *Salmonella* inactivation in peanut butter.

III.1.5 Survival study of *Salmonella* in unprocessed and high pressure processed peanut butter

Finally, in order to understand the survival pattern of *Salmonella* in inoculated unprocessed peanut butter as well as inoculated high pressure processed peanut butter, the survival of *Salmonella* in peanut butter over 10 weeks at room temperature was measured.

III.2. MATERIALS AND METHODS

III.2.1. Materials

The peanut butter and pathogenic strains of *Salmonella* used were the same as mentioned in Chapter II 2.1.2.

III.2.1.1 Nisaplin®

One kg of Nisaplin® was purchased from Danisco® (New Century, KS) in a polyethylene bottle with a tamper-proof seal. Nisaplin® is composed of nisin (minimum 1000 IU/mg) and sodium chloride (minimum 50%), and contains 2.5% pure nisin by weight. The recommended dosage level of Nisaplin® is 25-500 mg per kg or liter of food.

III.2.1.2 Peanut Oil

A 24 fl oz. bottle of Planters 100% Peanut Oil (New Century, KS) was purchased from a local supermarket. The nutrition facts label indicated 14 g of fat per 14 g-serving (1 Tbsp). The bottle of peanut oil was stored at room temperature before and after opening.

III.2.1.3 Peanut Flour

A 1 kg sample of 12% fat, light roast, partially defatted peanut flour was obtained from Golden Peanut Company (Alpharetta, GA).

III.2.1.4 Peanut Butter

16.3 oz. jars of Skippy® Creamy Peanut Butter were purchased from local supermarkets. The ingredients listed were roasted peanuts, sugar, hydrogenated vegetable oils (cottonseed, soybean, and rapeseed), and salt. The nutrition facts label indicated 16 g of fat and 7 g of protein per each 32 g serving, and each jar contained 462 g of peanut butter. Unopened jars of processed peanut butter were stored at room temperature. Once opened, the jars were stored at refrigeration to prevent rapid spoilage.

III.2.1.5 Almond Butter

16.3 oz. jars of Nature's Promise Organic Almond Butter were purchased from local supermarkets. The nutrition facts label indicated 16 g of fat and 5 g of protein per each 32 g serving, and each jar contained 462 g of almond butter.

III.2.2 Methods

III.2.2.1 *Salmonella* behavior in peanut oil after HHPP

Overnight culture of the six pathogenic strains *Salmonella enterica* serovar strains was prepared and cocktail prepared as described in Chapter II. One tenth ml of the cocktail was inoculated into 10 ml of peanut oil in plastic vials (Fisher Scientific, Pittsburgh, PA). The contents of the vials were vortexed, diluted with peptone water prepared and plated on XLT4 (BD, Sparks, MD) agar plates in duplicate to determine the initial load of *Salmonella* in the peanut oil. Control vials of *Salmonella* inoculated peanut oil were stored at room temperature for 2 hours (equivalent to the time to prepare samples

and process them under high pressure), and test vials of *Salmonella* inoculated peanut oil were vacuumed packed using a FoodSaver® vacuum sealer (Sunbeam Products, Inc., Boca Raton, FL) prior to high pressure processing. Vials were high pressure processed at 600 MPa for 18 min at room temperature. The ambient temperature control vials and high-pressure processed vials were vortexed, diluted in peptone water and plated on XLT4 agar plates. The high-pressure processed samples were diluted if needed, and plated in duplicate on XLT4 agar plates. All plates were incubated for 24 h at 37˚C. Black colonies were putatively identified as *Salmonella* and enumerated after incubation.

III.2.2.2 *Salmonella* behavior in peanut flour after HHPP

The other major component of peanut butter is the peanut meal or peanut protein. Due to food industry policy constraints, we were not able to obtain 100% defatted peanut flour (peanut meal) which is used as animal feed. We did obtain 12% fat, light roast peanut flour and used this for all subsequent experiments. Five hundred (500) g of partially defatted 12% fat light roast peanut flour was weighed into a 2000 ml blender jar. It was not possible to achieve 100% defatted peanut flour since it is against regulations for use in laboratories for experiments and is used as animal feed only. A cocktail of *Salmonella* strains was prepared as mentioned in Chapter II and 5 ml of the cocktail inoculated into the peanut flour (1% by weight of peanut flour) and blended. The inoculated peanut flour was distributed into four pouches (Fisher Scientific, Pittsburgh, PA) and high pressure processed at 600 MPa for 18 min at room temperature. Control (unprocessed inoculated peanut flour) and high-pressure processed samples were serially

diluted and plated on XLT4 agar plates. The plates were incubated at 37˚C for 24 hours and putative *Salmonella* (black colonies) enumerated after incubation.

III.2.2.3 Effect of HHPP on *Salmonella* in peanut butter at higher water activity

Different volumes of sterile distilled water were added and blended into peanut butter to increase its water activity. Peanut butter water activity was measured using the digital a_w meter (Rotronic Instrument Corp., Hauppauge, NY). Experiments were carried out with 10%, 15%, 25%, 35%, 50%, 75% and 90% (w/w) added moisture. For example for the 10% added moisture experiment, a 16.3 oz (462 g) jar of peanut butter was emptied into the blender jar and 10% of the weight of peanut butter, i.e., 46.2 ml of sterile distilled was water added and blended into the peanut butter until no phase separation was observed. This was repeated for other added moisture content experiments in a similar manner. For each added moisture content sample, the water activity was measured in replicates using the digital a_w meter.

A cocktail of *Salmonella* strains was prepared as described in Chapter II and inoculated into the modified peanut butter (1% by weight of the modified peanut butter). The inoculated peanut butter was distributed into pouches, the pouches were vacuum packed using the FoodSaver® vacuum sealer (Sunbeam Products, Inc., Boca Raton, FL) and high pressure processed at room temperature at 600 MPa for 18 min. Control and high pressure processed samples were diluted and plated on XLT4 agar. Plates were incubated at 37˚C for 24 hours and putative *Salmonella* colonies enumerated.

III.2.2.4 Effect of HHPP on *Salmonella* inoculated decreased water activity formulations of peanut butter

Measured volumes of peanut oil were added and blended into peanut butter to decrease its water activity. Resulting water activities were measured using the digital a_w meter. Experiments were carried out with 50% and 75% added peanut oil (w/w). For example for the 50% added peanut oil experiment, a 16.3 oz (462 g) jar of peanut butter was emptied into the blender jar and 50% of the weight of peanut butter, i.e., 231 ml of peanut oil added and blended into the peanut butter until no phase separation is observed. This was repeated for the 75% added peanut oil content experiment in a similar manner. A cocktail of *Salmonella* strains was prepared as described in Chapter II and inoculated (1% by weight) into the modified peanut butter. The inoculated peanut butter was distributed into pouches, vacuum packed and high pressure processed at room temperature at 600 MPa for 18 min. Samples were diluted and plated on XLT4 agar plates, which were incubated at 37˚C for 24 hours and then enumerated.

III.2.2.5 Effect of nisin in combination with HHPP on *Salmonella* inoculated peanut butter

Nisin was incorporated into peanut butter in its food grade formulation Nisaplin® (Danisco®, USA) at four concentrations: 100 ppm, 200 ppm, 500 ppm and 1000 ppm. This corresponds to 2.5 ppm, 5 ppm, 12.5 ppm and 25 ppm of pure nisin, which is within the range of recommended dosage levels for food applications (Delves-Broughton, 2005). Nisin shows increased solubility in an acidic environment and shows most stability in the pH range of 3.0 to 3.5 (Delves-Broughton, 2005). According to Friedman and Epstein in

1951, an accurately weighed quantity of nisin is dissolved was 0.02 N hydrochloric acid (HCl) for better solubility of nisin.

Calculations:

To prepare 0.02N hydrochloric acid, 37% hydrochloric acid was taken and the following calculations done:

37% HCl = 37 g / 100 ml = 370 g/ 1000 ml

Based upon the law of equivalents,

N1 X V1 = N2 X V2

Molecular weight of HCl = 36.5

N1 = Normality of 37% HCl = 370/36.5 N

V1 = ?

N2 = Desired normality = 0.02 N

V2 = 1000 ml

N1 X V1 = N2 X V2

(370/36.5) X V1 = 0.02 X 1000

V1 = 1.972 ml

1.972 ml of 37% HCl was needed to prepare 1000 ml of 0.02 N HCl

Hence, to prepare 50 ml of 0.02 N HCl, 0.099 ml i.e. ~0.1 ml of 37% HCl was needed.

To calculate the amount of Nisaplin® to add to peanut butter to have for example a 100 ppm concentration:

100 ppm Nisaplin® (2.5 ppm nisin) = 100 mg / 1000 g of peanut butter, i.e., 0.1 g /1000 g of peanut butter

Hence, for 462 g of peanut butter (weight of peanut butter per 16.3 oz jar), 0.046 g of Nisaplin® was dissolved in 0.02 N HCl. Similarly, the weight of Nisaplin® to be added to 462 g of peanut butter at 200 ppm (5 ppm nisin), 500 ppm (12.5 ppm nisin) and 1000 ppm (25 ppm nisin) was calculated to be 0.092 g, 0.23 g and 0.46 g respectively.

Experiment methodology:

A cocktail o*f Salmonella* strains was prepared as described in Chapter II. Appropriate amounts of Nisaplin® to produce the desired concentration in 462 g of peanut butter was weighed, added to 3 ml of 0.02 N HCl, vortexed to dissolve and filtered to produce a non-particulate solution. This Nisaplin® solution was added to peanut butter and blended until thoroughly mixed (~2 min). The inoculum was then added to the peanut butter (1% by weight of peanut butter) and blended. The inoculated nisin-containing peanut butter was then distributed into multiple pouches and high pressure processed at 600 MPa for 18 min. Twenty five (25) g of control samples (nisin-containing inoculated unprocessed peanut butter) and 2 pressure treated pouches was weighed out in filter bags 1 hour after high pressure processing. The 1 hour delay was to allow time for nisin to permeate through the ruptured membrane of the *Salmonella* cells after high pressure processing. Twenty-five ml of peptone water was added to each 25 g sample of peanut butter and stomached for 2 min followed by dilution and plating on XLT4 agar plates. Plates were incubated at 37˚C for 24 hours and colonies enumerated. Other pressure treated pouches were stored at room temperature to sample over a longer

time periods. The extent to which the experiment was carried out varied for each concentration of Nisaplin® differed as follows: High pressure processed pouches containing 2.5 and 5 ppm nisin were sampled 1 day after high pressure processing, samples containing 12.5 ppm nisin were sampled after 1 and 3 days and samples containing 25 ppm nisin were sampled 1, 3, 5 and 7 days.

Role of nisin alone

To determine if Nisaplin® alone played any role over time on inactivation of *Salmonella* in peanut butter without high pressure processing, an experiment was carried out using 12.5 ppm nisin (500 ppm Nisaplin®). A cocktail o*f Salmonella* strains was prepared as described in Chapter II. A quantity of Nisaplin® needed to produce 500 ppm concentration in 462 g of peanut butter was measured, added to 3 ml of 0.02 N HCl, vortexed and filtered to get a non-particulate solution. This solution was added to peanut butter and. The inoculum was then added to the peanut butter (1% by weight of peanut butter) and blended. The nisin containing inoculated peanut butter was then distributed into multiple pouches and sampled immediately after inoculation and after 1 hour, 1 day, 3 days, 5 days and 7 days. For each time interval, 25 ml of peptone water was added to each sample of peanut butter and stomached for 2 min. Samples were diluted and plated on XLT4 agar plates. Plates were incubated at 37˚C for 24 hours and black colonies of *Salmonella* enumerated after incubation.

III.2.2.6 Survival pattern of *Salmonella* in unprocessed and high pressure processed peanut butter over 10 weeks

An overnight culture of the pathogenic *Salmonella* strains was prepared described above. Two large 40 oz (1134 g) jars of peanut butter were purchased from a local supermarket. The peanut butter from both the jars was emptied into the blender bowl and 22.7 ml of inoculum added (1% of weight of peanut butter). The inoculum was blended on high speed for a few minutes until evenly dispersed in the matrix of peanut butter. Half of this inoculated peanut butter was distributed into pouches and vacuum packed for high pressure processing and the other half of inoculated peanut butter was put back into the Skippy® peanut butter jar to serve as the control. The inoculated peanut butter pouches were then high pressure processed at 600 MPa for 18 min. The control and high-pressure processed pouches were stored at 25˚C for 10 weeks. At time 0 and for every subsequent week for 10 weeks after, 25 g sample of unprocessed inoculated peanut butter and 25 g of high pressure processed inoculated peanut butter, in duplicate were weighed out. Twenty five ml of peptone water was added to each weighed out sample of peanut butter and put in the stomacher for 2 min. Samples were diluted, plated and colonies enumerated as described above.

III.2.2.7 Effect of HHPP on *Salmonella* inoculated almond butter

To understand better the survival pattern of *Salmonella* in foods of similar water activity and texture as peanut butter, an experiment to study the effect of HHPP on *Salmonella* inoculated almond butter which has a similar water activity and texture as peanut butter.

Overnight culture of the *Salmonella enterica* serovars strains at 37˚C was prepared and cocktail prepared after incubation as described earlier in Chapter II. 1% of the inoculum was inoculated into organic almond butter taken in the blender jar and blended until the inoculum was evenly distributed into the almond butter. Two pouches of inoculated almond butter were prepared and loaded into the HHPP vessel. HHPP was carried out at 600 MPa for 18 min. 25 g of the unprocessed inoculated almond butter samples (control) and the high pressure processed pouch samples were weighed out, diluted with 225 ml peptone buffer and put in the stomacher for 2 min. Ten-fold dilutions were made and 100 µl aliquots of the control and processed samples plated on XLT4 agar.

III.2.2.8 Microscopic images of peanut butter

Two glass slides were prepared with a smear of peanut butter matrix and smear of peanut butter matrix with a single water droplet added. These slides were put on an optical microscope and magnification adjusted to 4X and light adjusted until a clear microscopic image of the peanut butter smears was seen. A Nikon camera attached to the microscope was used to capture the microscopic images.

III. 3. RESULTS AND DISCUSSION

III.3.1 *Salmonella* behavior in peanut oil after HHPP

Figure 19 shows the effect of HHPP at 600 MPa for 18 min on *Salmonella*-cocktail inoculated peanut oil in plastic vials.

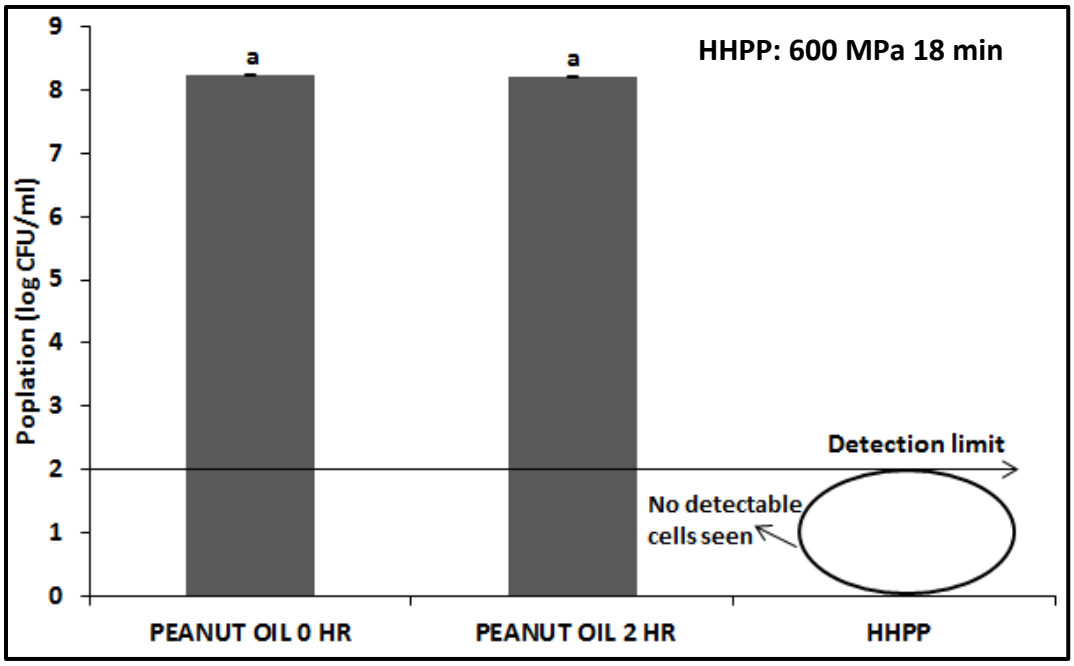


Figure 19: Effect of HHPP on *Salmonella* in peanut oil. Same lowercase letters indicate results are not significantly different ($p<0.05$).

The *Salmonella* cocktail–inoculated vials containing 100% peanut oil originally contained ~8.2 log CFU/ml. The unprocessed samples spread plated on XLT4 2 h after inoculation, the level of *Salmonella* remained at 8.2 log CFU/ml. No detectable cells were observed in the high-pressure processed samples, so processing inactivated *Salmonella* to below the detection limit of 100 CFU/ml. These results are quite interesting since peanut oil is a very low water activity environment, yet *Salmonella* is

inactivated as easily as in a high water activity environment, in contract to the moderately low water activity environment seen in peanut butter (Chapter II). This is in accordance with results obtained by Grasso et al. (2010) where there was only a 1 log CFU/g survival of *S.* Typhimurium ATCC 53647 in 100% peanut oil after high pressure processing at 600 MPa for 5 min at 45°C.

III.3.2 *Salmonella* behavior in peanut flour after HHPP

Figure 20 shows the effect of HHPP at 600 MPa for 18 min on *Salmonella*-cocktail inoculated 12% fat, light roast peanut flour in pouches.

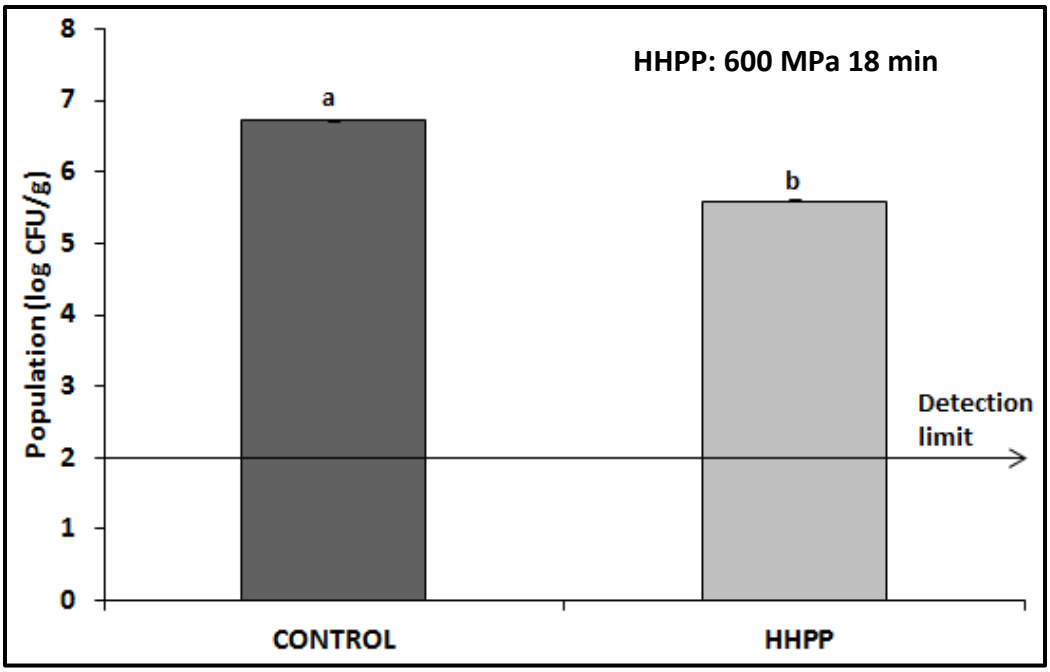


Figure 20: Effect of HHPP on *Salmonella* in 12% fat, light roast peanut flour. Different lowercase letters on the bars indicate significant difference ($p<0.05$).

The *Salmonella* recovered in the inoculated peanut flour was 6.7 log CFU/g. After HHPP at 600 MPa for 18 min, the level of *Salmonella* recovered in the pressure treated sample was 5.6 log CFU/g, indicating a 1.1 log CFU/g reduction of *Salmonella* in peanut flour was achieved. This suggests that the peanut flour component (peanut protein) of peanut butter may play a role in *Salmonella* survival during and after HHPP.

III.3.3 Effect of HHPP on *Salmonella* inoculated increased water activity formulations of peanut butter

Research has speculated three possible reasons for *Salmonella* survival in organic peanut butter. First, the low water activity may be inducing a vegetative state that makes *Salmonella* more resistant to pressure since it has been shown that stationary phase cells survive better when stressed than log phase cells (Patterson, 2005). Another possible reason is that low water activity may induce filamentation, in which the biomass of *Salmonella* increases, but cellular number does not increase due to a lack of septum formation between cells (Mattick et al., 2000). Filamentation in low water activity conditions may cause incorrect enumeration of *Salmonella.* The third possible reason for survival of *Salmonella* in organic peanut butter may be that the dense, lipid rich matrix may be forming protective pockets around the water droplets in the emulsion. *Salmonella* most likely resides in the water droplets in the peanut butter emulsion because it increases nutrient access as opposed to residing in the lipid portion where nutrient availability is limited (Ma et al., 2009). To investigate the exact affect water activity had on *Salmonella* survivability in organic peanut butter, it was decided that the water activity should be raised above 0.95 subsequently, because it is in this range that *Salmonella* will

proliferate. This would also ensure that filamentation does not occur, improving enumeration methods. It would also reduce the density of the matrix and create an emulsion that would be more favorable for growth and less favorable for protective pocket formation.

Figure 21 shows the effect of HHPP on *Salmonella* formulations of peanut butter of differing water activities. The initial level of *Salmonella* recovered in each different moisture content peanut butter is represented by the black bars. The light grey bars represent the level of *Salmonella* in the water activity modified samples of peanut butter after HHPP at 600 MPa for 18 min.

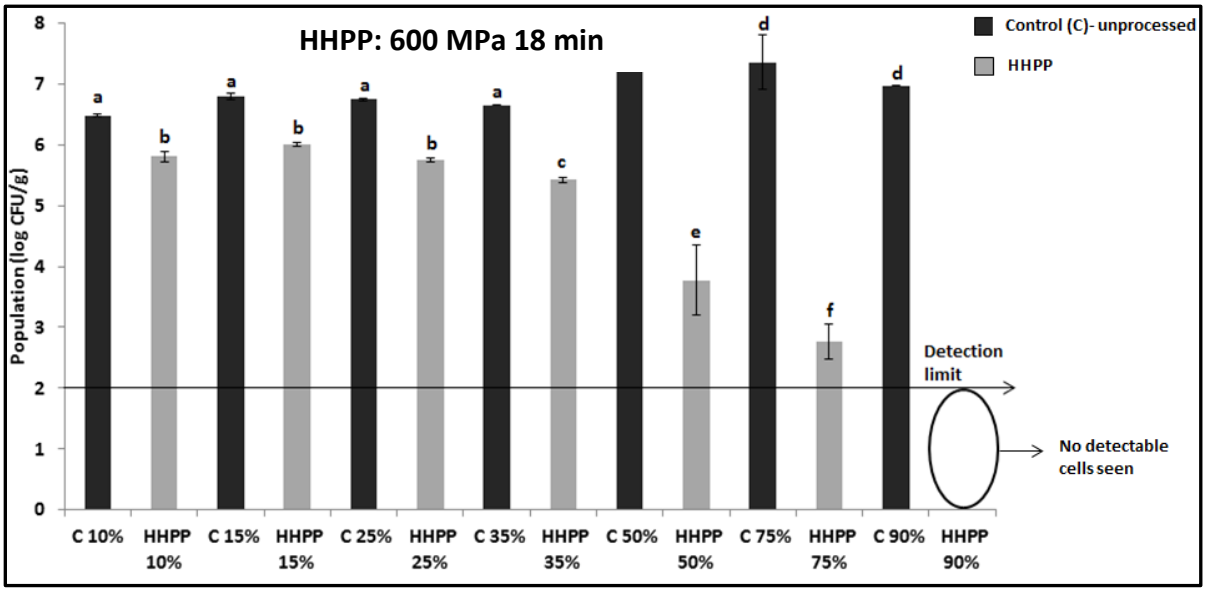


Figure 21: Populations of pathogenic *Salmonella* in control (recovered in inoculated, unprocessed, increased water activity peanut butter) and pouches of inoculated, increased water activity peanut butter samples high pressure processed at 600 MPa for 18 min. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As moisture contents increases, the water activity of peanut butter increases as shown in **Table 5**. However, as moisture content increases the texture and appearance of the peanut butter also changes. After high pressure processing, the color of all peanut butter samples containing added moisture contents darkens in color. **Figure 22** shows photographs of peanut butter with a) 10% added moisture, b) 90% added moisture and c) 50% added moisture after HHPP.

Figure 22: From left to right: Peanut butter with 10% added moisture, peanut butter with 90% added moisture, and peanut butter with 50% added moisture after HHPP at 600 MPa for 18 min.

Table 5 shows the log reductions of *Salmonella* obtained after HHPP as a function of water activity of peanut butter using the same data shown in **Fig. 21.**

Table 5: Moisture content (%), water activity and log reduction of *Salmonella* after HHPP at 600 MPa for 18 min data

ADDED MOISTURE CONTENT (%)	WATER ACTIVITY	LOG REDUCTION AFTER HHPP AT 600 MPa FOR 18 MIN
10	0.67	0.9
15	0.79	1.0
25	0.87	1.2
35	0.89	1.4
50	0.93	4.0
75	0.94	4.6
90	0.96	$\geq$ 4.9

As **Table 5** shows, as water activity of peanut butter was increased, the log reduction of *Salmonella* in peanut butter also increased. Peanut butter containing from 10% added moisture (a_w = 0.67) to 35% added moisture (a_w = 0.89), yielded a 1.0 – 1.5 log reduction after processing at 600 MPa for 18 min. When peanut butter contained 50% added moisture (a_w = 0.93), a significant increase in the log reduction (~4.0) was achieved. At 75% added moisture (a_w = 0.94), a 4.6 log reduction was achieved and at 90% added moisture (a_w = 0.96), inactivation of *Salmonella* to below detection limit of 100 CFU/g was achieved. It should be noted that in all of these experiments the texture of peanut butter changed significantly due to the excess moisture present. The texture of peanut butter resembled a slurry at added moisture contents at 50% and above. While these experiments show that as the moisture content of peanut butter increased the log reductions of *Salmonella* due to HHPP also increased, the texture and color of the resulting product make this technique impractical to say the least.

III.3.4 Effect of HHPP on *Salmonella* inoculated decreased water activity formulations of peanut butter

Figure 23 shows the effect of HHPP on *Salmonella* in formulations of peanut butter with lower water activities. Concentrations of *Salmonella* in unprocessed samples are represented by the black bars and concentration after high pressure processed at 600 MPa for 18 min are represented by the grey bars.

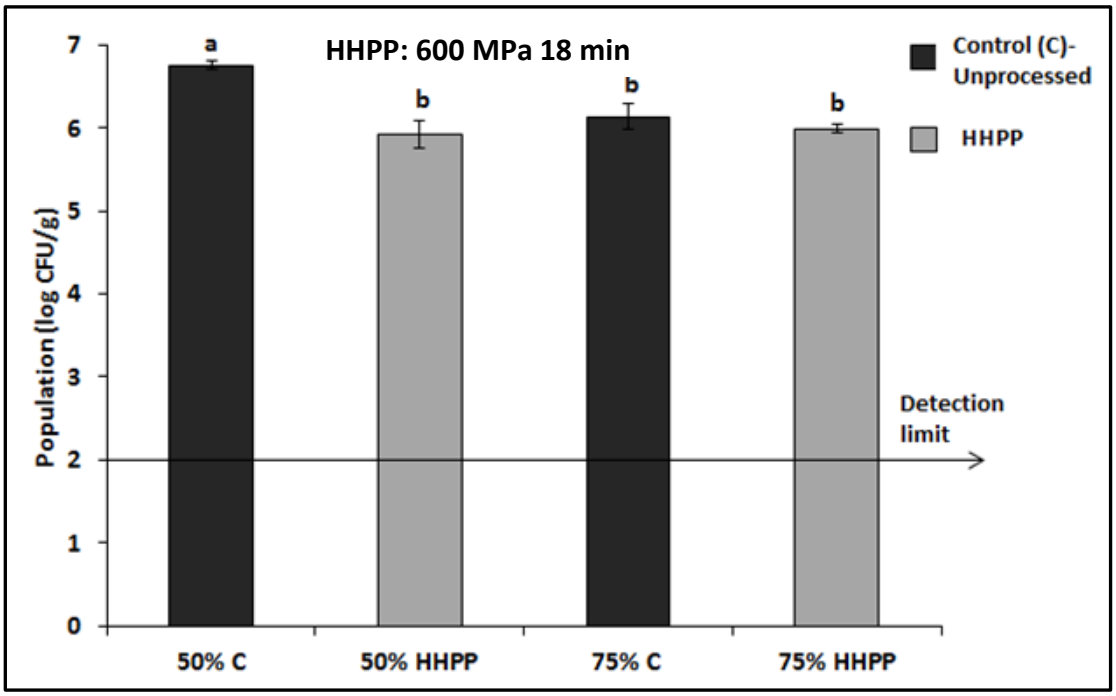


Figure 23: Populations of pathogenic *Salmonella* in control C (recovered in inoculated, unprocessed, reduced water activity peanut butter) and pouches of inoculated, reduced water activity peanut butter samples high pressure processed (HHPP) at 600 MPa for 18 min. Same lowercase letters indicate results are not significantly different ($p<0.05$).

At 50% added peanut oil (a_w = 0.16), less than a 1 log reduction was achieved. At 75% added peanut oil (a_w = 0.13), no significant log reduction was achieved. These

experiments showed that the addition of peanut oil to further lower the water activity of peanut butter further reduces the effectiveness of HHPP, in contrast to the results described above with peanut oil alone where *Salmonella* in HHPP peanut oil was inactivated to below the detection limit. *Salmonella* have increased heat tolerance in lipid-rich matrices, and thus it is reasonable to conclude that increased lipid content may provide a protective effect against pressure and temperature changes due to adiabatic heating (Shachar and Yaron, 2006). Studies have shown that lipids crystallize under high pressure, which may also contribute to the rigidity of the micelle-structure and the protective effects against high pressure and temperature (Schaschke et al., 2007). Hence, addition of lipid content as peanut oil to peanut butter further protects *Salmonella* inactivation by HHPP.

III.3.5 Effect of nisin in combination with HHPP on *Salmonella* inoculated peanut butter

The level of *Salmonella* recovered in inoculated peanut butter containing 12.5 ppm nisin (500 ppm Nisaplin®) was ~5.9 log CFU/g. Concentrations of *Salmonella* are unchanged from 1 h post-inoculation, up to at least 7 days post-inoculation as seen in **Fig. 24**. Nisin alone appears to have no effect on *Salmonella* concentration in peanut butter.

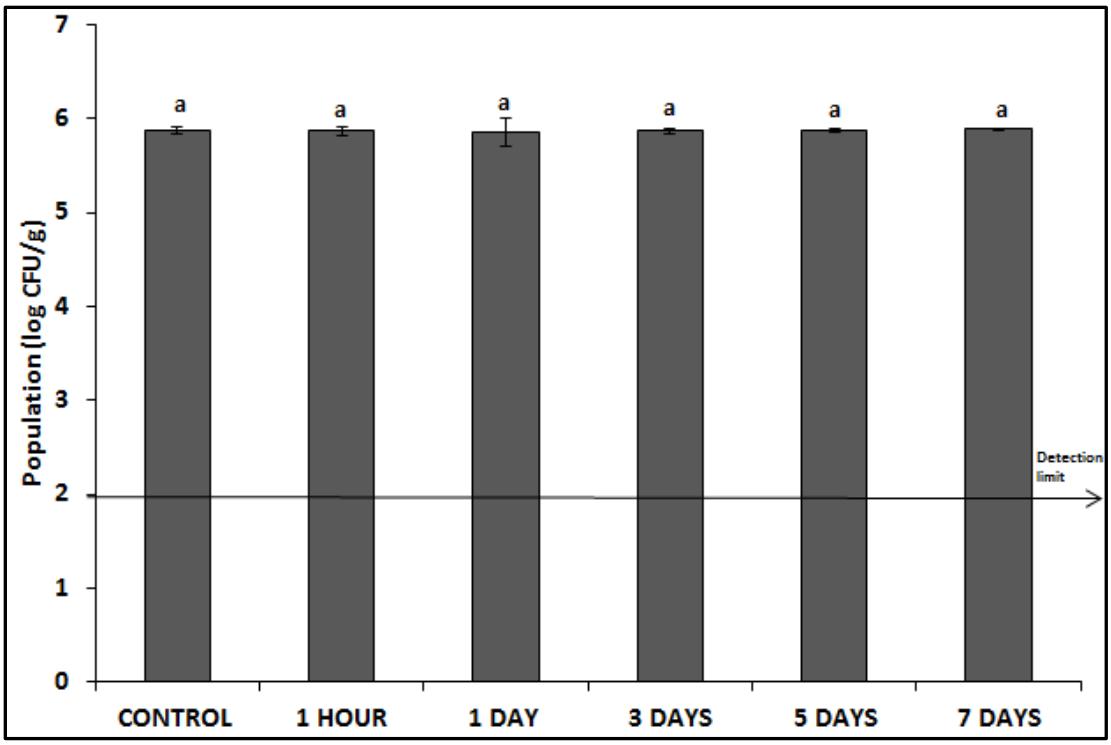


Figure 24: Populations of *Salmonella* recovered in control (inoculated peanut butter containing 12.5 ppm nisin (500 ppm Nisaplin®) at 1 hour, 1 day, 3 days, 5 days and 7 days after inoculation Same lowercase letters indicate results are not significantly different ($p<0.05$).

III.3.5.1 2.5 ppm nisin (100 ppm Nisaplin®):

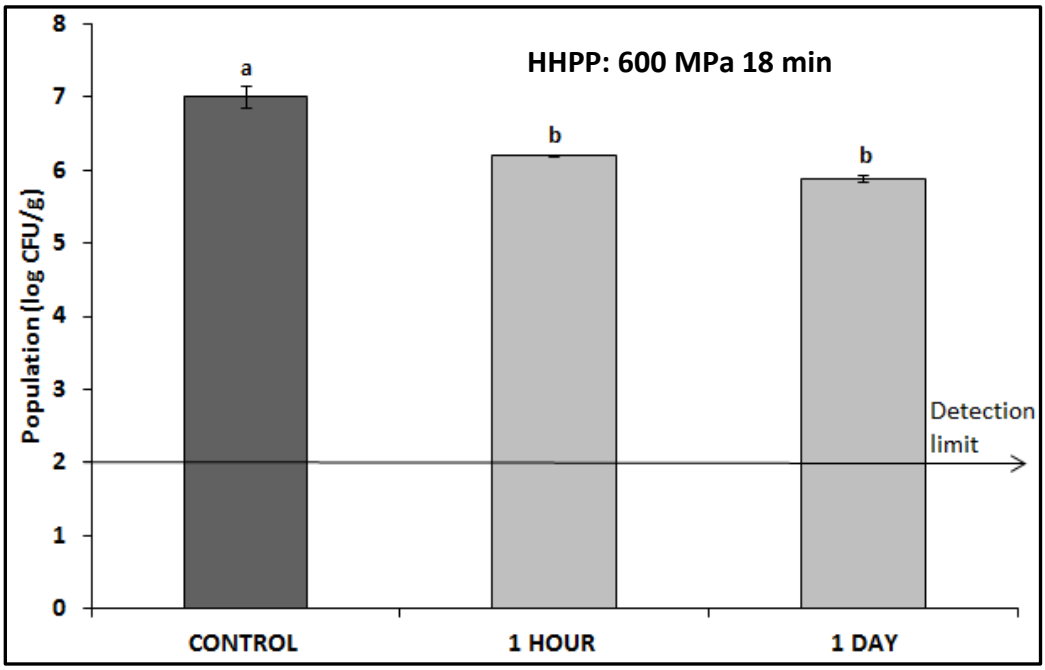


Figure 25: Populations of *Salmonella* recovered in control (inoculated peanut butter containing 2.5 ppm nisin), in HHPP sample plated after 1 hour and in HHPP sample plated after 1 day respectively. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As seen in **Fig. 25**, there was approximately a 1 log reduction of *Salmonella* in peanut butter containing 2.5 ppm nisin after HHPP treatment at 600 MPa and 18 min. This is similar to the log reductions seen in experiments discussed in Chapter II above where nisin was not used. There was no statistically significant difference in the level of *Salmonella* recovered in peanut butter 1 hour vs. 1 day after HHPP, although the observed concentration was slightly less.

III. 3.5.2 5 ppm nisin (200 ppm Nisaplin®):

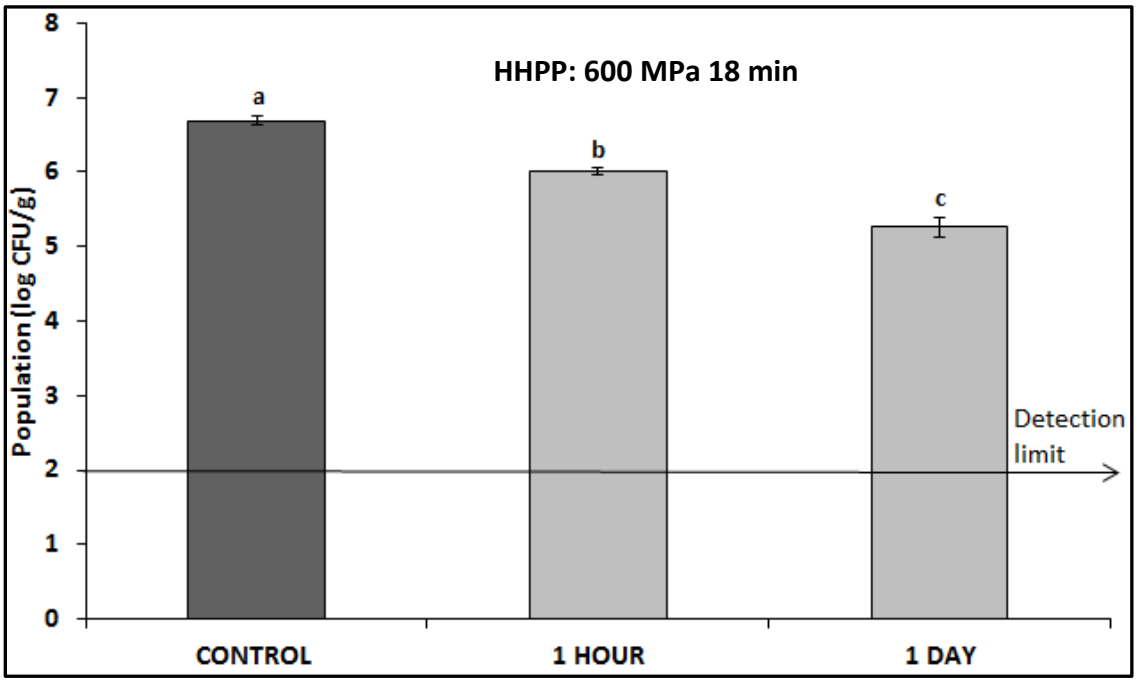


Figure 26: Populations of *Salmonella* recovered in control (inoculated peanut butter containing 5 ppm Nisaplin®), in HHPP sample plated after 1 hour and in HHPP sample plated after 1 day, respectively. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As seen in **Fig. 26**, there was a 0.6 log reduction of *Salmonella* in peanut butter was achieved with 5 ppm nisin and HHPP treatment (600 MPa and 18 min) in combination when plated after 1 hour. Samples plated 1 day after the 5 ppm nisin and HHPP treatment resulted in a 1.4 log reduction of *Salmonella* when compared with the control.

III.3.5.3 12.5 ppm nisin (500 ppm Nisaplin®):

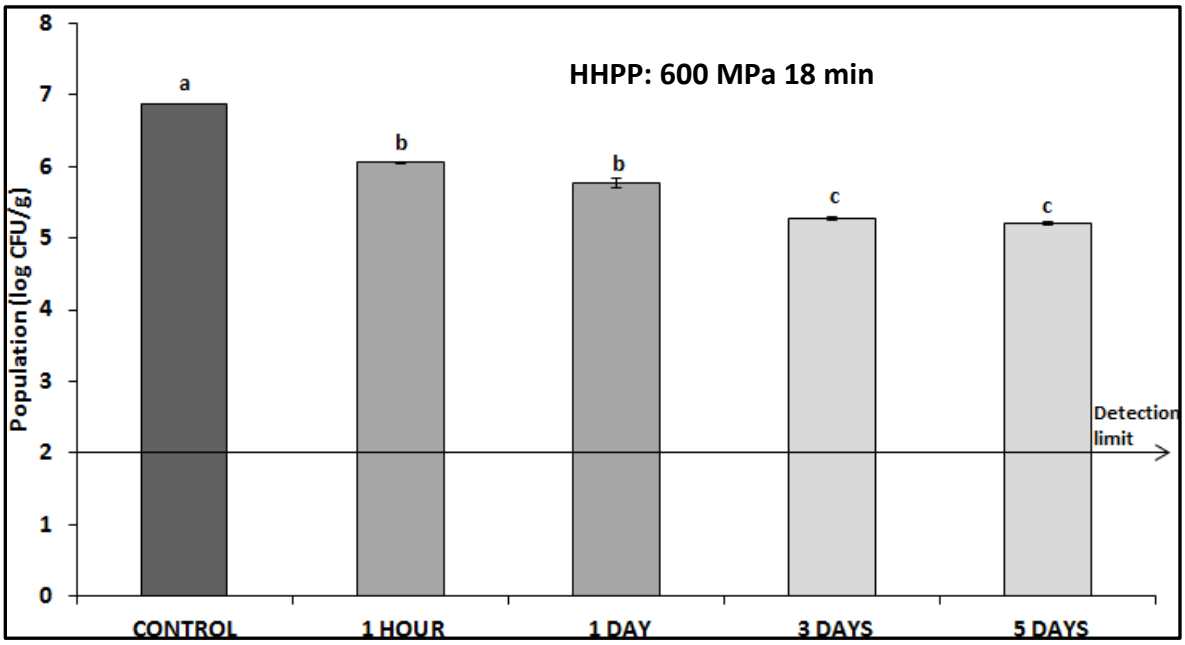


Figure 27: Populations of *Salmonella* recovered in control (inoculated peanut butter containing 12.5 ppm nisin), in HHPP samples plated after 1 hour, 1 day, 3 days and 5 days, respectively. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As seen in **Fig. 27**, there was a 0.7 – 1.0 log reduction of *Salmonella* in peanut butter was achieved with 12.5 ppm nisin and HHPP treatment in combination when plated 1 hour and 1 day after. Samples plated 3 days and 5 days after the 12.5 ppm nisin and HHPP treatment resulted in a 1.7 log reduction of *Salmonella* when compared with the control.

III. 3.5.4 25 ppm nisin (1000 ppm Nisaplin®):

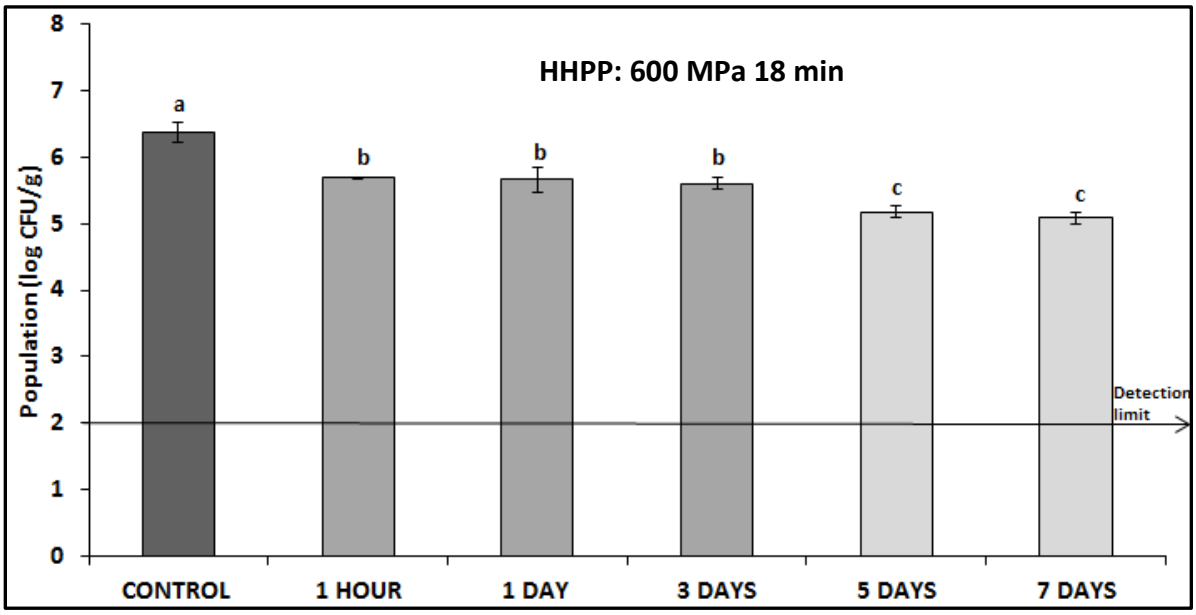


Figure 28: Populations of *Salmonella* recovered in control (inoculated peanut butter containing 25 ppm nisin, in HHPP samples plated after 1 hour, 1 day, 3 days and 5 days and 7 days, respectively. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As seen in **Fig. 28**, there was a 0.7 – 0.8 log reduction of *Salmonella* in peanut butter was achieved with 25 ppm nisin and HHPP treatment in combination when plated 1 hour, 1 day and 3 days after. Samples plated 5 days and 7 days after the 25 ppm nisin and HHPP treatment resulted in a 1.4 log reduction of *Salmonella* when compared with the control.

Overall, plating after nisin and HHPP treatment at varying time periods, did show some effect on increase in the log reduction of *Salmonella* with time. The maximum log reduction of *Salmonella* achieved was 1.7 log CFU/g, which was comparable to that achieved by pressure non-cycling alone as shown in Chapter II. Hence, it can be

concluded that the application of nisin in combination with HHPP does not enhance inactivation of *Salmonella* in peanut butter to the point where it would likely be worth the effort or cost.

Nisin is a water soluble molecule, but also is able to bind to cell membranes. It has been shown in micellar systems, that the didehydroalanine and leucine of ring A (residues 5 and 6) will insert themselves to the lipid phase (van den Hooven et al., 1996). Hence from the results of the experiments with nisin and HHPP on *Salmonella* inoculated peanut butter, it can be speculated that most of the nisin added to the peanut butter inserts itself into the lipid phase of peanut butter and hence has negligible effect on inactivation of *Salmonella* that possibly resides in the small water phase pockets of the peanut butter matrix.

III.3.6 Survival pattern of *Salmonella* in unprocessed and high pressure processed peanut butter over 10 weeks

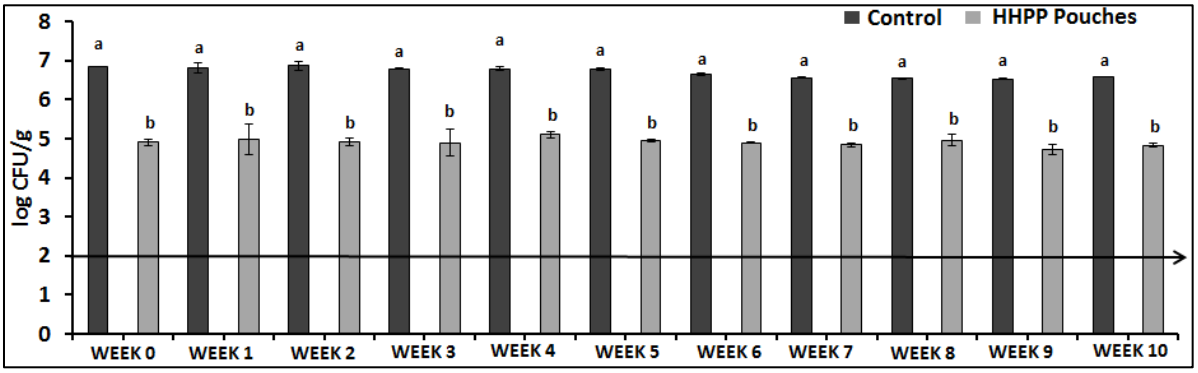


Figure 29: Levels of *Salmonella* in inoculated unprocessed peanut butter (control) and in high pressure processed (600 MPa for 18 min) pouches of peanut butter, when stored at 25˚C for 10 weeks. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As seen in **Fig. 29**, the level of *Salmonella* recovered in the control at week 0 was 6.8 log CFU/g. Thereafter, there was a very slight, but not statistically significant decrease in the level of *Salmonella* in the unprocessed peanut butter over the 10 week experiment. High pressure processing at 600 MPa for 18 min, produced a ~2 log reduction of *Salmonella*, and there was no statistically significant change in the level of *Salmonella* in the high pressure processed peanut butter over the 10 week experiment.

This ten-week survival study hence proved that the peanut butter matrix itself does not contribute to inactivation or allow growth of *Salmonella*. High pressure processing achieved an immediate 2 log reduction of *Salmonella,* which does not change over at least 10 weeks of observation.

III.3.7 Effect of HHPP on *Salmonella* inoculated almond butter

Almond butter is also a low water activity food having a similar high fat matrix to peanut butter. The water activity of the almond butter measured was approximately 0.4.

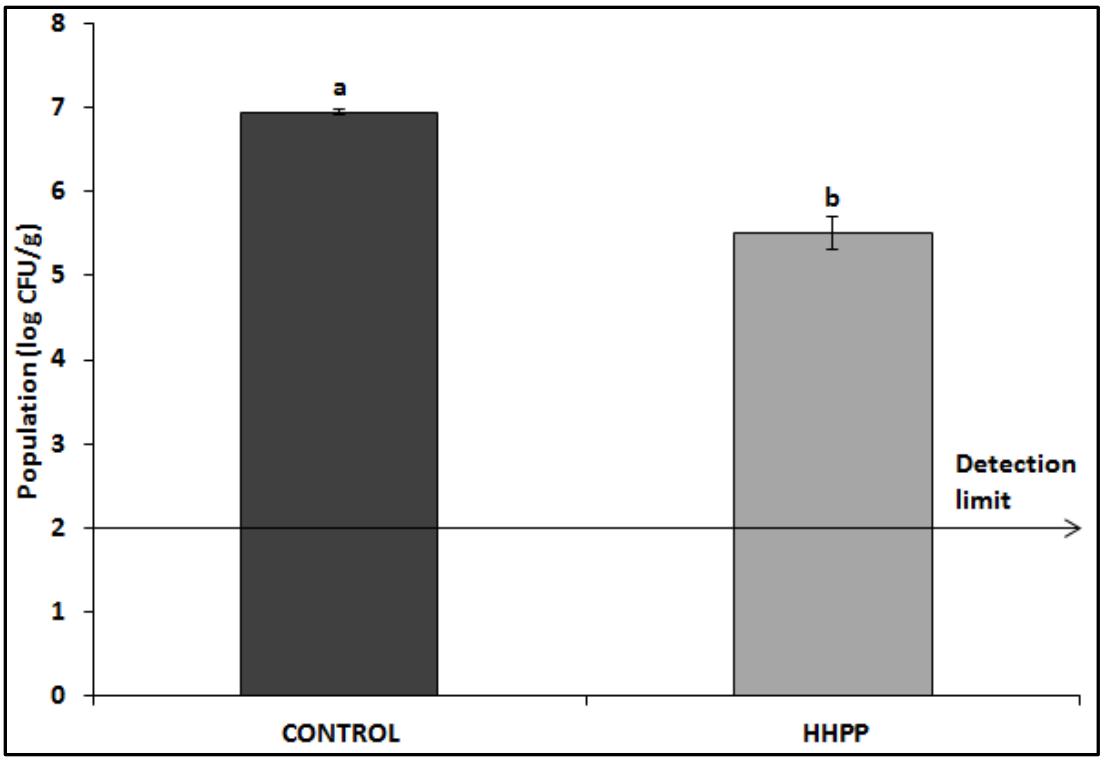


Figure 30: Effect of HHPP at 600 MPa for 18 min on *Salmonella* in almond butter. Same lowercase letters indicate results are not significantly different ($p<0.05$).

As seen in **Fig. 30**, the mean log reduction after HHPP obtained in pouches was ~1.4 log CFU/g which was comparable to that obtained with peanut butter. Thus, it can be estimated that food matrices similar to peanut butter with similar moisture and fat content, show similar levels of inactivation of *Salmonella* after HHPP and that such foods similar to peanut butter may also pose a food safety threat of *Salmonella*.

III.3.8 Microscopic images of peanut butter

The microscopic images of the peanut butter smear and the peanut butter smear with a water droplet, as photographed are seen below (**Fig. 31** and **Fig. 32**).

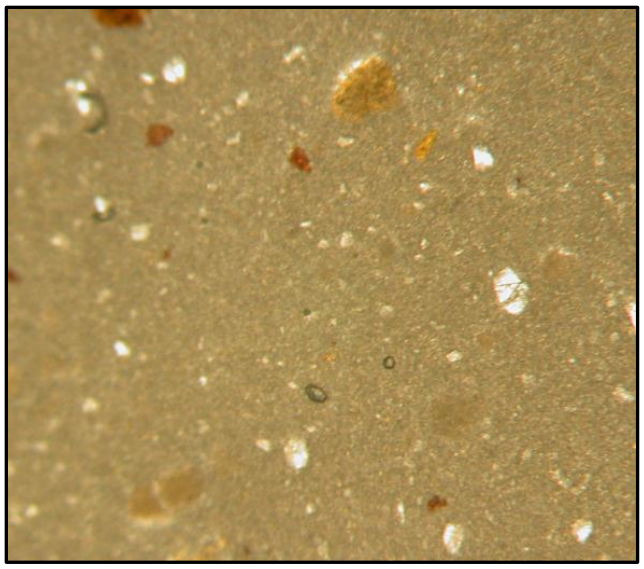


Figure 31: Microscopic image of Skippy® creamy peanut butter (Magnification 4X)

The particle size was between 50 – 100 microns. The matrix was very grainy. The brown irregular structures represent pieces of crushed peanut and the white rounded structures were estimated to be the carbohydrates.

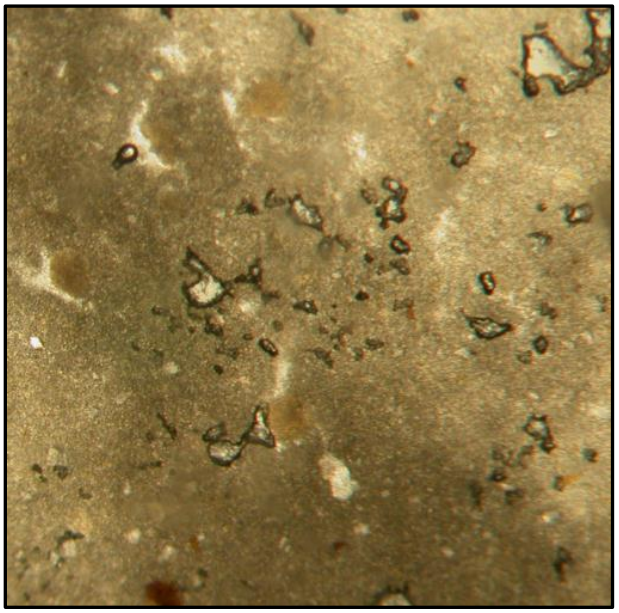


Figure 32: Microscopic image of Skippy® creamy peanut butter with water droplet dispersed (Magnification 4X)

Figure 32 shows the grainy peanut butter matrix with the water droplet dispersed as small aqueous pockets in the fat phase of peanut butter. Peanut butter contains 1.5 – 2% moisture. It can be estimated that *Salmonella* survives in the water pockets within the peanut butter matrix. Further research needs to be carried out to investigate the molecular mechanism of *Salmonella* survival in the peanut butter matrix.

IV. CONCLUSIONS

This research has contributed to our understanding of the effect of high pressure processing on inactivation of *Salmonella* in peanut butter. The hypothesis of this research was proved partially correct, *i.e.*, high hydrostatic pressure processing does show some ability to inactivate *Salmonella* in peanut butter, however, the effectiveness of HHPP is unlikely to justify the added complexity or costs for commercial implementation of HHPP as a risk mitigation measure for *Salmonella* contaminated peanut butter. The main conclusions drawn from the various experiments conducted can be summed up as follows:

- Various combinations of HHPP pressure and time can produce 1.6 to 1.9-log reductions of *Salmonella* in peanut butter. Neither pressure nor time significantly influenced the measured log reduction of *Salmonella* in peanut butter.
- Pressure cycling was no more effective than non-cycling for inactivation of *Salmonella* in peanut butter. The log reductions of *Salmonella* achieved by pressure cycling varied between 1.7 – 1.9 log CFU which was comparable to non-cycling HHPP. Extreme pressure cycling for 10 cycles with 6 min hold time each at 600 MPa did not enhance inactivation of *Salmonella* in peanut butter.
- Temperature played a synergistic role in combination with pressure to achieve inactivation of *Salmonella* in peanut butter during HHPP at room temperature. When processed at lower initial temperatures like 7˚C, a lower log reduction of *Salmonella* of approximately 1 log CFU/g was achieved. This reduction can likely be attributed to effect of pressure alone. HHPP at higher temperatures

(50°C) is comparable to results obtained at initial room temperature and does not enhance inactivation of *Salmonella* in peanut butter

- A ~2 log CFU/g reduction of *Salmonella* in organic, creamy peanut butter was obtained after HHPP at 600 MPa for 18 min which was comparable to that achieved with Skippy® creamy peanut butter. These results indicate that the ingredients other than peanuts present in processed peanut butter such as salt and hydrogenated vegetable oils, did not contribute to reducing or enhancing *Salmonella* inactivation by HHPP in peanut butter.
- *Salmonella* was inactivated to below detection limit in high water activity environments like peptone water. *Salmonella* is also inactivated to below detection limit in very low water activity environments like peanut oil, which is one of the major components of peanut butter. In 12% fat, light roast peanut flour a 1-log CFU/g reduction of *Salmonella* is achieved. Peanut flour or peanut meal, is the other major component of peanut butter. These results indicate water activity alone cannot explain *Salmonella* survival during HHPP of peanut butter.
- When peanut butter is modified to water activities of 0.96, *Salmonella* can be inactivated to below detection limit. Such a modified product is significantly different from peanut butter and darkens considerably after HHPP.
- Nisin, in combination with HHPP was not effective enhancing inactivation of *Salmonella* in peanut butter. The maximum log reduction after HHPP at 600 MPa and 18 min achieved was 1.7 log CFU/g which was comparable to the result obtained with HHPP alone. *Salmonella* concentrations after HHPP do differ significantly, but those differences are of little practical significance.

- *Salmonella* levels in unprocessed as well as HHPP peanut butter did not show any statistically significant change over a period of 10 weeks.

In summary, the peanut butter matrix supports the survival of *Salmonella* in peanut butter during and after HHPP. High pressure processing alone is not a suitable technology to manage the microbiological safety of *Salmonella* contaminated peanut butter. HHPP can be explored in combination with other technologies for achieving greater inactivation of *Salmonella* in peanut butter and/or be the final step of a multiple-hurdle approach to ensure the microbiological safety of peanut butter.

V. FUTURE WORK

- The effect of HHPP on *Salmonella* inoculated peanut butter acidified to pH values below 4.0 should be investigated. *Salmonella* is known to survive in a pH range of 4.1 to 9.5 with optimal growth in the pH range of 6.5 to 7.5 (Doyle and Beuchat, 2007). It would be interesting to study the effect of HHPP in combination with peanut butter of an acidic pH below 4.0 on *Salmonella* inactivation.
- The effect of HHPP on *Salmonella* inoculated other brands and other types of peanut butter like chunky peanut butter should be studied. Like almond butter, the patterns of *Salmonella* inactivation by HHPP in other low water activity foods should be compared with peanut butter to better understand the characteristics of a food matrix that allow *Salmonella* survival.
- The molecular mechanism behind the survival of *Salmonella* in peanut butter during HHPP needs to be researched and understood. Detailed microscopy such as the use of fluorescence microscope and *Salmonella* staining/labeling for inoculated peanut butter would be useful to understand the peanut butter matrix structure and the possible locations within the peanut butter matrix where *Salmonella* survives.

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