A Comparative Study of the Effects of High Hydrostatic Pressure and Ultraviolet Light on Stability, Health Related Constituents and Quality Parameters of Tiger Nut Milk

By
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ABSTRACT

A Comparative Study of the Effects of High Hydrostatic Pressure and Ultraviolet Light on Stability, Health Related Constituents and Quality Parameters of Tiger Nut Milk

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University of Guelph, 2016

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Tiger nut milk is classified as a low acid beverage characterized by having a high starch and protein content. In the following, a comparative study was performed to: 1) determine the efficacy of thermal Long Time Low Temperature treatment (LTLT), high hydrostatic pressure (HHP) and ultraviolet light at 254 nm (UV-C) to pasteurize tiger nut milk; 2) To evaluate thermal, HHP and UV-C effects on physicochemical properties, nutrients and bioactive constituents. Thermal (60ºC for 30 min), HHP (500 MPa and 600 MPa for 90, 120 and 180s), and UV-C (18.4 mJ/cm²) at 254 nm treatments achieved 5-log reduction of target bacteria. On the other hand, HHP decreased the measured protein, vitamin C and the antioxidant contents. In comparison, UV-C treatment did not result in significant changes in protein, vitamin C or antioxidant content of tiger nut milk. However, despite the microbial reduction supported by UV-C treatment the shelf-life of tiger nut milk under refrigerated conditions was not improved compared to non-treated controls. In conclusion, UV-C treatment would be the preferred approach for pasteurizing tiger nut milk to ensure adequate pathogen reduction whilst retaining nutrients content of the beverage.
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Chapter I

1.1. General Introduction

Nut milks have risen in popularity as consumer preference for low-acid beverages have increased. Due to the low acidity of nut milks, in combination with their high protein and carbohydrate content, the beverage is prone to spoilage and supporting the growth of pathogens. Therefore, a pasteurization step is required to ensure food safety and reasonable shelf-life (Cortes, Esteve, Frigola & Torregrosa, 2005; Corrales, Souza, Stahl & Fernandez, 2012; Makinen, Wanhalinna, Zannini & Arendt, 2015).

Although the appearance and name of Tiger Nuts would suggest similarities to almonds, in reality the vegetable is a tuber like potato. The tiger nut plant (*Cyperus sculentus lativum*) is commonly cultivated in the Far East though North America and Southern Spain. In a normal growing season, planting occurs in April with harvesting occurring from December through to Mark. Upon harvesting, the tiger nuts are washed to remove soil then sun dried over a 3-month period. The dried tiger nuts are then packed before being distributed. Tiger nuts can be made into flour or more commonly, hydrated in water then blended to make a milk-like beverage called Horchata (Cortes *et al.*, 2005). The health benefits of tiger nut milk are thought to be derived from the high energy (starch and fat) and fibre content.

Thermal pasteurization of tiger nut milk results in gelatinization of starch and protein denaturation to form a gel structure (Cortes *et al.*, 2005). As a consequence, non-thermal pasteurization methods are required to reduce microbial loading whilst retaining sensory and nutritive characteristics. HHP and UV-C light are approved by FDA and Health Canada, non-thermal pasteurization methods with a history of treating a wide spectrum of beverages.
However, to date the main focus has been with high acid juices or those with high UV transmission. Little work has been undertaken with respect to non-thermal processing of opaque, low-acid beverages such as tiger nut milk.

The proposed research will provide important information for the treatment of low-acid juices with alternative non-thermal technologies. The following reports on a comparative study to evaluate High Hydrostatic Pressure (HHP) and Ultraviolet Light (UV-C) as non-thermal pasteurization technologies.

1.2. Research Hypothesis

Non-thermal technologies based on high pressure or UV light can support a 5-log reduction of pathogens in tiger nut milk without causing a significant change in the nutritive value of the beverage.

1.3. Objectives

The objectives of this thesis are as follows:

1) Validate methods for determining nutrients, bioactive and microbiological contents, and overall quality parameters of tiger nut milk.

2) Identify processing parameters of both thermal and non-thermal interventions to achieve a 5-log inactivation of the surrogate bacteria and yeast.

3) Determine the impact of thermal, HHP and UV-C intervention technologies on nutrient content and quality characteristics of tiger nut milk.
4) Undertake shelf-life studies at the refrigerated storage temperature (4°C) on selected treatments to assess extended microbiological and nutrient stability of tiger nut milk held under the same conditions after HHP and UV-C light treatments.

1.4. Literature Review

High acid juices (pH<4.6) are generally considered safe given that the acidity significantly contributes to inhibiting the growth of pathogens during storage. Moreover, the acidity also enhanced lethal effects of interventions such as thermal, UV and high pressure to inactivate microbes resulting in a stable product with a shelf-life of over 60 days (Lukas, 2013). However, low-acid juices do not have the pH hurdle that ultimately can limit the efficacy of pasteurization methods along with compromising shelf-life due to the growth of spoilage microbes.

According to the US. Food and Drug Administration (USFDA, 2007) the guidance pertains to low-acid juice products subject to the pathogen reduction provisions of the Hazard Analysis and Critical Control Point (HACCP), requirements of 21 CFR Part 120 (the juice HACCP regulations). It is to determine where potential hazards can occur in their juices processing operations, and to implement control measures at points where hazards can occur to prevent safety problems with their products. The juice HACCP regulations includes: juice processors must evaluate their processing operations using HACCP principles; the HACCP plan and other records of sanitation standard operating procedures (SSOPs) and HACCP operations must be available for official inspection and copying; employees involved in developing, or in certain aspects of implementing a HACCP plan, must be trained in HACCP principles to achieve 5-log pathogen reduction; fruit surface treatments, cleaned and undamaged tree-picked fruit, and; shelf stable juices made using a
single thermal processing step and juice concentrates made using a thermal concentration process (USFDA, 2007).

1.4.1. Plant-Derived Milk

Plant-derived milks can be sustainable alternatives to dairy milk. Plant-derived milk substitutes are water extracts of legumes, oil seeds, nuts or cereals that resemble cow’s milk in appearance. There are a variety of traditional plant-derived beverages around the world, such as Horchata (tiger nut milk), coconut milk and almond milk (Makinensch et al., 2015)

Juices have been implicated in several food pathogenic outbreaks over the past few decades primarily involving high acid juices such as apple and orange (Makinensch et al., 2015). Lukas (2013) stated that most juices are acidic (pH<4.6); however low acid juices (pH > 4.6) are considered the riskiest, with characteristics to support greater pathogen survival and growth (Lukas, 2013). Low-acid juices have also been implicated in food-borne illness cases, but less so, due to the inherent susceptibility to spoilage. Consequently, low acid juices are UHT processed to be shelf-stable, thereby representing a low food safety risk (Echavarria, Torras, Pagan &Ibarz, 2011). However, given the perceived detrimental effects of thermal processing, there is a move towards lower thermal processing regimes or non-thermal interventions (Echavarria et al., 2011).

Therefore, the FDA considers these juices as well as their original fruits to be hazardous because of their association with food borne outbreaks (Vojdani, Beuchat &Tauxe, 2007). One low acid juice that is gaining popularity is tiger nut milk (Udeze, 2014). However, a major problem with low acid plant-derived beverages in the industry is browning reaction.
Browning reactions of foods during processing and storage, includes: caramelization, ascorbic acid degradation and the Maillard reaction. It affects the sensory characteristics of foods such as flavour, aroma and color because it affects product quality (Echavarria et al., 2011).

### 1.4.2. Tiger Nut Milk

Tiger nut milk is a non-alcoholic beverage with a milky appearance derived from the tubers of tiger nuts mixed with sugar and water. Tiger nut (*Cyperus esculentus* L., family: Cyperaceae) is a highly nutritive nut. Udeze (2014) mentioned that tiger nut is commonly produced in the Northern part of Nigeria, and has various names in different Nigerian languages, such as: Zulu nut, yellow nut grass, ground almond, chafu, edible rush and rush nut. Among these, only yellow and brown tiger nuts are available on the international market. Tiger nut also contains a high-quality oil, which accounts for about 25.5% of its content (Udeze, 2014). The protein in tiger nut is also of high biological value because of the many essential amino acids which exceed the recommended intake by the Food and Agriculture Organization/World Health Organization (FAO/WHO) (Udeze, 2014). Moreover, according to Corrales et al. (2012), tiger nut can be eaten raw, roasted, dried, baked or in the form of a beverage called Horchata De Chufas (tiger nut milk).

Tiger nut milk has a high nutritive value (Table 1.1). According to Udeze (2014), the nutritional profile of tiger nut milk as follows: 5.8% moisture, rich in protein (8%) and minerals (sodium, potassium, iron, calcium, magnesium, zinc, and copper), carbohydrate including reducing sugar content (7.4%), soluble polysaccharides (7.4%), and starch (86.4%). Tiger nut milk is a nutritional and energetic drink containing high amounts of fibre, starch, glucose, protein, minerals, vitamins C and E, and oleic acid (Adejuyitan, 2011). According to Codina-Torrella, Guamis and Trujillo (2014), its general composition (as % dry matter) corresponded to 8.66± 0.04 moisture, 35.21 ±3.07 fat, 8.45±0.20 protein, and 45.05 ± 3.13 nitrogen-free material (2014).
During processing, it does not heat above 72°C, preventing starch jellification, and it has a pH in the range 6.3–6.8 (Cortes *et al*., 2005).

**Table 1.1- Proximate Compositions of Dried Tigernut (Oladele & Aina, 2007)**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Yellow Variety (%)</th>
<th>Brown Variety (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3.50</td>
<td>3.78</td>
</tr>
<tr>
<td>Crude protein</td>
<td>7.15</td>
<td>9.70</td>
</tr>
<tr>
<td>Lipid</td>
<td>32.13</td>
<td>35.43</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>6.26</td>
<td>5.62</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>46.99</td>
<td>41.22</td>
</tr>
<tr>
<td>Ash</td>
<td>3.97</td>
<td>4.25</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1343</td>
<td>1511</td>
</tr>
</tbody>
</table>

In preparing tiger nut milk, the tuber nuts are first sorted and washed (Figure 1.1). Soaking is considered the most important step. The tubers are soaked in water, wet milled, sieved, sweetened, and flavoured at ambient temperature (25-30°C) for 5h to 12h. The soaked nuts are blended in a high-speed blender and extracted using a cheese cloth bag. The extracted milk is then homogenized and bottled for future use (Adekanmi, Oluwatooyin & Yemisi, 2009). However, changes in the organoleptic characteristics of fresh tiger nut milk, a short shelf-life, and jellification after treatment above 72°C, lead researchers to try different treatment conditions with various methods to overcome these challenges (Corrales *et al*., 2012).
1.4.3. Outbreaks Associated with Low Acid Unpasteurized Juices

Consumers are becoming increasingly aware of the relationship between diet and disease prevention. There has been an increase in the consumption of unpasteurised fruit and vegetable juices in recent years due to their freshness, low calorie contribution, and good nutritional quality. However, unpasteurised fresh juices with low acidity (pH >4.6) and high water activity (aw > 0.85) can support the growth of pathogenic microorganisms (Pilavtepe-Celik, 2012).

It has been reported that enteric food-borne pathogens including *Escherichia coli* O157:H7 and *Salmonella enterica* serovars *Typhimurium*, and Gram-positive pathogens such as *Clostridium botulinum* and *Listeria monocytogenes* can survive in raw fruit and vegetable juices (Pilavtepe-Celik, 2012).
In addition, outbreaks of *Salmonella spp.*, *E. coli* O157:H7 and *L. monocytogenes* have been linked with the consumption of fresh-cut melon and watermelon, as well as their juices (Pilavtepe-Celik, 2012). According to the Juice HACCP Hazards and Controls Guidance, low acid juices, such as carrot juice and watermelon, do not fall under the low acid canned foods regulation, and need to be used under refrigeration (Pilavtepe-Celik, 2012).

**Table 1.2 - Outbreaks due to Low Acid Juices (CDC, 2006; Danyluk, Goodrich-Schneider, Schneider, Harris & Worobo 2012)**

<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Pathogen</th>
<th>Year</th>
<th>Location</th>
<th>Cases (death)</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>Unpasteurized</td>
<td><em>E. coli</em> O157:H7</td>
<td>2010</td>
<td>USA (MD)</td>
<td>7</td>
<td>Retail</td>
</tr>
<tr>
<td>Carrot</td>
<td>Pasteurizes</td>
<td><em>C. botulinum</em></td>
<td>2006</td>
<td>USA &amp; Canada</td>
<td>6</td>
<td>Retail</td>
</tr>
<tr>
<td></td>
<td>Home made</td>
<td><em>C. botulinum</em></td>
<td>1993</td>
<td>USA</td>
<td>1</td>
<td>Home</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Home made</td>
<td><em>Salmonella spp.</em></td>
<td>1993</td>
<td>USA</td>
<td>18 (0)</td>
<td>Home</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Home made</td>
<td><em>Norovirus</em></td>
<td>2005</td>
<td>USA</td>
<td>0</td>
<td>Home</td>
</tr>
<tr>
<td></td>
<td>Home made</td>
<td><em>Campylobacter</em></td>
<td>2006</td>
<td>USA</td>
<td>1 (0)</td>
<td>Home</td>
</tr>
<tr>
<td>Coconut</td>
<td>Milk</td>
<td><em>Vibrio cholera</em></td>
<td>1991</td>
<td>USA</td>
<td>4</td>
<td>Home/picnic</td>
</tr>
</tbody>
</table>
1.4.4. General Preservation Methods of Foods

There are different thermal and non-thermal preservation methods to process food products includes:

1.4.4.1. Thermal Processing

Thermal processing is a combination of temperature and time treatment required to eliminate a desired number of microorganisms from a food product to a designated F value, and is intended primarily for preserving and extending the shelf-life of foods. For example, in the case of juice the main pathogens of concern are *Salmonella, L. monocytogenes* and *E. coli* O157:H7 that need to be decreased by at least 5-log CFU (Colony-forming unit) (Zemser, 2015).

The FDA requirement for pasteurization of juice is a minimum of 160°F (71°C) for 6 seconds. Hot filling and retorting a beverage support a 5-log reduction of the most relevant pathogens and helps it to remain shelf-stable for a long time at refrigerator temperature (Zemser, 2015). There are other levels of heat treatment include aseptic, ultra-high temperature (UHT), and extended shelf-life (ESL). UHT is required to produce a shelf-stable product. Yet, thermal treatments based on pasteurization are preferred due to the lower impact on sensory and nutritive content of the juice (Zemser, 2015).

Pasteurisation is a relatively mild heat treatment at temperatures less than 100°C and is the main technique used for beverage preservation and microbiological safety (Rupasinghe & Yu, 2012). It is an appropriate method to inactivate pathogenic microorganisms and extend shelf-life in low acid foods (pH>4.6). On the other hand, it is also used to extend shelf-life and inactivate spoilage microorganisms and enzymes in acidic foods (pH<4.6) (Rupasinghe & Yu, 2012).
There are two types of thermal treatment process, as follows:

Low temperature long time (LTLT):

Foods are treated at lower temperatures for a longer time to kill pathogenic bacteria. Treatment occurs at temperatures between 63-65°C for 30 minutes (the holder process). Milk, for example, is pasteurized at 145°F (63°C) for 30 min (USFDA, 1998). However, this method causes significant changes in flavour and lead to a loss of vitamins (Rupasinghe & Yu, 2012).

High temperature short time (HTST):

This method reduces the change in food quality that occur in the LTLT technique. Lately, HTST is the most commonly used method for pasteurization of fruit juice (Rupasinghe & Yu, 2012). This method involves heating beverage particles to at least 72°C and holding for at least 15 seconds; this is carried out as a continuous process (Rupasinghe & Yu, 2012).

Unfortunately, while inactivating microorganisms, thermal treatment can also adversely influence product quality characteristics (Balasubramaniam, Farkas & Turek, 2008). Thermal processing methods used for juices include flash pasteurization (80°C for 20s), hot filling (87°C), in-pack pasteurization (74°C for 17 min) and aseptic processing (Damar, Balaban & Sims, 2009).

D- and Z-values can be used to calculate the heat required to destroy microorganisms in the product, which is defined as Thermal Death Time (TDT). These values are dependent on the heat resistance of microorganisms (Ewoldt, 2012). The D-value is defined as the time at a specific temperature required to reduce a particular number of microorganisms by 90% or to result in a 1-log reduction (Equation 1.1). The Z-value indicates the change in the death rate based on temperature, and is the number of degrees between a 10-fold change (1-log cycle) in an organism’s resistance (Equation 1.2) (Ewoldt, 2012).

\[
D = t_2 - t_1 / \log_{10} (A) - \log_{10} (B) \tag{1.1}
\]
Where A and B indicate the survivor counts following heating for times $t_1$ and $t_2$ in minutes.

\[ Z = T_2 - T_1 / \log_{10}(D_1) - \log_{10}(D_2) \]  

(1.2)

Where $D_1$ and $D_2$ are D values at temperatures $T_1$ and $T_2$, respectively.

Ukwuru and Ogbodo (2011) studied effects of processing treatments on the quality of tiger nut milk. Pasteurization was conducted at 75°C for 15 min and ultra-high temperature treatment (UHTM) was conducted at 145°C for 14 seconds. However, heat treatment affected nutrient content and the quality of tiger nut milk (Ukwuru & Ogbodo, 2011). The pasteurization resulted in a pH of 6.2; UHTM resulted in the lowest pH. UHTM had lower total solids (20.0%), and the crude fat extract of the milk samples (5.5%) was below the minimum standard of (8%) for dairy milk. In terms of microbial stability, there was no survival growth of microorganisms after pasteurization and UHTM (Ukwuru & Ogbodo, 2011). In general, thermal processing methods have been shown to change the quality and sensory characteristics for many plant-derived beverages, such as almond milk and coconut milk (Ukwuru & Ogbodo, 2011).

**Effects of Thermal Processing on Microbial Content and Juice Quality**

The consumption of unpasteurized beverages has been recently linked to food-borne outbreaks due to several serotypes of *Salmonella* spp., *Escherichia coli* O157:H7 and other pathogens (Fontan, Santos, Bonomo, Lemos, Ribeiro & Veloso, 2009). Therefore, the USFDA published the federal Juice Hazards Analysis Critical Control Point(HACCP) regulation. Thermal treatment is still considered the more traditional but effective method of inactivating food pathogenic and spoilage microorganisms (Fontan et al., 2009).

Landl, Abadias, Sárraga, Viñas and Picouet (2010) studied the effects of a mild pasteurization treatment (75°C/10 min) and high pressure on physicochemical properties and nutrient contents of apple purée.
Results indicated that mild treatment did not affect its physicochemical properties, but that there was a significant reduction in vitamin C content from 354±34 mg kg\(^{-1}\). In addition, total polyphenol compounds (TPC) were affected significantly by pasteurization. Indeed, after pasteurization, 87% of the TPC was retained in the purée product (Landl et al., 2010).

Additionally, Zhang et al. (2011) evaluated the effect of thermal treatments (60°C for 5, 20, 40, and 60 min) using atmospheric pressure and high pressure (HP) (300, 600, and 900 MPa/60°C/5, 20, 40, and 60 min) on the color of watermelon juice. The results showed that thermal treatment for 60 min led to a significant change in the color of watermelon juice with ΔE 6.94. Tola and Ramaswamy (2014a) found that thermal treatment (70 to 90°C) caused degradation in vitamin C content of watermelon juice, and the \(D\) value of vitamin C ranged from 40 to 176 min.

Table 1.3 - Advantages and Limitations of Thermal Treatment (Pasteurization) (Rupasionghe & Yu, 2012)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>- To eliminate pathogens</td>
<td>- Changes in texture, color and appearance of food</td>
</tr>
<tr>
<td>- To eliminate or reduce spoilage organisms</td>
<td>- Changes in the nutritional quality of product</td>
</tr>
<tr>
<td>- To extend the shelf-life of products.</td>
<td>- Formation of off-flavors in the heated product</td>
</tr>
<tr>
<td>- To improve palatability of the food</td>
<td>- Causes negative consumer perception in terms of freshness and nutritional quality</td>
</tr>
<tr>
<td>- Accepted by consumers, predictable process</td>
<td></td>
</tr>
<tr>
<td>- An inexpensive technology</td>
<td></td>
</tr>
</tbody>
</table>

1.4.4. 2. Non-Thermal Processing

In addition to thermal methods, emerging non-thermal food treatment technologies such as irradiation, pulsed electric field processing (PEF), high pressure processing (HPP), and ultraviolet light (UV), can also be used for the preservation of foods (Balasubramaniam et al., 2008).
1.4.4.2.1. High Pressure Processing

High pressure, or ‘clean’ processing is considered an emerging alternative technology to traditional food processing techniques that can support the inactivation of pathogens without resulting in significant changes to the product quality or nutritive value (Jordan, Pascual, Bracey & Mackey, 2001). High pressure techniques include high hydrostatic pressure (HHP) and high pressure homogenization (HPH), which are considered to be suitable, and safe methods for food processing. This includes, changing protein structure, enzyme inactivation, contents of chemical constitutes and microbiological stability. The solid or liquid foods are pressurized to 400 – 600 MPa at room temperature or higher, or refrigerated conditions, to achieve pasteurization due to pressure rather than heat. Thus, pressure pasteurization can result in the destruction of vegetative cells, preventing spoilage and pathogenic microorganisms, and leading to the inactivation of many enzymes, allowing most foods to be preserved with minimal negative impact on nutritional and sensory quality characteristics (Balasubramaniam et al., 2008).

In sum, HPP allows for the production of safe products with a longer shelf-life because it inactivates vegetative microorganisms and preserves the quality of products. Thus, while thermal pasteurization degrades vitamins, antioxidants, and polyphenol, HPP process retains them at levels almost as high as that of the fresh, unprocessed juice (Zemser, 2015).

The antimicrobial properties of high pressure were first reported in 1899, but at that time the technology was unreliable along with more focus on thermal pasteurization as an intervention. Consequently, high pressure processing was no pursued as a food preservation method although did find utility in material sciences to develop novel ceramics and artificial diamonds (Dalai & Sahu, 2010). Yet, the application of high pressure processing of foods was revisited in the 1990’s when the first HHP products were marketed in Japan.
The purpose of HHP at that time was as a mean of extended the shelf-life of jams and jellies as opposed to pathogen control. Shortly after, HHP was adopted for preserving guacamole, first in the US but then in South America thereby introducing the technology into more mainstream processing (Dalai & Sahu, 2010). However, it was not until HHP was applied as a pathogen reduction step that growth in the use of the technology occurred (Dalai & Sahu, 2010).

**Main Principles of High Hydrostatic Pressure**

There are three basic principles of HHP processing. The first is the Le Chatelier- Braun Principle, which explains that HPP stimulates some favourable phenomenon which results in a decrease in volume. Moreover, the breaking of ionic bonds is also enhanced by HPP, as this leads to a volume decrease (Rupasinghe & Yu, 2012; Koutchma, 2014a). Secondly, the Isostatic Principle states that pressure is instantaneously and uniformly transmitted throughout the sample, independent of the size and shape of food. A uniform pressure will be applied to the sample in all directions. As such, the pressure will not damage the product, which will return to its original shape when the pressure has been released. Thus, it has been found that HPP delivers very subtle energy in comparison to thermal process and thus the covalent bonds (other than disulfide bonds) usually remain unaltered (Rupasinghe & Yu, 2012). HPP is a flexible processing technology, which does not depend on product type, shape and size, and type of packaging materials. HPP can disrupt large molecules and microbial cell structures, such as enzymes, proteins, lipids, and cell membranes, and leave small molecules such as vitamins and flavour components unaffected (Koutchma, 2014a).
High Pressure Processing Equipment

HPP is primarily carried out using batch equipment, although semi-continuous equipment is also available. According to Hogan, Kelly and Sun (2005), the main components of a high pressure processing system include:

- A cylindrical pressure vessel.
- A low pressure pump.
- Two end closures for sealing the vessel.
- A restraining device for the end closures including: yoke, threads, and pin.
- An intensifier pump that uses liquid to generate high pressure process fluid for system compression.
- A system for controlling and monitoring pressure and temperature.
- A product-handling system (Figure 1.2).

Commercial High Pressure Systems

High pressure food processing is commonly performed as a batch process to pasteurize pre-packaged foods (Koutchma, 2014a). The cycle time based on the type of food and the processing (Dalai & Sahu, 2010).

The batch system is the most commonly used method for high pressure food processing especially for packaged foods (Koutchma, 2014a). Food is prepared and aseptically filled/sealed in plastic containers, then placed in a pressure chamber for pressurizing and the chamber is then decompressed. The cycle time is based on the type of food and the processing (Dalai & Sahu, 2010).
The system consists of a process vessel that filling with product and they may operate in a vertical, horizontal, or tilting mode at pressures over 400 MPa; closure for sealing the vessel which bringing the vessel to pressure process conditions, decompressing the vessel and removing the product; outer and inner cylinders and the inner cylinder and all other parts exposed to water or food must be made of stainless steel to avoid corrosion (USFDA, 2014); a low pressure pump; a system for controlling and monitoring the pressure and temperature; and a pressure generation system (direct piston type compression and indirect and indirect compression method) (Dalai & Sahu, 2010). For batch operation, packaged food is placed into the pressure vessel, the vessel is sealed, and water is pumped into the vessel to remove any air. The pressure relief valve is closed after the vessel is full and water is pumped into the vessel until the process pressure is reached (Figure 1.4) (Fellows, 2009; USFDA, 2014).

![Figure 1.2 - Schematics of High-Pressure Food Processing Techniques (Balasubramanum, 2008)](image-url)
Figure 1.3 - Horizontal High Pressure System

- Packed food in sterilized containers
- Load in pressure chamber
- Fill chamber with water
- Pressurized chamber
- Hold under pressure
- Depressurized the chamber
- Remove treated food

Figure 1.4 - Batch Operation Processing in HHP
High Pressure Mechanisms of Inactivation

According to the FDA (2014), HPP mechanisms of inactivation can be classified into four groups: cell envelope-related effects, pressure-induced cellular changes, biochemical aspects, and effects on genetic mechanisms. Cellular morphology is changed by pressure and its division slows with increasing pressure application at pressures of 100-300 MPa. In addition, high pressure treatments inhibit reactions that increase volume and activate reactions that decrease volume, causing damage to the cell membrane. Moreover, HPP affects enzyme inactivation by changing the intermolecular structures and causing conformational changes at the active site. Enzymes differ in their sensitivity to denaturation, although this is defined by the matrix being treated (USFDA, 2014). Generally, the damage caused by HPP is essentially equivalent to the damage caused by high temperature and oxidative stress in microbes (USFDA, 2014).

Lauric Arginate

Lauric Arginate (LAE) (C_{20}H_{41}N_{4}O_{3}Cl) is a novel antimicrobial that has been approved by the FDA (Figure 1.5). It is an antimicrobial agent that acts on the cell membranes and the cell cytoplasm, detaining growth of the bacterial population (Pattanayaiying, H-Kittikun & Cutter, 2014). It has a broad spectrum of activity (bacteria & fungi), is effective over a wide pH range (3-7), and is easy to use and handle. Further, it is a cost-effective and has no influence on the organoleptic characteristics of food, and it breaks down to ethanol, carbon dioxide and glycine. Therefore, it does not need to be included on labels (Suksathit & Tangwatcharin, 2013).
Figure 1.5 – Lauric Arginate

Lavieri et al. (2014) studied effects of LAE, Octanoic Acid, thermal treatment and high hydrostatic pressure (400-600 MPA for 4 min) to control *Listeria monocytogenes* on alternatively-cured frankfurters. Results showed that LAE, Octanoic Acid, thermal treatment and high hydrostatic pressure at 400 MPa reduced *L. monocytogenes* by 2.28, 2.03, and 1.88-log$_{10}$CFU/g. However, *L. monocytogenes* grew in all post-lethality intervention treatments, with the exception of a 600 MPa high hydrostatic pressure and LAE treatment applied for 4 min.

**High Pressure Mediated Inactivation of Microbes**

The inactivation of microorganisms, such as vegetative bacteria, yeasts, and viruses is dependent on the amount of pressure, treatment temperature and, time. The history of the microbe in terms of growth phase, pre-imposed stress and strain related factors also contribute to pressure resistance. Spores resist combination of standard high pressure (400-600 MPa), and treatment at room temperature. However, pressure-assisted thermal sterilization (PATS) at pressure (500-900 MPa) combined with thermal treatment (90-120°C) causes inactivation of spore microorganisms (Koutchma, 2014a).
During HHP treatment, there is a linear inactivation in the microbial load. The rate of microbial inactivation decreases with time progress, resulting in a survival curve with a 'pressure-resistant tail' due to inherent phenotype differences in pressure resistance. As a result, HHP and HT applied for the inactivation (Koutchma, 2014a).

Varela-Santos et al. (2012) examined the effects of HHP on the microbial count of pomegranate juice, showing that HHP treatment over 350 MPa for 150s reduced the microbial load around 4-log cycles (Varela-Santos et al., 2012). Moreover, Moody, Marx, Swanson and Bermudez-Aguirre (2014) studied the effect of HHP on the inactivation of *E. coli* in apple juice. The study showed that the most effective treatment was HHP at 600 MPa at 21°C, which inactivated 7-log of bacteria cell in less than 1 min (Moody et al., 2014). On the other hand, the combination of high pressure carbon dioxide and high pressure as a non-thermal processing technique has also been used to improve the safety and shelf-life of carrot juice (Park, Lee & Park, 2002). The combined treatment of carbon dioxide 4.90 MPa and high-pressure treatment at 300 MPa resulted in complete destruction of aerobic microbes at initial count of the control was 8.0-log CFU/mL. The effect of carbon dioxide on the microbial inactivation was due to a decrease in pH inside the cells and increased permeation rate of carbon dioxide molecules, which changed to bicarbonate or carbonate and caused malfunctions in the cell membrane and even cell death (Park et al., 2002). (Table1.4) summarizes the effect of HHP treatment on the microbial inactivation in juices.
### Table 1.4 - Effect of HHP Treatment on Microbial Inactivation in Juices

<table>
<thead>
<tr>
<th>Treatment HHP/time</th>
<th>Juice</th>
<th>Microorganism</th>
<th>Effect of HHP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>350 - 550 MPa/ 30, 90 and 150s</td>
<td>Pomegranate</td>
<td>Total aerobic mesophilic bacteria, mold and yeast</td>
<td>4-log cycles reduction at 350 MPa or more for 150s</td>
<td>Varela-Santos et al. (2012)</td>
</tr>
<tr>
<td>300 - 600 MPa/ 0.5 - 7 min</td>
<td>Apple</td>
<td><em>Escherichia coli</em></td>
<td>7-log cycle reduction at 600 MPa in less than 1 min.</td>
<td>Moody <em>et al.</em> (2014)</td>
</tr>
<tr>
<td>200 - 400 MPa 10- 15 min</td>
<td>Orange</td>
<td><em>Salmonella. Typhimurium</em></td>
<td>7.04-log CFU/mL inactivation at 400 MPa in 10 min. 7.04-log CFU/mL inactivation at 400 MPa in 15 min.</td>
<td>Erkmen (2011)</td>
</tr>
<tr>
<td>400 MPa/ 1 min</td>
<td>Orange</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>2.4-log CFU/mL inactivation at 400 MPa in 1 min.</td>
<td>Yoo <em>et al.</em>, (2015)</td>
</tr>
<tr>
<td>400, 500 and 600 MPa/ 120s</td>
<td>Coconut water</td>
<td><em>Escherichia coli</em> <em>Salmonella Listeria</em></td>
<td>More than 6-log CFU/mL inactivation at 500 and 600 MPa in 120s.</td>
<td>Lukas (2013)</td>
</tr>
<tr>
<td>300 and 600 MPa/ 5 and 10 min and CO₂ at 0.3, 0.5 and 0.8 mol/L</td>
<td>Carrot</td>
<td>Total aerobic mesophilic</td>
<td>4-log CFU/mL inactivation at 300 MPa in 5 mins at 0.8 mol/L CO₂</td>
<td>Park <em>et al.</em> (2002)</td>
</tr>
</tbody>
</table>

**Effects of High Pressure Processing on Juice Quality**

HPP has the potential to maintain high-quality foods that improving food safety, preventing food spoilage, preserving food quality and extending product shelf life that display characteristics of fresh products (Considine, Kelly, Fitzgerald, Hill & Sleator, 2008). One of the most important advantages of HPP of food is the extension of shelf life, while retaining the sensory characteristics of fresh food products (Considine *et al.*, 2008). Varela-Santos *et al.* (2012) studied the effects of HHP on the physiochemical properties, bioactive compound and shelf life of pomegranate juice.
The study showed that HHP treatment over 350 MPa for 150s extended the shelf-life of pomegranate juice stored at 4 °C for more than 35 days with a non-significant reduction in antioxidant capacity (Varela-Santos et al., 2012). In addition, Landl et al. (2010) compared at an industrial scale between effect of high pressure treatments (400 and 600 MPa/5 min/20°C) and a mild conventional pasteurization at (75°C/10 min) on total vitamin C, ascorbic acid, total phenolic, physicochemical properties of an acidified apple purée product that stored for 3 weeks at refrigerated temperatures (5°C ± 1°C). The researchers reported that high pressure treatment did not have any significant effects on physicochemical properties and pressure at 400 MPa did not affect total vitamin C, ascorbic acid and total phenolic contents, but it increased viscosity. However, treatment at 600 MPa combined with heat treatment decreased the total phenolic content and the retention of TPP was 75%, whereas vitamin C from 354 ± 34 to 278 ± 17 mg kg⁻¹ (Landl et al., 2010).

Zhang et al. (2011) evaluated the effect of thermal treatment (60°C for 5, 20, 40, and 60 min) and HP (300, 600, and 900 MPa/60°C/5, 20, 40, and 60 min) using hydraulic press U101 in the color of watermelon juice. Results demonstrated that there was a significant change on the color of watermelon juice after HP treatment at 900 MPa for 60 min with ΔE 8.01. However, HP treatment was an effective way to preserve the color of the treated watermelon juice compared to the thermal and UV-C treatments (2011). Tola and Ramaswamy (2014a) found that high pressure treatment (400-600 MPa) caused no significant degradation in vitamin C content of watermelon juice in comparison to thermal treatment. The D value of vitamin C degradation ranged from 4 to 24 h (zp= 257 MPa). Moreover, the combination of high pressure carbon dioxide and high pressure is a non-thermal processing technique used to improve the safety and shelf-life of carrot juice.
The combined treatment of high pressure (600 MPa) and 4.90 MPa-HPCD resulted in 11.8% PPO inactivation (Park et al., 2002). Liu, Hu, Zhao and Zhang (2011) studied the effect of HPCD treatment on the polyphenol oxidase (PPO) inactivation in watermelon juice. HPCD treatment was at 30 MPa and 50 °C for 30 min, which caused a 95.8% reduction in polyphenol oxidase activity (Liu et al., 2011). Yaldagard, Mortazavi and Tabatabaie (2008) showed that high pressure treatment at less than 800 MPa of strawberry jam retained fresh flavour much more than traditional thermal processing. Further, HHP treatment of milk and dairy products at 300-400 MPa for 3-15 min had a low impact on flavour resulted in a shelf-life comparable to that of thermal pasteurization (Yaldagard et al., 2008). Finally, Jung, Samson and Lamballerie (2011) stated that the volatile components of guava juice treated at 600MPa and 25°C for15 min were similar to those of fresh guava juice, and suggested that pressurized treatment preserved the original juice flavour (Jung et al., 2011). (Table 1.5) summarises effect of HHP treatment on juice quality.
Table 1.5-Effect of HHP Treatment on Juice Quality

<table>
<thead>
<tr>
<th>Treatment HHP/time</th>
<th>Juice</th>
<th>Quality</th>
<th>Effect of HHP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 MPa/ 1 min</td>
<td>Orange</td>
<td>- pH</td>
<td>- No significant difference in pH after treatment at 400 MPa for 1 min</td>
<td>Yoo et al., (2015)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Color</td>
<td>- No significant difference in color</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Vitamin C</td>
<td>- Vitamin C reduced by 35.1% after 400 MPa for 1 min. treatment</td>
<td></td>
</tr>
<tr>
<td>400, 500 and 600 MPa/ 120s</td>
<td>Coconut water</td>
<td>Phenoloxidase</td>
<td>No significant difference in phenol oxidase after treatment at 500 and 600 MPa for 1 min</td>
<td>Lukas, A. (2013)</td>
</tr>
<tr>
<td>350 - 550 MPa/ 30, 90 and 150s</td>
<td>Pomegranate</td>
<td>- Phenolic compounds</td>
<td>- Increased significantly at 550 MPa for 150s</td>
<td>Varela-Santos et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Antioxidant</td>
<td>- Decreased at 550 MPa for 150s, but in significant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Color</td>
<td>- Difference in color was significant after HHP treatment at 550 MPa for 150s</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Shelf-life</td>
<td>- HHP treatment at 550 MPa for 150s extended shelf-life to 35 days’ storage at 4°C</td>
<td></td>
</tr>
<tr>
<td>300, 600, 900 MPa/ 5, 20, 40, and 60 min at 60°C</td>
<td>Watermelon</td>
<td>Color</td>
<td>Significant change in color characteristics</td>
<td>Zhang et al. (2011)</td>
</tr>
<tr>
<td>400 - 600 MPa/ 5 min</td>
<td>Apple purée</td>
<td>- TPC</td>
<td>- Significant decreasing in TPC after treatment at 600 MPa for 5 min</td>
<td>Landl et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Vitamin C</td>
<td>- No significant difference in Vitamin C after treatment at 600 MPa for 5 min</td>
<td></td>
</tr>
<tr>
<td>600 MPa/ 15 min</td>
<td>Guava</td>
<td>- Volatile components</td>
<td>- No significant difference in volatile components after treatment at 600 MPa for 15 min</td>
<td>Jung et al. (2011)</td>
</tr>
<tr>
<td>HPCD, 30 MPa for 30 min</td>
<td>Watermelon</td>
<td>PPO</td>
<td>- PPO activity inactivated by 95.8% after HPCD treatment</td>
<td>Liu et al. (2011)</td>
</tr>
<tr>
<td>300 and 600 MPa/ 5 and 10 min and CO₂ at 0.3, 0.5 and 0.8 mol/L</td>
<td>Carrot</td>
<td>Phenoloxidase</td>
<td>- Phenoloxidase reduced by 11.8% after 600 MPa for 10 min. with 0.8 mol/L CO₂ treatment</td>
<td>Park et al. (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Color</td>
<td>- Significant color changes</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.6 - Advantages and Limitations of HHP Process (Koutchma, 2014a; Yaldagard et al., 2008)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Eliminate pathogens</td>
<td>- HPP sterilization and pasteurization are relatively expensive technology</td>
</tr>
<tr>
<td>- Eliminate or reduce spoilage organisms</td>
<td>- processing must be done in a sealed, pressured vessel</td>
</tr>
<tr>
<td>- Extend the shelf-life of products</td>
<td>- Enzymes and spore microorganisms require very high pressure for inactivation</td>
</tr>
<tr>
<td>- Improve palatability of food</td>
<td>- Pressure processed foods require low temperature storage to preserve their sensory and nutritional qualities</td>
</tr>
<tr>
<td>- Independent of product mass and size</td>
<td></td>
</tr>
</tbody>
</table>

1.4.4.2. Ultraviolet Light Processing

UV light has a long history of use for the decontamination of foods and is generally accepted by consumers (Koutchma, Forney & Moraru, 2009). UV has a wavelength range from 100-400 nm. There are four UV light ranges shown in (Figure 1.6): UV-A 315-400 nm causes changes in human skin, UV-B 280-315 nm causes skin burning and cancer, and UV-C 200-280 nm inactivates bacteria and viruses. In addition, the UV vacuum range is 100-200 nm (Koutchma et al., 2009).

![Figure 1.6 - UV Electromagnetic Spectrum (courtesy of UVDGM, 2003)](image-url)
UV light is a product of photons resulting from electrons transitioning from a high to lower energy state. Within gas discharge of UV lamps, this light interacts with the constituents of the UV reactor, such as lamp sleeves and reactor wall, affecting the intensity and wavelength of the UV light that illuminates the microorganisms and chemical components of the liquid (Koutchma et al., 2009).

**Table 1.7 - Relative Resistance of Microorganisms to UV-Light (254 nm) (Nicholson & Galeano, 2003; Gabriel & Nakano, 2009)**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Medium</th>
<th>D Value (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>phosphate-buffered saline</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Escherichia coli</em> K-12</td>
<td>phosphate-buffered saline</td>
<td>0.22</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>phosphate-buffered saline</td>
<td>0.1</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>phosphate-buffered saline</td>
<td>0.26</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>phosphate-buffered saline</td>
<td>0.28</td>
</tr>
<tr>
<td><em>B. subtilis</em> ATCC</td>
<td>Water</td>
<td>120J/m²</td>
</tr>
</tbody>
</table>

**UV Reactors Design**

UV light is considered an effective technology to reduce the level for microbial contamination for many liquid foods and beverages. However, the penetration of UV light is limited in fruit juices and beverages due to color compounds, organic solutes and suspended matter, which reduce UV efficiency (Koutchma, 2009). Consequently, to facilitate the UV treatment of such beverages, it is necessary to ensure efficient mixing in which each part of the fluid is exposed to UV, or to make a sufficiently thin film to account for poor photon penetration.

**Laminar Poiseuille Flow Reactor**

Laminar Poiseuille Flow Reactor is a thin film with a velocity profile parabolic in nature (Koutchma et al., 2009). It consists of four chambers with different lamp lengths and fluence rates on the quartz surface, a protective quartz sleeve, and a remote power (Koutchma et al., 2009).
However, the main limitation of thin film reactors is the low flow rates that are achieved with some fluids (Koutchma et al., 2009). However, (Figure 1.7a) the Cider Sure Unit can efficiently mix a fluid while being exposed to UV. This reactor consists of three chambers connected to outside tubing and 8 low pressure mercury (LPM) quartz lamps inside a cylinder. The apple juice is pumped through a 0.8 mm annular gap ring-shaped opening located between the inner chamber surface and the outside surface of the quartz sleeve (Koutchma, 2014b). Moreover, Matak et al. (2005) found that the flow pattern within this reactor had a significant impact on the reduction of L. monocytogenes via UV light treatment. The Cider Sure model FPE regulated the UV fluencies by using three flow rate settings (Koutchma, 2014b).

![Schematics of (a) A Laminar Thin Film Reactor (Cider Sure) and (b) A Laminar Taylor Couette UV Reactor](image-url)
**Turbulent Flow Reactor**

Turbulent Flow Reactor uses a high flow rate to increase fluid turbulence, which allows for processing of high product volumes. In the application of turbulent flow UV reactors for food pasteurization, fluid flows through a stainless steel cylindrical chamber contains parallel low pressure mercury (LPM), low pressure amalgam (LPA), or medium pressure mercury (MPM) lamps inside a quartz sleeve. The intensity of UV light is detected using a sealed UV monitor. PureLine UV models (PQ 0200-1100) have been used in the disinfection of sugar syrups (Koutchma, 2014b).

**A Bench Scale Apparatus "Collimated Beam"**

The collimated beam apparatus is specifically designed to measure microbial inactivation kinetics. The main components of this design, illustrated in (Figure 1.8) are as follows. **Lamp:** lamps may be either low pressure mercury vapour (monochromatic) at 253.7 nm or medium pressure mercury vapour polychromatic UV light. **Collimating tube:** this tube provides a spatially homogeneous irradiation field on a given surface area, and in order to prevent reflection, the inner surface of the collimating tube must be roughened and painted with a flat black paint. **Window:** the lamp enclosure should be thermally stable because the output of many UV lamps is quite temperature sensitive. The most useful type is a quartz window which prevents changes in air drafts. **Shutter:** shutters are used to regulate the time of exposure factor in the fluence UV dose calculation by blocking or allowing exposure to UV energy. **Platform:** this is platform on which the Petri dish and stirring motor are placed for UV exposure; it should be thermally and physically stable and easily controllable. **Stirring:** it is important to assure equal fluence UV dose for all microorganisms in the suspension.
**Radiometer detector**: this accurately measures the irradiance in the beam. **Power supply**: it is very important to maintain a constant emission from the UV lamp over exposures that may be as long as 1 or 2h (Bolton & Linden, 2003).

The uses of a Bench Scale Collimated Beam Apparatus are as follows: 1) development of standardized fluence UV dose inactivation response; 2) generation of fundamental fluence ~UV dose inactivation response data for different pathogens; and 3) investigation of the photochemical degradation of contaminants (Bolton & Linden, 2003).

![Diagram of Bench Scale Devices for Conducting UV Experiments](image)
1. **Reflection Factor**: When the beam of light passes from one medium to another, the refractive index changes and a small fraction of the beam is reflected off the interface between the media. The fraction reflected $R$ is given by the Fresnel Law, wherein, $R$ for water is 1.000 and 1.372, $R = 0.025$. The reflection Factor is $(1 - R) = 0.975$, and represents the fraction of the incident beam that enters the water Equation (1.3) (Bolton & Linden, 2003).

\[
\text{Reflection factor} = 1 - R \quad \text{(1.3)}
\]

2- **Petri Factor**: The ratio of the average of the incident irradiance over the area of the Petri dish to the irradiance at the centre of the dish. It is used to correct the irradiance reading at the centre of the Petri dish to more accurately reflect the average incident fluence rate over the surface area. It is determined by the radiometer detector every 5 mm over the area of the Petri dish, by dividing the irradiance at each point by the centre irradiance, and taking an average of these ratios Equation (1.4) (Bolton & Linden, 2003).

\[
\text{Petri factor} = \frac{\text{central intensity}}{\text{intensity of each 5mm}} \quad \text{(1.4)}
\]

3- **Water Factor**: The Water Factor is defined as:

\[
1 - 10^{-AL/AL \times \ln(10)} \quad \text{(1.5)}
\]

where $A =$ decadic absorption coefficient (cm$^{-1}$) or absorbance for a 1 cm path length and, $L =$ vertical path length (cm) of the water in the Petri dish (Bolton & Linden, 2003)

4- **Divergence Factor**: the divergence and the water absorbance effects, which need to be considered together. However, for path lengths less than 5 cm, the errors involved in treating them separately are negligible Equation (1.6) (Bolton & Linden, 2003).

\[
\text{Divergence Factor} = \frac{L}{L + d} \quad \text{(1.6)}
\]
D value (mJ/cm²) per 1 log reduction for different microorganisms in tiger nuts milk sample using equation (1.7):

\[ D \text{ value} = \frac{\text{Delivered dose}}{\log \text{ reduction}} \]  

(1.7)

**Hydrogen Peroxide**

Hydrogen peroxide is a disinfectant that works by oxidizing the cell membranes and inner cell structures of microbes and produces reactive oxygen species (ROS), which can oxidize susceptible organic materials, metal ions, and cellular contents (known as "Advanced Oxidative Process") (Nederhoff, 2000). The advantages of hydrogen peroxide include the lack of disinfection by products and complete degradation. Moreover, hydrogen peroxide is easy to use and inexpensive (LDHP, 2015).

Advanced oxidation process (AOPs) is characterized by the production of highly reactive and non-selective hydroxyl radicals, which capable of oxidizing nearly all organic compounds to water, carbon dioxide, and mineral (Nederhoff, 2000). UV/H₂O₂ is an effective technology for the destruction of Endocrine Disrupting Compounds (EDCs) and Pharmaceuticals and Personal Care products (PPCPs), and wastewater treatments with high turbidity to enhance action of UV. This technology combines the effects of direct and indirect UV photolysis and is very energy intensive process. Many radicals based reactions take place (Caris & Beerendonk, 2011).

**Effects of Ultraviolet Light Processing on the Microbial Content of Juice**

The antimicrobial effect of UV-C light is a result of its ability to damage microbial deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and prevent reproduction via cross-linking and strand breakage, as shown in (Figure 1.9).
Certain microorganisms are able to repair the damage caused by UV light and regain infectivity. Repair of UV light-induced DNA damage can be accomplished by photo reactivation and/or dark repair (Knudson, 1985). Photo reactivation is dependent on many factors, including types, species, and strains of the target microorganism, inactivation levels following irradiation, the photo reactivating light and the nutrient state of the microorganism (1985).

![Figure 1.9 - The Effectiveness of UV-C on the DNA Structure in the Microorganisms](image)

Some research has studied the impact of UV light treatment on the microbiological stability of food. Koutchma et al. (2009) studied the effect of UV irradiation on the microorganism activity of apple cider using a thin film Cider Sure™ UV reactor, which was used to examine the qualification of UV on 5-log reduction of *E. coli* O157:H7. Results indicated that a greater than 5-log reduction was achieved after one pass of treatment (Koutchma et al., 2009).
A study examined the effect of decontamination on quality attributes of fresh tiger nut milk beverage (Horchata) with short wave ultraviolet treatments (UV-C). UV-C treatment of tiger nut milk was conducted in a stainless-steel UV chamber with one low pressure mercury lamp (9W output and maximum peak radiation of 253.7 nm). Results showed that there was a significant effect on the microbial count, which achieved up to 3-log_{10} CFU/mL of spoilage microorganisms (yeast and mold, mesophilic flora, and psychotropic bacteria) (Corrales et al., 2012). Moreover, Müller, Reub, Greiner and Stahl (2013) studied effects of UV treatment on carrot juice at UV-C dose (4.3 kJ L^{-1}), combined with thermal treatment (65°C, 5 min). The results showed that UV-C treatment achieved a 3-log reduction of total aerobic microbial count.

Effects of UV Light Processing on Juice Quality

Koutchma et al. (2009) discussed the effect of UV irradiation on the organoleptic properties of apple cider by using a thin film cider sure™ UV reactor. The study showed that UV light had minimal impacts on the flavour quality of apple cider because there was a poor transmission of UV light through the cider. Moreover, Koutchma et al. (2009) also examined UV treatment of reconstituted orange juice, including its effect on shelf-life, pH, color, vitamin C, and degradation of pectin methylestrase (PME) during exposure of UV light of 73.8–100 mJ/cm². Results indicated no significant impact on color, PME and pH of the orange juice; however, there was significant loss in vitamin C to 17% at a UV dose of 100 mJ/cm². UV light of 73.8 mJ/cm² extended shelf-life to 5 days. Koutchma et al. (2009) also reported the effect of UV irradiation on quality attributes of apple juice using sterile Pyrex oven dishes (25 cm diam.) and exposed to a 30-W UV-light source, pulsed electric field (PEF), and heat treatment at 72, 94ºC. Exposure of the juice to UV light of 30 W led to the greatest decrease in TPC and antioxidants after heat treatment. There was a significant loss in phenolic contents after UV and PEF treatment (Koutchma et al., 2009).
On the other hand, UV treatment affected the vitamin C concentration in apple juice. Table (1.8) shows that the degradation of vitamin C after exposure to a UV dose of 600mJ/cm² ranged from 30-40 %, and 18-25% in orange and carrot juice, respectively (Koutchma et al., 2009).

Tikekar, Anantheswaran, Elias and LaBorde (2011) demonstrated the effects of UV light treatment on ascorbic acid degradation in juices. Results reported that AA treated with UV light continued to degrade during dark storage, and there was a significant positive relationship between initial UV treatment dose and dark storage degradation rate suggests that amore stable reactive compound was formed during UV exposure.

Table 1.8 - Vitamin C Degradation in Juices After UV Light Treatment

<table>
<thead>
<tr>
<th>Vitamin C in juices</th>
<th>Degradation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple juice</td>
<td>30 - 40%</td>
</tr>
<tr>
<td>Orange juice</td>
<td>18%</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>25%</td>
</tr>
</tbody>
</table>

Corrales et al. (2012) studied the impacts of UV-C light on quality characteristics of tiger nut milk (pH, total soluble solids, color, antioxidant, TPC, peroxidase, and shelf-life). The study was conducted using a Collimated beam scale constructed by UV-Consulting Peschi España (Burjassot, Valencia, Spain), with a low pressure mercury lamp with 9W output, peak radiation at 253.7 nm, and an average UV radiation intensity (2.35 mW/cm²). Samples were 12 mL volume and 0.2 cm height were placed in Petri dishes (60.3 cm²) and treated in batches under constant stirring. The result showed that UV-C light extended its shelf life to 11 days at refrigerator temperature compared to the untreated samples.
Additionally, finding indicated that UV-C treatment had a low impact on the total soluble solids, pH and antioxidant at residual content 71%. Color, which was beneficial as these are the main quality parameters indicative of product freshness of tiger nut milk remained almost unchanged after UV-C treatments, and there was no significant increase in a* value and decrease in L* value compared to the fresh tiger nut milk samples. On the other hand, there was a significant decrease in the peroxidase activity at residual content 14%. However, there was a significant loss of antioxidant and TPC due to low UV light penetration and turbidity in the tiger nut milk beverage, and there was thiobarbituric acid reactive substances, further provided an estimation of the extent of the oxidation generated by UV-C, specifically concerning the oxidation of lipids and browning (Corrales et al., 2012). Additionally, Zhang et al. (2011) studied the effect of UV treatment on the color of watermelon juice by using a helically wound Teflon tubing wrapped around a quartz glass tube with a UV lamp (9W UV-C low pressure mercury lamp at 254 nm) (Dean vortex reactor). The watermelon juice passed through the coiled tubing for 3, 6, 9, and 12 cycles at a flow rate of 8.4 l/h, and UV-C doses of 2421, 4843, 7264, and 9685 J/l. The result showed that UV treatment had a significant impact on color change on watermelon juice with ΔE 7.84 at UV dose 9685 J/l.

Alexandra-Müller et al. (2013) studied effects of UV-C treatment on carrot juice at a UV-C dose (4.3 kJ L⁻¹), combined with thermal treatment (65°C, 5 min). The result showed that combined treatment extended carrot juice shelf-life to 12 days, with no significant loss in Carotenoids after UV-C treatment.
Table 1.9 - Advantages and Limitations of Using UV Technique (Health Canada, 2006; Bolton & Cotton, 2008)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>- It is a low cost non-thermal technology</td>
<td>It is expensive in terms of energy requirements and hardware</td>
</tr>
<tr>
<td>- It inactivates pathogenic and spoilage microbes</td>
<td>- It may cause serious health problems for the users, including blindness</td>
</tr>
<tr>
<td>- It causes little changes in the food's nutritional quality</td>
<td>- Generation of photoproducts</td>
</tr>
<tr>
<td>- It has low residuals and hazardous</td>
<td>- High dose to inactivate spores and limited efficacy for opaque samples</td>
</tr>
<tr>
<td></td>
<td>- No residual antimicrobial activity</td>
</tr>
</tbody>
</table>

Therefore, the focus of the study was to evaluate nutrients, enzymes, bioactive constituents and microbiological stability of fresh tiger nut milk, in relation to the treatment under different processing conditions or parameters with thermal, HHP and UV-C light technologies. The hypothesis was that High Hydrostatic Pressure and UV-C treatment will be more effective methods to preserve tiger nut milk quality and microbiological stability than traditional thermal processing treatment. This is because, as discussed above, findings from the literature indicate that HHP and UV-C light are particularly effective method for treating and preserving the quality and microbiological stability of tiger nut milk and other low acid beverages.
Chapter II

2. Method

2.1. Materials

The materials used in this research study were purchased from Sigma-Aldrich, (Oakville, ON, Canada), and include: phenolphthalein indicator (C$_{20}$H$_{14}$O$_{4}$), sodium hydroxide (NaOH), trichloromethane (Chloroform) (CHCl$_{3}$), methanol (CH$_{4}$O); (MeOH), sodium chloride (NaCl), sodium carbonate (Na$_{2}$CO$_{3}$), copper sulfate penthydrate (CuSO$_{4}$.5(H$_{2}$O)), sodium trisulfate (Na$_{2}$trisulfate.2 (H$_{2}$O)), Folin reagent (C$_{10}$H$_{5}$NaO$_{5}$S), 2,6dichlorophenolindophenol(C$_{12}$H$_{7}$Cl$_{2}$NO$_{2}$), 2,4 dinitrophenylhydrazine (C$_{6}$H$_{6}$N$_{4}$O$_{4}$) meta phosphoric acid (HPO$_{3}$)$_{n}$, Gallic acid (C$_{7}$H$_{6}$O$_{5}$), 2,2-diphenyl-1-picrylhydrazyl(DPPH)(C$_{18}$H$_{12}$N$_{5}$O$_{6}$), sodium nitrite (NaNO$_{2}$), guaiacol substrate (C$_{7}$H$_{8}$O$_{2}$) and hydrogen peroxide (H$_{2}$O$_{2}$).

2.2. Sample Preparation:

2.2.1. Tiger Nut Milk Preparation

Tiger nuts (250 g) from Spain were soaked overnight at room temperature in potable water at a 1:3 ratio of nuts to water. The nuts and water were then homogenized using a Q-Link Blender (Model 365XG) to obtain a smooth consistency that was subsequently filtered through cheese cloth to extract the vegetable milk. The resulting tiger nut milk was then subdivided into 50 mL lots and frozen at -20°C until required (Figure 2.1).
Fresh Tiger Nuts

Sorted and washed with distilled water

Mixture soaked (1: 3 nuts: water) overnight at a room temperature

Homogenized using a high speed blinder

Extracted using cheese cloth

Bottled for further use

Tiger Nut Milk

Figure 2.1 - Preparation of Tiger Nut Milk

The control samples were left untreated and were physic-chemically and microbiologically tested at the same time as the treated samples.

2.3. Physico-chemical Properties

**Determination of pH and Conductivity**

In the current study, pH and conductivity of untreated and treated samples were measured by a digital Oakton pH meter (pH/CON serial # 531157, Eutech instrument, Singapore) at room temperature.
The pH meter was calibrated using pH 4 and pH 7 standard solutions (Fisher Scientific, NJ) on each test day. The pH and conductivity measurements was conducted in triplicate, as per Okyere and Odamtten (2014).

**Determination of Total Soluble Solid (Brix)**

Brix, or total soluble solid was measured using a digital hand-held refractometer (Atago Co., Ltd., Tokyo, Japan). Two to three drops of tiger nut milk were placed onto the prism and the reading was recorded, and measurements were performed in triplicate, as per Okyere and Odamtten (2014).

**Determination of Titratable Acidity**

Twenty mL of the sample were placed into a conical flask and 2 drops of 1% phenolphthalein indicator were added to the mixture and titrated with 0.1N NaOH. The results were recorded as soon as a pink colour was observed and persisted, using the Equation 2.1. (Awonorin & Udeozor, 2014).

\[
T.T.A = \frac{\text{number of mL of NaOH used}}{\text{sample taken}} \quad (2.1)
\]

**Determination of Viscosity**

The viscosity of untreated and treated samples (250 mL) were measured using a Brookfield viscometer (LVLD - 1 Prime, Brookfield Engineering Laboratory Inc., SN: 8670850, USA) using spindle 3 at speed 100 rpm. Measurements were performed at room temperature (25°C) (Okyere & Odamtten, 2014).

**Determination of Crude Protein**

The protein content of tiger nut milk sample was determined by the Lowry method (Lowry, Rosebrough, Farr &Rondall, 1951). Multiple sample concentrations were prepared. First, a total of 0.5 mL of the sample was taken, and 0.7 mL of freshly mixed complex reagent of (NaOH+
Na₂CO₃, CuSO₄·5(H₂O) and Na₂tratrate (H₂O) (100:1:1) was added to the sample. The solution was vortexed (Fisher Scientific 120V, SN: 120223008, USA) and stored in a dark place for 20 minutes. After 20 minutes, 0.1 mL of Folin reagent was added to each tube (5mL of Folin reagent + 6 mL distilled water). The solution was then vortexed and stored in a dark location for 30 min. The protein content was measured at 750 nm using UV-VIS spectrophotometer (BIO-BAD, Smart Spect Plus, SN: 273 BRO 5206, USA) (Equations 2.2).

Protein was calculated by using the slope of the standard curve equation

\[
Y = 0.0056X + 1.8625
\]  

(2.2)

**Determination of Ascorbic Acid (Vitamin C)**

Ascorbic acid (vitamin C) content of the sample was determined using the titration with a (2,6-dichloro-phenolindophenol) (2,6 DPIP) reagent. A sample of 30-40 mL was added to 0.2 g solid oxalic acid, and was swirled until it dissolved. A 2,6 DPIP titration solution was prepared in 1 liter distilled water, dissolved 1.25 g of 2.6 DPIP reagent and 1.05 g NaHCO₃, and this solution was diluted in the ratio of 1:4. Finally, take 5-10 mL of prepared sample was titre with 2.6 DPIP solution until the appearance of the first pink permanent color.

The vitamin C content of tiger nuts milk was calculated using the formula (2.3)

\[
\text{Mass of vitamin C mg/100ML} = \frac{T}{St} \times 2 \times \text{dilution}
\]  

(2.3)


**Determination of Total Phenolic Compounds (TPC)**

Total phenolic compounds were determined using the Folin-Ciocalteau method. The total phenolic concentration was calculated using the standard curve of gallic acid (r=0.9987). One mL of each four samples of varying concentrations (10%, 12%, 14%-20%) were incubated at room
temperature in 5 mL of Folin-Ciocalteau reagent and 4 mL of 1 mol/L Na$_2$CO$_3$. After 15 min of incubation, absorbance was measured at 765 nm by UV-VIS spectrophotometer (BIO- BAD, Smart Spect Plus, SN: 273 BRO 5206, USA) (Corrals et al., 2012).

**Determination of Antioxidants**

The antioxidant activity of the tiger nut milk samples was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Oxygen Radical Absorbance Capacity (ORAC).

**DPPH Assay:** The reagents used in this assay were DPPH 3.5 mM stock (MW 394.32). The following procedure was followed: dissolved 0.0345 g in 25 mL MeOH, DPPH 350 μM working solution: Added 2.5 mL of 3.5 mM stock solution to 22.5 mL MeOH, Trolox standard: Trolox (Mw 250.29) stock solution (20 mM): 25 mg of Trolox is dissolved in 5 mL MeOH and Trolox working solutions: Labelled six 1.5 mL microfuge tubes with Trolox with different concentrations. Samples were centrifuged for 30 s at 3000 rpm to deposit the starch. In a blank well 225 μL of MeOH was added and in a control well 25 μL of the sample was mixed with 200 μL of 0.1 mM DPPH. The mixture was gently swirled, mixed and sealed the mixture. The mixture was kept at room temperature for 6 hours in the dark place to react. Antioxidants activity of the supernatant were determined by monitoring the decrease in absorbance of the DPPH during 30 min at 517 nm in a UV-Vis spectrophotometer (BIO- BAD, Smart Spect Plus, SN: 273 BRO 5206, USA) **Equation 2.4** (Herald, Gadgil & Tilley, 2012).

\[
\text{% DPPH quenched} = \left\{ 1 - \frac{(A_{\text{sample}} - A_{\text{blank}})}{(A_{\text{control}} - A_{\text{blank}})} \right\} \times 100
\]

**ORAC assay:** The reagents used in the assay were Phosphate buffer (75 mM, pH 7.4), Fluorescein stock solution (144.65×10$^{-3}$ mM), Fluorescein working solution (8.68×10$^{-5}$mM), Trolox (Mw 250.29) standard stock solution (0.02M), Trolox working solution, and AAPH solution. Approximately, 25 μL of appropriately diluted samples, 25 μL of buffer, 25 μL of Trolox dilution...
(6.25, 12.5, 25.0, 50.0, 100μM), and 150 μL of 8.68×10^{-5} mM Fluorescein working solution were added into all experimental 96 well plates. Plates were incubated for about 30 min in a Synergy HT Multi-Detection Micro Plate Reader (FLx800, Bio-Tek Instruments Inc., Winooski, VT, USA) at 37°C. The reaction was then initiated by adding 25 μL of AAPH reagent, and shaken at maximum intensity for 10s. Fluorescence was monitored kinetically with data every minute for up to 2 h (Prior et al., 2003).

**Determination of Enzymes Activity**

Enzymes that were measured in the current study include peroxidase (POD), polyphenol oxidase (PPO) and amylase. **Peroxidase activity:** About 4 mL of tiger nut milk was centrifuged at 4300 rpm for 15 min, the supernatant was filtered through filter paper (110 mm), and diluted 1:100 with deionized water. POD activity was assayed using 2 mL of the filtrate and by adding 2 mL of 2% guaiacol in methanol (v/v) and 1 mL of 0.16% (v/v) H₂O₂. The absorbance was measured at 480 nm, with the time recorded, using spectrophotometer (BIO- BAD, Smart Spect Plus, SN: 273 BRO 5206, USA) (Cortes et al., 2005). **Polyphenol oxidase activity:** Polyphenol oxidase activity in tiger nut milk was measured using the method outlined by Cano, Hernandez and Ancos (1997) with a slight modification in the amount of solutions used. 1 mL of a solution of 0.07M catechol in 0.05M sodium phosphate buffer (pH 6.5) were added to 1 mL of tiger nut milk. The activity was measured with the spectrophotometer at 420 nm (BIO- BAD, Smart Spect Plus, SN: 273 BRO 5206, USA) at 25°C (Cano, Hernandez & Ancos, 1997). **Amylase Activity:** 1-50 μL of sample in 96 well plates were added to 100 μL of master reaction mix (50 μL amylase assay buffer + 50 μL amylase substrate mix). After mixture was incubated in a dark place at room temperature for 2-3 min, (T_{initial}) absorbance at 405 nm was measured using spectrophotometer (BIO- BAD, Smart Spect Plus, SN: 273 BRO 5206, USA) (A_{405}^{initial}).
Plates were incubated out of light and absorbance was measured every 5 min \( (A_{405})_{\text{final}} \). Continued taking measurement until the value of the most active sample was greater than the highest value of standard. Amylase activity was calculated using (Equations 2.5; 2.6)

\[
A_{405} = (A_{405})_{\text{final}} - (A_{405})_{\text{initial}}
\]

\[
\text{Amylase Activity} = B_{\text{sample}} \times \text{dilution factor} / \text{reaction time} \times V
\]

Where \( B \) = amount (nmole) of nitrophenol generated between \( T_{\text{initial}} \) and \( T_{\text{final}} \), \( V \) = sample volume (mL) added to well (Fisher Scientific kit assay, ON., Canada).

**Color**

The color of untreated and treated tiger nut milk was determined using a portable colorimeter (Chroma Meter CR400, Minolta, Japan) measuring the L*\( a^* \)\( b^* \) color space in reflectance mode before and after thermal, HHP and UV-C light treatment using equation (2.7) (Kizzie-Hayford, Jaros, Schneider & Rohm, 2014).

\( L^* \) indicates lightness

\( a^* \) is the red/green coordinate

\( b^* \) is the yellow/blue coordinate

\( \Delta E^* \) Total color difference

\[
\Delta E^* = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}
\]

2.4. **Bacteria, Cultivation Conditions and Enumeration**

The bacteria used in this study were *Escherichia coli* P36, *Listeria innocua* ATCC 51742 and *Salmonella Typhimurium* WG49 obtained from The American Type Culture Collection (ATCC).

*E. coli* P36 suspensions were cultivated from overnight culture grown aerobically at 37°C in Tryptone Soy Broth (TSB; OXOID Ltd, Basingstoke, Hampshire, England) containing 30 µg/mL filtered sterilized kanamycin, *Salmonella Typhimurium* WG49 was cultivated in (XLD; OXOID
L. innocua ATCC 51742 was cultivated in Listeria Selective Agar Base (Oxford Formulation, Oxoid Ltd, Basingstoke, United Kingdom). Additionally, the cells were harvested by centrifugation (Avanti J-20 XPI centrifuge; Beckman Coulter, California, USA) at 5000 rpm for 10 min in 50 mL centrifuged tube at room temperature and resuspended in sterile saline to give a final optical density at 600 nm of 0.8 % w/v (7-log CFU/mL). For enumeration, a 0.1 mL of tiger nut milk was mixed thoroughly, added to the sterile saline 0.9%, and then serially diluted to $10^{-5}$ (serial dilutions of the bacteria, treated and untreated samples are 0, -1, -2, -3, -4 and -5). A 0.1 mL aliquot of each dilution was plated onto Tryptone Soy Agar (TSA) containing 30μg/mL kanamycin, Oxford Formulation (Oxoid Ltd.), and Oxford Formulation (Oxoid Ltd,) containing 2mL/500mL Modified Listeria Selection Supplement (SRO206B), respectively for the three tested bacteria. Finally, the plates were incubated at 37°C for 24 h and colonies were counted using Equation (2.8).

\[
\text{CFU/mL} = \frac{1}{\# \text{ of colonies} \times \text{aliquot plated (0.1mL)} \times \text{dilution factor}}
\]  

(2.8)

After the incubation, the colonies were counted and converted into $\log_{10}$ before calculation of Log Count Reduction (LCR) in microbial number. The LCR was calculated using Equation (2.9; 2.10).

\[
LCR = \log_{10}\text{cfu/carries cm}^2 N_i - \log_{10}\text{cfu/carries cm}^2 N_o
\]  

(2.9)

Log reduction = Initial $\log_{10}$ CFU/mL − Final $\log_{10}$ CFU/mL  

(2.10)

Where the: $N_i = \text{the initial loading (pre-treatment count)}$

$N_o = \text{the surviving numbers (post-treatment count)}$
2.5. Thermal and Non-Thermal Treatment of Tiger Nut Milk

2.5.1. Thermal Treatment

There were 2 thermal treatment trials of tiger nut milk conducted using a serological water bath (Boekel, 148007, Markham, Ontario, Canada) as shown in (Figure 2.2); the temperatures were adjusted using a digital thermometer (S/N 80583726, Fisher Scientific, Canada). The first trial was conducted at temperatures between 72-75°C for 15 seconds (HTST). The second performed trial was performed at 60°C for 30 min (LTLT). Both trials were undergoing the same procedure.

Sterile glass test tubes each containing 10 mL of tiger nut milk samples were spiked with the appropriate concentration of target microbes 0.1 mL of $10^7$ CFU/mL of three different types of bacteria (E. coli P36, Salmonella WG 49 and Listeria innocua ATCC 51742). Samples were subjected to thermal treatment at 60°C for 30 min by immersing tubes in a serological water bath (Boekel, 148007, Markham, Ontario, Canada). A tube containing tiger nut milk sample was inserted using a digital thermometer and immersed in the bath for temperature monitoring (S/N 80583726, Fisher Scientific, Canada). The tubes were removed from the hot water bath after exposure to the specific temperature and time treatments, and then immediately immersed and stored in an ice bath until physicochemical properties were assessed.

Consequently, the fresh tiger nut milk samples were treated at condition 60°C for 30 min, which achieved the 5-log reduction for the tested bacteria after the treatment. The preparation of the sample for nutrient analysis was same as the microbial samples preparation, but without microbial inoculation. After the samples were thermally treated, all nutritional analyses were done.
2.5.2. High Hydrostatic Pressure Treatment

The HHP unit used in this study was the Hyperbaric 55 illustrated in (Figure 2.3). The treatment chamber was 55 L in volume, 200 mm in diameter, and 22 m² in internal surface. The temperature of the incoming water was 11°C and was monitored, along with vessel pressure, throughout the treatment cycle. Based on the FDA recommendations for unpasteurized beverages and on previous studies of low acid beverages (Jung et al., 2011; Lukas, 2013), the HHP treatment parameter of tiger nut milk was 400, 500 and 600 MPa for different time durations (90, 120 and 180 seconds).

Fresh prepared tiger nut milk samples were inoculated with $10^7$ CFU/mL of three tested bacteria strains (E. coli P36, Listeria innocua ATCC 51742, and Salmonella typhimurium WG49). The levels of the target bacteria in samples was determined as followed:10 mL of the untreated sample was inoculated with 0.1 µL of each bacterium. The samples were dispensed into sterile plastic pouches (Size 23 × 15.5 cm) (Winpak, MB, Canada) at -20°C. The frozen samples were transported to the pilot plant facility and temperature was taken prior to HHP processing.
The samples were loaded into the HHP unit and treatments were performed at set pressure and time at Gridpath Solutions Inc. (328 Glover Road, Stoney Creek, ON., Canada), the temperature of HHP treatment was 11°C ± 1. The temperature of the water and sample following HPP treatment was recorded. The samples were then returned to the laboratory and kept frozen at -20°C for analysis.

Consequently, processing pressures of 500 MPa and 600 MPa for 60, 90 and 180 seconds for each pressure were used to treat the tiger nut milk for quality and nutrients analysis because the 5-log reduction for three types of tested bacteria was achieved at these conditions. Samples of fresh tiger nut milk (150 mL) were dispensed into sterile plastic pouches at -20°C. The frozen samples were transported to HHP processing under the same previous conditions. The samples were then returned to the laboratory and kept frozen at -20°C for analysis.

Figure 2.3 - Hyperbaric 55L High Pressure Equipment (http://www.hiperbaric.com/en/hiperbaric55)
2.5.2.1. Shelf-life Study of HHP Treated Samples

In the shelf-life study there were two separate samples: non-inoculated and inoculated samples with $10^7$ CFU/mL of all tested bacteria. The non-inoculated and inoculated samples were held at 4°C with samples withdrawn periodically over a period of 8 days to evaluate the shelf-life according to the physical assessment and microbial count.

2.5.3. UV-C Light Treatment

The UV-C treatment of tiger nut milk was conducted using Collimated Beam and Dean Flow Reactor.

2.5.3.1. Collimated Beam UV Unit

A collimated beam set up offers the possibility to determine the effect of the UV dose on the inactivation kinetic of microorganisms (Figure 2.4). The system consisted of a 30 W low pressure mercury vapour UV lamp emitting at 254 nm (Trojan Technologies Inc., London, ON, Canada) and a magnetic stirrer placed on the Petri dish (50 x 35mm; Kimax, Kimble Chase, Vineland, NJ, USA). The UV intensity at the surface of the sample (incident intensity ($I_0$) or irradiance at the surface) was measured using a radiometer with UVX-25 sensor (UVX, UVP Inc., CA, USA). The radiometer was placed at the same level as the liquid interface. A high adjuster was used to control the distance between the UV lamp and sample. The degree to which the destruction or inactivation of microorganisms occurs by the UV radiation is directly related to the UV dose. The UV lamp was switched on for 45 min to reach the optimal working temperature (22°C) prior to irradiating the tiger nut milk sample.
Figure 2.4 - Lab-Scale Collimated Beam UV Unit
Table 2.1 - Technical Characteristics of the Collimated Beam UV Unit

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflection Factor, $R_f$</td>
<td>0.97</td>
</tr>
<tr>
<td>Petri Factor, $P_f$</td>
<td>1.09</td>
</tr>
<tr>
<td>Absorbance coefficient, $A$, cm$^{-1}$</td>
<td>193</td>
</tr>
<tr>
<td>Vertical path length, $d$, cm</td>
<td>0.5</td>
</tr>
<tr>
<td>Distance from the UV lamp to the cell suspension, $L$, cm</td>
<td>32</td>
</tr>
<tr>
<td>Water Factor, $W_f$</td>
<td>0.004</td>
</tr>
<tr>
<td>Divergence Factor</td>
<td>0.98</td>
</tr>
</tbody>
</table>

2.5.3.1.1. Sample Preparation for Collimated Beam

Fresh prepared tiger nuts milk samples were separately inoculated with three bacterial strains ($E. coli$ P36, $Listeria innocua$ ATCC 51742 and $Salmonella Typhimurium$ WG49) to provide a final inoculum of $10^7$ CFU/mL. Five mL of inoculated samples were added to the Petri dish to obtain a sample depth of 0.5 cm and exposed to direct UV-C light at a distance 5 cm from the collimated tube, 32 cm from the UV-C lamp and at 0.107 mW/cm$^2$ UV incident intensity level for different exposure times and D-values. Adequate stirring was applied during treatment in order to ensure mixing, but without vortices to reduce variation in liquid. The microbial inoculated samples were cultivated and incubated for a specific time for each bacterium at 37°C in the incubation, and the surviving cells were enumerated.

2.5.3.1.2. Absorbance Coefficient of Tiger Nut Drink

The absorbance coefficient of the samples was measured using a Cary 300 UV/V Spectrophotometer (Varian Inc. Walnut Creek CA) equipped with a Labsphere DRA-CA-3300 Integrating Sphere (Labsphere, North Sutton NH).
Each sample was tested in triplicate both before and after each UV treatment. The absorbance coefficient was determined from the slope of the linear plot, and it was measured at 193/cm\(^{-1}\).

**Calculation of UV dose from Collimated Beam Apparatus**

The degree to which the destruction or inactivation of microorganisms occurs by UV radiation is directly related to the UV dose. The UV dosage can be calculated using *(Equation 2.11)*: (White, 1992)

\[
D = I \times t \tag{2.11}
\]

where \(D\) = UV Dose, mW*\(s/cm^2\)

\(I\) = intensity, mW/cm\(^2\)

\(t\) = time of exposure, s

The UV dose (mJ/cm\(^2\)) has been defined as the product of the irradiation time (t in seconds) and the irradiation intensity (wavelength dependent UV output of the lamp) in mW/cm\(^2\).

The UV dose calculated using *(Equation 2.12)*:

\[
D = I_0 \times P_f \times (1 - R) \times (L / (L + d)) \times (1 - 10^{-A_d / A_d \ln(10)}) \times \text{time (s)} \tag{2.12}
\]

\(D\) is the UV irradiance, \(I_0\) is the UV intensity as measured using a radiometer at a location corresponding to the centre of the sample surface, \(P_f\) is termed the Petri factor and defined as the ratio of the UV intensity measured at the centre of the sample surface to the average intensity measured across the sample surface, \(1-R\) is termed the reflection factor where \(R\) is the reflection of UV light at 254 nm at the air-surface interface (typically \(R = 0.025\)), \(L/(d + L)\) is termed the divergence factor where \(L\) is the distance from the lamp centerline to the sample surface and \(d\) is the sample depth, and \((1-10^{A_d})/(A_d \ln (10))\) is termed the absorbance factor where \(A\) is the 1 cm UV absorbance of the suspension at 254 nm (Bolton & Linden, 2003). The Collimated beam UV unit characteristics are shown in *(Table 2.1)*.
2.5.3.2. Dean Flow Apparatus

Collimated beam UV unit was modified to incorporate UV treatment of tiger nut milk in a continuous flow regime, and a quartz coil tube was installed around the UV lamp (Figure 2.5). A helically cavity Quartz-coil is used to cause a secondary eddy flow. This type of liquid flow results in secondary vortices (Dean Vortices) (Müller, Stahl, Graef, Franz, & Huch, 2011).

In the laboratory, the continuous flow system was conducted to treat tiger nut milk. The main component is a 30 W low- pressure mercury vapour UV lamp emitting at 254 nm (Trojan Technologies Inc., London, ON, Canada). The device has a quartz-coil with helically wound cavity. The irradiance of the lamp was measured using a radiometer with UVX-25 sensor (UVX, UVP Inc., CA, USA), and was (1.5 mW/cm²). The fluid from the inlet tube was pumped into the coiled tube by a peristaltic pump (Masterflex L/S®, model HV-77202-60, USA) at speed 100 rpm to the outlet tube. The Dean flow UV unit characteristics are shown in (Table 2.2).

![Figure 2.5 - Dean Flow UV Reactor](image_url)
Table 2.2 - Technical Characteristics of the Dean Flow Reactor

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Flow rate, cm$^3$/s</td>
<td>1.8</td>
</tr>
<tr>
<td>- Coil tube volume, cm$^3$</td>
<td>6.3</td>
</tr>
<tr>
<td>- Inner diameter of quartz tube, d. mm</td>
<td>29.3</td>
</tr>
<tr>
<td>- Hydraulic diameter of quartz tube. mm</td>
<td>1.95</td>
</tr>
<tr>
<td>- Number of coil rotors</td>
<td>23</td>
</tr>
<tr>
<td>- Length of coil tube, h. cm</td>
<td>211.6</td>
</tr>
<tr>
<td>- Absorbance coefficient, cm$^{-1}$</td>
<td>193</td>
</tr>
<tr>
<td>- UV light irradiance ($I_0$), mW/cm$^2$</td>
<td>1.5</td>
</tr>
<tr>
<td>- Velocity m/s</td>
<td>0.6</td>
</tr>
<tr>
<td>- Reynolds number</td>
<td>110.8</td>
</tr>
<tr>
<td>- Dean number</td>
<td>28.6</td>
</tr>
<tr>
<td>- Absorbed UV dose, mJ/cm$^2$</td>
<td>18.4</td>
</tr>
<tr>
<td>- Resident time, s</td>
<td>3.5</td>
</tr>
<tr>
<td>- Applied UV dose J/mL</td>
<td>3.6</td>
</tr>
</tbody>
</table>

The Dean flow was calculated using a formula that is based on the Reynolds number (Equation 2.13). The Dean number (De) is based on the Reynolds number and the geometric data (dh and D) of the coiled tube (Equation 2.14) where dh is the diameter of the tube which corresponds to 1.95 mm in the reactor used. Using this term, data can be calculated in the same way as for a round tube.
D is the inner diameter of the coil and this corresponds to 29.3 mm, u is the velocity (m/s), v is the kinematic viscosity (m²/s), n is the dynamic viscosity (Pa/s) and p is the density (kg/m³).

\[ R_e = \frac{U \times d}{V} = u \times d \times \frac{p}{n} \]  \hspace{1cm} (2.13)

Dean number = \( R_e \sqrt{\frac{d h}{D}} \)  \hspace{1cm} (2.14)

The UV-C dose was calculated based on the energy delivered per volume of juice. The dose, expressed as joules per millilitre using **Equation (2.15)**, and 30% efficiency of the UV-C lamp need to be considered along with 70% of UV transmission of quartz coil (Müller et al., 2011).

Absorbed UV-C dose = 0.7 \times \text{Intensity} (I_0) \times \text{Residence time} t_s \hspace{1cm} (2.15)

UV-C Applied dose J/mL = Total UV-C output power W / Flow rate mL/s  \hspace{1cm} (2.16)

Residence time = Volume in the coil (V) / Flow rate (Q)  \hspace{1cm} (2.17)

**2.5.3.2.1. Sample Preparation for Dean Flow Reactor Treatment of Tiger Nut Milk**

Fresh prepared tiger nut milk samples were inoculated with three bacterial strains (\textit{E. coli} P36, \textit{Listeria innocua} ATCC 51742 and \textit{Salmonella Typhimurium} WG49) separately to provide a final inoculum of 10⁷ CFU/mL. Twenty mL of inoculated samples were added in sterile containers in the inlet tube and another sterile container to collect samples from the outlet tube. Samples were exposed to direct UV-C light by passing them through the Dean flow reactor for 5 passes by a Masterflex L/S pump (HV-77202-60, assembled in USA) into outlet tube. The UV intensity (incident intensity (I₀) at the surface of the quartz rotor was measured using the digital UVX radiometer (UVP LLC, Canada). The radiometer was placed at the same distance of the coil tube from the lamp. Consequently, after 5-log CFU reduction of three tested bacteria was achieved at UV dose 18.4 mJ/cm² for 5 cycles. Therefore, the sample was treated for 5 cycles for nutrients analysis.
2.5.3.2.2. Shelf-life Study of UV Light Treated Samples

In the shelf-life study, there were two separate samples: non-inoculated and inoculated samples. Non- inoculated samples were exposed to the UV-C light treatment at UV-C dose 18.4 mJ/cm$^2$, and hydrogen peroxide (Fisher Scientific, Whitby, ON, Canada). H$_2$O$_2$ was diluted from a 30% stock-solution to a different concentration (0.5-1.0-1.5- % v/v) using the following formula:

\[ C_1V_1 = C_2V_2 \]

Where C1 is concentration of the stock solution, V1 is the volume of the stock solution required, C2 is the final concentration and V2 is the volume of the reaction. Inoculated samples were subjected to inactivation by combined UV-C dose 18.4 mJ/cm$^2$ with hydrogen peroxide (H$_2$O$_2$) as described above. Finally, the non-inoculated and inoculated samples were held at 4°C with samples withdrawn periodically over a 7-day period to evaluate shelf-life according to physical assessment and microbial count.

2.6. Experimental Design and Statistics

All trials were performed at least three times using two replicate samples in each treatment. The data obtained from different analyses were subjected to various statistical analyses using SPSS software program (IBM SPSS Statistics, Armonk, NY, USA). The data were compared using one-way Analysis of Variance (ANOVA) and Tukey’s test. Also, means and standard deviations (SD) of triplicate observations were calculated. A value of P ≤ 0.05 was considered statistically significant measurements in this research study.
Chapter III

3. Results

3.1. Thermal Treatment

Thermal treatment of tiger nut milk was conducted at 60°C for 30 min. Temperature of treatment was monitored using data-logger (time/temperature profile), and the time needed for samples to reach 60°C was around 5 min (Figure 3.1).

3.1.1. Thermal Effects on Inactivation of Surrogate Organisms in Tiger Nut Milk

The starting population of *Listeria innocua ATTC 51742, E. coli P36* and *Salmonella Typhimurium* WG49 before thermal treatment were on average 7.33 ± 0.06, 7.34 ± 0.04 and 7.40 ± 0.09-log $10 \text{ CFU mL}^{-1}$, respectively. A thermal treatment of 60°C for 30 min supported a >5-log CFU reduction of the introduced bacteria with no significant (P>0.05) difference in the log count reduction of the test bacteria being recorded (Table 3.1). However, there were significantly differences in the microbial inactivation between control and LTLT treated tiger nut milk.

**Table 3.1 - Effect of Thermal Treatment on Microbial Inactivation of Tiger Nut Milk**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Temperature</th>
<th>Time (min)</th>
<th>D Value (min)</th>
<th>Initial</th>
<th>Log cfu/ml*</th>
<th>LCR*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria</em></td>
<td>60 C°</td>
<td>30</td>
<td>11.9</td>
<td>7.33 ± 0.06$^A$</td>
<td>2.2 6± 0.01</td>
<td>5.07 ± 0.01$^{AB}$</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>60 C°</td>
<td>30</td>
<td>12</td>
<td>7.34 ± 0.04$^A$</td>
<td>2.31 ± 0.03</td>
<td>5.03 ± 0.03$^{AB}$</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>60 C°</td>
<td>30</td>
<td>11.9</td>
<td>7.40 ± 0.09$^A$</td>
<td>2.33 ± 0.11</td>
<td>5.07 ± 0.11$^{AB}$</td>
</tr>
</tbody>
</table>

$^*$Values are the Mean ± SD of three duplicates, LCR = Log Count Reduction. CFU= Colony Forming Unit. Identical superscripts in the same column indicate non-significantly different at the 95% confidential level.
3.1.2. Thermal Effects on Physicochemical Properties of Tiger Nut Milk

Thermal treatment effects on physicochemical properties and nutrients quality showed in (Table 3.2). Thermal treatment did not significantly (P>0.05) alter the pH or total soluble solids of the tiger nut milk (Table 3.2). However, there was a significant increase in titratable acidity from 22.5% to 25% and conductivity from 3.2 ± 0.03 to 4.1 ± 0.15, and significant decrease in the measured protein content of the beverage from 5.53 ± 0.2 to 3.6 ± 0.02 mg/L of thermally treated tiger nut milk compared to non-treated controls. In comparison, heating of tiger nut milk resulted in a non-significant (P>0.05) decrease in the viscosity to 9.3 ± 0.1cP (Table 3.2).
Table 3.2 - Effects of Thermal Treatment on Physicochemical Properties of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control sample</th>
<th>*Thermal treated sample</th>
<th>% RC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5 ± 0.01&lt;sup&gt;A&lt;/sup&gt;</td>
<td>6.5 ± 0.01&lt;sup&gt;A&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>TSS (Brix)</td>
<td>4.5 ± 0.04&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.5 ± 0&lt;sup&gt;A&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>TA (%)</td>
<td>22.5 ± 0.01&lt;sup&gt;A&lt;/sup&gt;</td>
<td>25 ± 1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>111</td>
</tr>
<tr>
<td>Conductivity</td>
<td>3.2 ± 0.03&lt;sup&gt;A&lt;/sup&gt;</td>
<td>4.1 ± 0.15&lt;sup&gt;B&lt;/sup&gt;</td>
<td>128</td>
</tr>
<tr>
<td>Viscosity (cP*)</td>
<td>10.4 ± 0.01&lt;sup&gt;A&lt;/sup&gt;</td>
<td>9.3 ± 0.1&lt;sup&gt;B&lt;/sup&gt;</td>
<td>89.4</td>
</tr>
<tr>
<td>Protein (mg/L)</td>
<td>5.53 ± 0.2&lt;sup&gt;A&lt;/sup&gt;</td>
<td>3.6 ± 0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>65.0</td>
</tr>
</tbody>
</table>

<sup>*</sup>Values are the Mean ± SD of three duplicates *TSS = Total Soluble Solids, cP = centipoise. Identical superscripts in the same raw indicate non-significantly different at the 95% confidential level.

3.1.3. Thermal Effects on Nutrients Quality and Enzymes Activity of Tiger Nut Milk

Thermal treatment at 60ºC for 30 min significantly (P>0.05) decreased the measured vitamin C content from 8.22 ± 0.02 mg/100mL to 5.99 ± 0.4 mg/100mL (Table 3.3). There were also significant losses in phenolics from 139.14 mg GAE/100ml to 99.17 mg GAE/100ml, antioxidants (DPPH & ORAC) with residual contents 67.7% and 62.8%, respectively, and enzymes (peroxidase, polyphenol oxidase) activity with residual contents of 40% and 17.5% after the heating process; however, there was no significant increase in the amylase activity with residual content 133.3% (Figure 3.2).
### Table 3.3 - Effects of Thermal Treatment on Nutrients Quality and Enzymes Activity of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control sample</th>
<th>*Thermal treated sample</th>
<th>% RC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mg/100mL)</td>
<td>8.22 ± 0.02 A</td>
<td>5.99 ± 0.4 B</td>
<td>72.8</td>
</tr>
<tr>
<td>TPC (mg GAE/100 mL) *</td>
<td>139.14 ± 2.02 A</td>
<td>99.17 ± 6.9 B</td>
<td>71.2</td>
</tr>
<tr>
<td>DPPH *</td>
<td>533.71 ± 2.4 A</td>
<td>361.44 ± 0.1 B</td>
<td>67.7</td>
</tr>
<tr>
<td>ORAC *</td>
<td>1.21 ± 0.1 A</td>
<td>0.76 ± 0.4 B</td>
<td>62.8</td>
</tr>
<tr>
<td>Peroxidase (Unit/mL)</td>
<td>0.2 ± 0.01 A</td>
<td>0.08 ± 0.01 B</td>
<td>40</td>
</tr>
<tr>
<td>Polyphenol oxidase (Unit/mL)</td>
<td>0.4 ± 0.05 A</td>
<td>0.07 ± 0.05 B</td>
<td>17.5</td>
</tr>
<tr>
<td>Amylase (nmole/min/mL)</td>
<td>0.3 ± 0.01 A</td>
<td>0.4 ± 0.01 B</td>
<td>133.3</td>
</tr>
</tbody>
</table>

* Values are the Mean ± SD of three replicated. TPC = Total Phenolic Compounds, RC = Residual content. Identical superscripts in the same raw indicate non-significantly different at the 95% confidential level. DPPH expressed as μmol L⁻¹ Trolox equivalent (TE) mL⁻¹. ORAC expressed as Trolox Equivalent mM

![Figure 3.2 - Peroxidase, Polyphenol oxidase and Amylase Residual Activity](image-url)
3.1.4. Thermal Effects on The Color Characteristic of Tiger Nut Milk

Color perception is a key indicator of physicochemical changes. A change in the shade of foods as a result of food processing can be perceived by the consumers as a low quality product, and consequently rejected. The ΔE values, which are an indicator of total color difference, showed that there were significant differences (p<0.05) in color between untreated and treated samples become less bright (low L* values), and more red (high a* values) after thermal treatment (Table 3.4), (Figure 3.3).

Table 3.4 - Effect of Thermal Treatment on The Color Characteristic of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control sample</th>
<th>*Thermal treated sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color L*</td>
<td>72.5 ± 0.02 A</td>
<td>68.45 ± 0.1 B</td>
</tr>
<tr>
<td>a*</td>
<td>-0.16 ± 0.02 A</td>
<td>-0.74 ± 0.05 B</td>
</tr>
<tr>
<td>b*</td>
<td>11.67 ± 0.3 A</td>
<td>6.56 ± 0.1 B</td>
</tr>
<tr>
<td>ΔE*</td>
<td></td>
<td>6.5</td>
</tr>
</tbody>
</table>

L* indicates lightness, a* is the red/green coordinate, b* is the yellow/blue coordinate, ΔE is the total color difference. Identical superscripts in the same raw indicate non-significantly different at the 95% confidential level.

Figure 3.3 - Thermal Treatment Effects on Color Characteristics of LTLT Treated Tiger Milk
3.2. HHP Treatment

3.2.1. HHP Effects on Inactivation of Surrogate Organisms in Tiger Nut Milk

Effects of HHP treatment at 400, 500 and 600 MPa on inactivation of pathogen and surrogate organisms in tiger nut milk represented in (Table 3.5).

- *Listeria innocua* ATCC51742

Initial count of *Listeria* before HHP treatment and with no added LAE was on average 7.48 ± 0.04-log_{10} CFU mL^{-1}. The inactivation kinetics were characterized as having an initial rapid decline in *Listeria* levels followed by tailing at longer treatment times especially at 500 or 600 MPa (Figure 3.4). The extent of *Listeria* inactivation was dependent on the applied pressure and treatment time. A 5-log CFU reduction of the bacterium was achieved at 500 MPa (5.06 ±1.1 and 5.11± 0.3-log_{10} cycles) and 600 MPa (5.51± 0.9 and 6.14± 0.6-log_{10} cycles) with treatments of 120 and 180s, respectively (Figure 3.4). The inclusion of LEA (0.1 – 2.0% v/v) did not significantly increase the lethality of HHP or lead to less tailing. A greater than 5-log_{10} reduction as recommended in pasteurized products by FDA was accomplished within the HHP treatment at 500 MPa and 600 MPa for time 120 and 180 seconds.
Figure 3.4 - Effect of HHP Treatment of Tiger Nuts Milk on Listeria Inactivation

- E. coli P36

Initial count of E. coli P36 in control sample before HHP treatment and with no adding LAE was in average 7.46 ± 0.06-log_{10} CFU mL^{-1}. The inactivation kinetics were characterized as having an initial slow and then rapid decline in E. coli P36 levels followed by tailing at longer treatment times especially at 500 and 600 MPa, respectively (Figure 3.5). The extent of E. coli inactivation was dependent on the applied pressure and treatment time. A 5-log CFU reduction of the bacterium was achieved at 500 MPa 90, 120 and 180s (5 ±0.2, 5.03 ±0.2 and 5.74 ±0.6 -log_{10} cycles) and 600 MPa (5.92± 0.3, 6.88± 0.2 and 7.08 ± 0.2-log_{10} cycles) with treatment times 90, 120 and 180s (Figure 3.5). The inclusion of LEA (0.1 % v/v) did not significantly increase the lethality of HHP or lead to less tailing. More than 5-log_{10} reduction as recommended in pasteurized products was accomplished within the HHP treatment at 500 MPa and 600 MPa for time 120 and 180 seconds.
Figure 3.5 - Effect of HHP Treatment of Tiger Nut Milk on *E. Coli* Inactivation

- *Salmonella Typhimurium* WG49

Initial count of *Salmonella Typhimurium* WG49 before HHP treatment was in average $7.06 \pm 0.1\log_{10}$ CFU mL$^{-1}$. The inactivation kinetics were characterized as having an initial rapid decline in *Salmonella* levels followed by tailing at longer treatment times especially at 500 and 600 MPa, respectively (Figure 3.6). The extent of *Salmonella* inactivation was dependent on the applied pressure and treatment time. Greater than 5-log CFU reduction of the bacterium was achieved at 500 MPa, 180s ($5.49 \pm 0.5\log_{10}$ cycles) and 600 MPa ($5.9 \pm 0.4$, $6.1 \pm 0.5$ and $6.6 \pm 0.3\log_{10}$ cycles) with treatment times 90, 120 and 180s (Figure 3.6). Greater than 5-$\log_{10}$ reduction as recommended in pasteurized products was accomplished within the HHP treatment at 500 MPa and 600 MPa for time 90, 120 and 180 seconds. Indeed, effects of HHP treatment at the same pressure on all tested bacteria represented in (Figures 3.7; 3.8 and 3.9).
Figure 3.6 - Effect of HHP treatment on *Salmonella* inactivation

Figure 3.7 - Effect of HHP Treatment at 400 MPa on Microbial Inactivation
Figure 3.8 - Effect of HHP Treatment at 500 MPa on Microbial Inactivation

Figure 3.9 - Effect of HHP Treatment at 600 MPa on Microbial Inactivation
Table 3.5- Effect of HHP Treatment on Microbial Inactivation of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (s)</th>
<th>Initial D value (s)</th>
<th>Log CFU/mL</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>L. innocua ATCC 51742</em></td>
<td>90s</td>
<td>7.48 ± 0.04A</td>
<td>55.5</td>
<td>1.93 ± 0.1 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>2.01 ± 0.2 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>2.08 ± 0.2 Ab</td>
</tr>
<tr>
<td>400 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.48 ± 0.04A</td>
<td>21.7</td>
<td>4.82 ± 0.1 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>5.06 ± 1.1 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>5.11 ± 0.3 Ab</td>
</tr>
<tr>
<td>500 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.48 ± 0.04A</td>
<td>20.8</td>
<td>5.08 ± 0.8 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>5.51 ± 0.9 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.48 ± 0.04A</td>
<td>16.6*</td>
<td>5.92 ± 0.9 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>6.88 ± 0.3 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>7.08 ± 0.2 Ab*</td>
</tr>
<tr>
<td>2. <em>E. coli P36</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.46 ± 0.06A</td>
<td>55.5</td>
<td>1.84 ± 0.04 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>2.0 ± 0.1 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>2.37 ± 0.5 Ab</td>
</tr>
<tr>
<td>500 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.46 ± 0.06A</td>
<td>22.2</td>
<td>5.00 ± 0.2 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>5.03 ± 0.2 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>5.74 ± 0.6 Ab</td>
</tr>
<tr>
<td>600 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.46 ± 0.06A</td>
<td>16.6*</td>
<td>5.92 ± 0.9 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>6.88 ± 0.3 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>7.08 ± 0.2 Ab*</td>
</tr>
<tr>
<td>3. <em>S. Typhimurium WG49</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.06 ± 0.2A</td>
<td>62.5</td>
<td>2.46 ± 0.4 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>2.26 ± 0.2 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>2.83 ± 0.1 Ab</td>
</tr>
<tr>
<td>500 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.06 ± 0.2A</td>
<td>24.3</td>
<td>5.09 ± 0.1 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>5.28 ± 0.3 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>5.49 ± 0.5 Ab</td>
</tr>
<tr>
<td>600 MPa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90s</td>
<td>7.06 ± 0.2A</td>
<td>17.3*</td>
<td>5.95 ± 0.4 Ab</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td></td>
<td></td>
<td>6.19 ± 0.5 Ab</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td></td>
<td></td>
<td>6.67 ± 0.3 Ab*</td>
</tr>
</tbody>
</table>

*Values are the Mean ± SD of three replicated. LCR = Log Count Reduction. CFU= Colony Forming Unit. Identical superscripts in the same column indicate non-significantly different at the 95% confidential level. * More sensitive bacteria for HHP treatment at 600 MPa for 18s. - More resistance bacteria for HHP treatment at 600 MPa for 18s.
3.2.2. HHP Effects on Physicochemical Properties and Composition of Tiger Nut Milk

The effects of HHP treatment on the physicochemical properties and composition of tiger nuts milk represented in (Table 3.6). There were no significant (P>0.05) changes in the pH, total solids, or titratable acidity following high pressure treatment except treatment at 500 MPa for 180s which decreased titratable acidity significantly to 17.5% (Table 3.6). There was a significant (P<0.05) decrease in the conductivity from 3.2 to 2.6 and 2.3 ms/cm\(^{-1}\) after HHP treatment at 500 and 600 MPa, respectively. There was significant increase in the viscosity of tiger nut milk treated at 600 MPa for >90s. Moreover, there was a significant decrease in the measured protein content from 5.53 ± 0.2 to 4.06 ± 0.1 mg/L under the same treatment regimes (Table 3.6).

Tables 3.6 - Effect of HHP Treatment on Physicochemical Properties of Tiger Nut Milk

<table>
<thead>
<tr>
<th>HHP treatment</th>
<th>PH</th>
<th>TSS* (Brix)</th>
<th>Conductivity ms/cm(^{-1})</th>
<th>Titratable Acidity (%)</th>
<th>Viscosity (cP)*</th>
<th>Protein (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.5±0.01(^{A})</td>
<td>4.5±0.04(^{A})</td>
<td>3.2±0.03(^{A})</td>
<td>22.5±0.01(^{A})</td>
<td>10.4±0.01(^{A})</td>
<td>5.53±0.2(^{A})</td>
</tr>
<tr>
<td>500MPa 90s</td>
<td>6.5±0(^{A})</td>
<td>4.5±0.01(^{A})</td>
<td>2.6±0.01(^{B})</td>
<td>22 ±0(^{A})</td>
<td>11.1±0.5(^{A})</td>
<td>5.44±0.02</td>
</tr>
<tr>
<td>500MPa 120s</td>
<td>6.4±0.05(^{A})</td>
<td>4.5±0(^{A})</td>
<td>2.6±0.01(^{B})</td>
<td>22 ±0(^{A})</td>
<td>11.9±0(^{B})</td>
<td>5.15±0.05(^{B})</td>
</tr>
<tr>
<td>500MPa 180s</td>
<td>6.4±0.06(^{A})</td>
<td>4.5±0(^{A})</td>
<td>2.6±0(^{B})</td>
<td>17.5 ±0(^{B})</td>
<td>12.6±0.2(^{B})</td>
<td>5.13±0.06(^{B})</td>
</tr>
<tr>
<td>600MPa 90s</td>
<td>6.4±0.05(^{A})</td>
<td>4.5±0(^{A})</td>
<td>2.6±0(^{B})</td>
<td>22.5 ±0(^{A})</td>
<td>13.6 ±0.6(^{B})</td>
<td>5.53 ±0.03(^{A})</td>
</tr>
<tr>
<td>600MPa 120s</td>
<td>6.5±0(^{A})</td>
<td>4.5±0(^{A})</td>
<td>2.3±0(^{B})</td>
<td>22.5 ±0(^{A})</td>
<td>14.7 ±0.5(^{B})</td>
<td>4.42 ±0.08(^{B})</td>
</tr>
<tr>
<td>600MPa 180s</td>
<td>6.5±0.01(^{A})</td>
<td>4.5±0.04(^{A})</td>
<td>2.3±0.01(^{B})</td>
<td>22.5 ±0.01(^{A})</td>
<td>12.2 ±0.1(^{B})</td>
<td>4.06 ±0.1(^{B})</td>
</tr>
</tbody>
</table>

3.2.3. HHP Effects on The Nutrients Quality and Enzymes Activity of Tiger Nut Milk

There was a significant loss in vitamin C content of treated tiger nut milk (8.22-5.93 mg/100mL) at 600 MPa 180s. Phenolic content was significantly (P<0.05) decreased from 139.14mg GAE/100mL to 95.85 after HHP treatment at 600 MPa for 120s (Table 3.7). The antioxidant content (DPPH& ORAC) was non-significantly (P<0.05) and significant changed as a result of pressure treatment with residual content 88.3% and 73.5%, respectively.
Moreover, the activity of peroxidase was significantly (P<0.05) decreased by HHP treatment at 600 MPa for 180s but was increased when lower pressure or treatment times were applied (Table 3.7). In comparison, there was a significant decrease in polyphenol oxidase activity at the pressure treatments tested (Figure 3.11).

Tables 3.7 - Effect of HHP Treatment on The Nutrients Quality and Enzymes Activity of Tiger Nut Milk

<table>
<thead>
<tr>
<th>HHP Treatment</th>
<th>Time (s)</th>
<th>Vitamin C (mg/100mL)</th>
<th>TPC* (mg GA/100mL)</th>
<th>% RC*</th>
<th>Antioxidant (DPH, ORAC)</th>
<th>% RC</th>
<th>Peroxidase (Unit/mL)</th>
<th>% RC</th>
<th>Polyphenol oxidase (Unit/mL)</th>
<th>% RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>8.22± 0.02</td>
<td>139.14± 2.02</td>
<td>100</td>
<td>533.71 ± 2.4</td>
<td>100</td>
<td>0.2± 0.01</td>
<td>100</td>
<td>0.4± .05</td>
<td>100</td>
</tr>
<tr>
<td>500 MPa</td>
<td>90s</td>
<td>6.21± 0.06</td>
<td>100.33± 72.1</td>
<td>%</td>
<td>507.67 ± 6.4</td>
<td>%</td>
<td>0.3±0.02</td>
<td>%</td>
<td>0.2±0.01</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>6.17± 0.01</td>
<td>11.9</td>
<td></td>
<td>506.20±4.4</td>
<td></td>
<td>0.3±0.05</td>
<td></td>
<td>0.2±0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td>6.06± 0.02</td>
<td>107.18±9.9</td>
<td></td>
<td>494.55±7.2</td>
<td></td>
<td>0.4±0.05</td>
<td></td>
<td>0.2±0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>107.21±19.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.02 ± 0.07</td>
<td></td>
<td>84.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.21± 0.2</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.14± 0.2</td>
<td></td>
<td>94.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>600 MPa</td>
<td>90s</td>
<td>6.23± 0.06</td>
<td>98.92±2.03</td>
<td>71.0</td>
<td>486.07 ± 2.1</td>
<td>91.0</td>
<td>0.3±0.02</td>
<td>150</td>
<td>0.1±0.03</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>120s</td>
<td>5.94± 0.06</td>
<td>95.85±14.3</td>
<td>68.8</td>
<td>481.65 ± 2.6</td>
<td>90.2</td>
<td>0.2±0.01</td>
<td>100</td>
<td>0.1±0.05</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>180s</td>
<td>5.93± 0.02</td>
<td>120.89±6.1</td>
<td>86.8</td>
<td>471.65 ± 2.7</td>
<td>88.3</td>
<td>0.1±0.05</td>
<td>50</td>
<td>0.1±0.01</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.03 ± 0.2</td>
<td></td>
<td>85.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.08 ± 0.3</td>
<td></td>
<td>89.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.89 ± 0.3</td>
<td></td>
<td>73.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are the Mean ± SD of three replicated TPC = Total Phenolic Compounds. RC = Residual content. Identical superscripts in the same column indicate non-significantly different at the 95% confidential level. DPPH expressed as μmol L⁻¹ Trolox equivalent (TE) mL⁻¹. ORAC expressed as Trolox Equivalent mM
Figure 3.10 - Peroxidase Residual Activity after HHP Treatment

Figure 3.11– Polyphenol oxidase Residual Activity after HHP Treatment
3.2.4. HHP Effects on The Color Characteristic of Tiger Nut Milk

The ΔE values, which are an indicator of total color difference, showed that there were no significant differences (p<0.05) in color between untreated and treated samples at 500 MPa for 90, 120 and 180s and 600 MPa for 90s. Samples become brighter (higher L* values), and less red (lower a* values) at 600MPa for 180s (Table 3.8). However, a significant decrease(p<0.05) in the lightness (L* value) was detected for tiger nuts milk at 500MPa for 180s. In general, the ΔE was significantly higher in treated samples at 500 MPa for 180s (6.7). Thermal effects on color characteristics of tiger nut milk showed in (Figure 3.12).

Table 3.8 - Effect of HHP Treatment on The Color Characteristic of Tiger Nut Milk

<table>
<thead>
<tr>
<th>HHP</th>
<th>Time (s)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>72.5 ± 0.02</td>
<td>-0.16 ± 0.02</td>
<td>11.67 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>500MPa</td>
<td>90</td>
<td>69.6 ± 0.05</td>
<td>-0.11 ± 0</td>
<td>9.6 ± 0.1</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>69.8 ± 0.07</td>
<td>-0.19 ± 0.03</td>
<td>9.3 ± 0.2</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>66.4 ± 0.3</td>
<td>-0.59 ± 0.02</td>
<td>8.7 ± 0.08</td>
<td>6.7</td>
</tr>
<tr>
<td>600MPa</td>
<td>90</td>
<td>69.4 ± 0.1</td>
<td>-0.05 ± 0.01</td>
<td>9.3 ± 0.2</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>71.8 ± 0.3</td>
<td>0.63 ± 0.01</td>
<td>9.6 ± 0.4</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>72.8 ± 0.2</td>
<td>-0.05 ± 0.06</td>
<td>8.9 ± 0.4</td>
<td>2.7</td>
</tr>
</tbody>
</table>

L* indicates lightness, a* is the red/green coordinate, b* is the yellow/blue coordinate, ΔE is the total color difference.
3.2.5. Shelf-life Study

The fate of *E. coli* P36 and *L. innocua* ATCC 51742 introduced into tiger nut milk, and then HHP treated at 600MPa for 180s then stored at 4°C for 8 days was determined (Table 3.9). The HHP treatment decreased *E. coli* and *Listeria* levels by 6.2 ± 0.05 and 5.18 ± 0.1-log CFU, respectively. *E. coli* levels decreased from 5.93 to 1.37-log CFU/mL during the 8-day storage period. In a similar manner, *Listeria* counts in HHP treated tiger nut milk was decreased from 5.18 ± 0.1 to 1.89 ± 0.02-log CFU/mL during storage at 4°C for 8 days. In inoculated but non-HHP treated controls, there was no significant difference in *E. coli* or *Listeria* levels following the 8-day storage period. Inclusion of LEA (0.1%) in tiger nut milk neither affected the efficacy of HHP or the levels of *E. coli* or *Listeria* at the end of storage at 4°C (Table 3.9). With respect to the endogenous microflora, yeast & moulds or spore forming bacteria were not recovered from prepared tiger nut milk (Table 3.9). The total aerobic count was decreased by HHP treatment to 4.8 log CFU during
storage at 4°C for 8 days. The final total aerobic count was significantly (P<0.05) lower compared to non-treated control.

At the end of the storage period yeast and moulds along with spore formers were significantly lower compared to non-treated controls (Table 3.9).

Table 3.9 - Effect of HHP Treatment on Natural Micro-Flora and Surrogate Organisms of HHP

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Microorganisms</th>
<th>Control</th>
<th>600MPa/180s</th>
<th>Control</th>
<th>600MPa/180s</th>
<th>Control</th>
<th>600MPa/180s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total aerobic count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>600MPa/180s</td>
<td>Control</td>
<td>600MPa/180s</td>
<td>Control</td>
<td>600MPa/180s</td>
<td>Control</td>
</tr>
<tr>
<td>Total aerobic count</td>
<td>4.94 ± 0.03</td>
<td>4.24 ± 0.1</td>
<td>0.8 ± 0.05</td>
<td>3.74 ± 0.01</td>
<td>6.6 ± 0.02</td>
<td>4.8 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Mold &amp; Yeast</td>
<td>ND</td>
<td>ND</td>
<td>2.3 ± 0.1</td>
<td>1.4 ± 0.01</td>
<td>5.2 ± 0.1</td>
<td>4.4 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Spore</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>4.1 ±0.02</td>
<td>3.6 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>E. coli (no inoculated)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>E. coli P36</td>
<td>7.48 ± 0</td>
<td>5.93* ± 0.02</td>
<td>6.42 ± 0</td>
<td>3.96* ± 0.05</td>
<td>5.93 ± 0</td>
<td>1.37 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>E. coli P36 + 0.1% LAE</td>
<td>7.48 ± 0</td>
<td>6.2* ± 005</td>
<td>6.05 ± 0</td>
<td>3.92* ± 0.1</td>
<td>5.87 ± 0</td>
<td>1.25 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Listeria innocua ATCC 51742</td>
<td>7.48 ± 0</td>
<td>5.18*± 0.1</td>
<td>6.68 ± 0</td>
<td>4.08*± 0.02</td>
<td>6.19 ± 0</td>
<td>1.89 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Listeria innocua ATCC +0.1% LAE</td>
<td>7.48 ± 0</td>
<td>5.34*± 0.1</td>
<td>6.23± 0</td>
<td>23.93*± 0.1</td>
<td>6.02 ± 0</td>
<td>1.87 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

ND= Not detected (parameters were not estimated because control sample was not inoculated with E. coli P36 and Listeria innocua), * more than 5-log cycles reduction and _ less than 5-log cycles reduction

Changes were observed when the color characteristics were analyzed by colorimetric during the storage period. There was not a significant (p>0.05) effect of pressure on color characteristics of tiger nuts milk with adding 0.1% LAE preservative (Table 3.10). There was significant color change in the HHP treated tiger nuts milk in storage days (0, 4 and 8).
Table 3.10 - Effect of HHP Treatment on The Quality Parameters of HHP Treated Tiger Nut Drink
Storage 8 Days.

<table>
<thead>
<tr>
<th>Storage days</th>
<th>Control</th>
<th>600MPa/180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Viscosity</td>
</tr>
<tr>
<td></td>
<td>ΔE</td>
<td>S*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S+0.1% LAE*</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>10.4 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.9</td>
<td>10.9 ± 0.05</td>
</tr>
<tr>
<td>8</td>
<td>4.1</td>
<td>11.2 ± 0.05</td>
</tr>
</tbody>
</table>

S = sample of tiger nuts milk, S + 0.1% LAE = sample of tiger nuts milk with 0.1% Lauric Arginate

3.3. UV-C Light Treatment

3.3.1. Collimated Beam Effects on Inactivation Kinetic of Surrogate Organisms in UV Treated Tiger Nut Milk

The starting population of *Listeria*, *E. coli* and *Salmonella* before UV light treatment were on average 7.33 ± 0.06, 7.90 ± 0.05 and 7.40 ± 0.05-log₁₀ CFU ml⁻¹, respectively. The inactivation kinetics were characterized as having an initial rapid decline in bacterial levels followed by tailing at longer treatment. A bacterial inactivation of 5.18 ± 0.03, 5.03 ± 0.02 and 5.42 ± 0.09-log₁₀ cycles reduction were achieved after UV light treatment at D values 0.40, 0.44 and 0.43 mJ/cm² in all tested bacteria with no significant (P>0.05) difference in the log count reduction of the test bacteria being recorded (Figures A 3.1, 3.2 and 3.3 in the Appendix). However, there were significantly different in the microbial inactivation between control and UV light treated tiger nuts milk.

Table 3.11 - Effect of Collimated Beam Unit Treatment on Inactivation Kinetic of UV Treated Tiger Nut Milk

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>D value mJ/cm²</th>
<th>Initial</th>
<th>Log cfu/ml</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria innocua</em></td>
<td>0.40</td>
<td>7.33 ± 0.06</td>
<td>2.15 ± 0.03</td>
<td>5.18 ± 0.03</td>
</tr>
<tr>
<td><em>E. coli P36</em></td>
<td>0.44</td>
<td>7.90 ± 0.05</td>
<td>2.87± 0.02</td>
<td>5.03± 0.02</td>
</tr>
<tr>
<td><em>Salmonella WG49</em></td>
<td>0.43</td>
<td>7.40 ± 0.05</td>
<td>1.98± 0.09</td>
<td>5.42± 0.09</td>
</tr>
</tbody>
</table>

*Values are the Mean ± SD of three replicated, LCR = Log Count Reduction. CFU= Colony Forming Unit. Identical superscripts in the same column indicate non-significantly different at the 95% confidential level
3.3.2. Dean Flow Effects on Inactivation Kinetic of Surrogate Organisms in UV Treated Tiger Nut Milk

The initial population of *Listeria, E. coli* and *Salmonella* before UV light treatment were in average $7.08 \pm 0.01$, $7.04 \pm 0.02$ and $7.24 \pm 0.01$-log$_{10}$ CFU mL$^{-1}$, respectively (Table 3.12.). The inactivation kinetics were characterized as having an initial rapid decline in bacteria levels followed by tailing at longer treatment (Figure 3.13). A bacterial inactivation of $5.74 \pm 0.1$, $5.53 \pm 0.4$ and $6.06 \pm 0.02$-log$_{10}$ cycles reduction were achieved after UV light treatment at D values 3.02, 3.3 and 3.2 mJ/cm$^2$ in all tested bacteria with no significant (P>0.05) difference in the log count reduction of the test bacteria being recorded (Figures A 3.4, 3.5 and 3.6 in the appendix). However, there were significantly different in the microbial inactivation between control and UV light treated tiger nuts milk.

Table 3.12 - Effect of Dean Flow Reactor Treatment on Inactivation Kinetic of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Bacteria (18.4mJ/cm$^2$)</th>
<th>D value mJ/cm$^2$</th>
<th>initial</th>
<th>Log cfu/ml</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria innocua</em></td>
<td>3.02</td>
<td>7.08 ± 0.01$^A$</td>
<td>1.34 ± 0.1</td>
<td>5.74 ± 0.1$^B$</td>
</tr>
<tr>
<td><em>E. coli P36</em></td>
<td>3.3</td>
<td>7.04 ± 0.02$^A$</td>
<td>1.51 ± 0.4</td>
<td>5.53 ± 0.4$^B$</td>
</tr>
<tr>
<td><em>Salmonella WG49</em></td>
<td>3.2</td>
<td>7.24 ± 0.01$^A$</td>
<td>1.18 ± 0.02</td>
<td>6.06 ± 0.02$^B$</td>
</tr>
</tbody>
</table>

*Values are the Mean ± SD of three replicated, LCR = Log Count Reduction. CFU= Colony Forming Unit. Identical superscripts in the same column indicate non-significantly different at the 95% confidential level.

Figure 3.13 - Effects of UV-C Light Treatment on Microbial Inactivation Kinetic
3.3.3. UV Light Effects on Physicochemical Properties of Tiger Nut Milk

UV light treatment of tiger nut milk was at UV dose 18.4 mJ/cm² represented in (Table 3.13). pH, total soluble solids, conductivity values and protein content did not show significant differences (p>0.05) after the exposure to UV-C light treatment of tiger nuts milk at 18.4 mJ/cm². There were significantly losses in the titratable acidity of tiger nuts milk after UV-C light treatment from 22.5% to 17.6 %. Moreover, there were significant increase in the viscosity of treated tiger nuts milk from (10.4 to 12.1 cP).

Table 3.13 - Effects of UV-C Treatment on Physicochemical Properties of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control sample</th>
<th>*UV-C treated sample (18.4 mJ/cm²)</th>
<th>% RC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5 ± 0.01 A</td>
<td>6.7± 0.01 A</td>
<td>103</td>
</tr>
<tr>
<td>*TSS (Brix)</td>
<td>4.5 ± 0.04 A</td>
<td>4.5 ± 0 A</td>
<td>100</td>
</tr>
<tr>
<td>TA (%)</td>
<td>22.5 ± 0.01 A</td>
<td>17.6 ± 0.1 B</td>
<td>78.2</td>
</tr>
<tr>
<td>Conductivity (ms/cm³)</td>
<td>3.2 ± 0.03 A</td>
<td>3.3 ± 0.01 A</td>
<td>103</td>
</tr>
<tr>
<td>Viscosity (cP*)</td>
<td>10.4 ± 0.01 A</td>
<td>12.1 ± 0.2 B</td>
<td>116.3</td>
</tr>
<tr>
<td>Protein (mg/L)</td>
<td>5.53± 0.2 A</td>
<td>5.28 ± 0.03 B A</td>
<td>95.4</td>
</tr>
</tbody>
</table>

* Values are the Mean ± SD of three replicated TSS Total Soluble Solids, cP A centipoise. Identical superscripts in the same row indicate non-significantly different at the 95% confidential level.

3.3.4. UV Light Effects on Nutrient Quality and Enzymes Activity of Tiger Nut Milk

There was no significant loss of vitamin C content (8.22-7.84 mg/100mL) and phenolic. Moreover, there was a significant increase in the antioxidant (DPPH), and no significant (ORAC) of UV treated tiger nuts milk with 117% and 103 %, respectively (Table 3.14).
The activity of peroxidase and polyphenoloxidase significantly decreased in treated tiger nuts milk beverage, in comparison to the values observed in the control sample \( (p<0.05) \) from 0.2 to 0.1 Unit/mL and 0.4 to 0.1 Unit/mL, respectively. *(Figure 3.14)* showed the residual content of POD and PPO after UV-C light treatment.

**Tables 3.14 - Effect of UV-C Light Treatment on Nutrient Quality of Tiger Nut Milk**

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control sample</th>
<th>*UV-C treated sample (18.4 mJ/cm²)</th>
<th>% RC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C mg/100mL</td>
<td>8.22 ± 0.02A</td>
<td>7.84 ± 0.3A</td>
<td>95.3</td>
</tr>
<tr>
<td>TPC (mg GAE/100 mL)*</td>
<td>139.14 ± 2.02A</td>
<td>137.4 ± 1.52A</td>
<td>98.7</td>
</tr>
<tr>
<td>DPPH *</td>
<td>533.71 ± 2.4A</td>
<td>626.8± 29.3B</td>
<td>117</td>
</tr>
<tr>
<td>ORAC *</td>
<td>1.21 ± 0.1A</td>
<td>1.25 ± 1.06A</td>
<td>103</td>
</tr>
<tr>
<td>Peroxidase (Unit/mL)</td>
<td>0.2 ± 0.01A</td>
<td>0.1 ± 0.01B</td>
<td>50</td>
</tr>
<tr>
<td>Polyphenol oxidase (Unit/mL)</td>
<td>0.4 ± 0.05A</td>
<td>0.1 ± 0.01B</td>
<td>25</td>
</tr>
</tbody>
</table>

* Values are the Mean ± SD of three replicated TPC = Total Phenolic Compounds. RC = Residual content. Identical superscripts in the same column indicate non-significantly different at the 95% confidential level. DPPH expressed as µmol L⁻¹ Trolox equivalent (TE) mL¹. ORAC expressed as Trolox Equivalent mM.
3.3.5. Effects of UV-C Treatment on The Color Characteristics of Tiger Nut Milk

The ΔE values, which are an indicator of total color difference, showed that there were no significant differences (p>0.05) in color between untreated and treated samples, samples preserve their bright (higher L* values), and less red (lower a* values) after UV-C light treatment (Figure 3.15).

Table 3.15 - Effect of UV-C Light Treatment on The Color Characteristic of Tiger Nut Milk

<table>
<thead>
<tr>
<th>Properties</th>
<th>*Control sample</th>
<th>*UV-C treated sample (18.4 mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color L*</td>
<td>72.5 ± 0.02 A</td>
<td>71.97 ± 0.1 A</td>
</tr>
<tr>
<td>a*</td>
<td>-0.16 ± 0.02 A</td>
<td>-0.10 ± 0.02 A</td>
</tr>
<tr>
<td>b*</td>
<td>11.67 ± 0.3A</td>
<td>11 ± 0.3A</td>
</tr>
<tr>
<td>ΔE*</td>
<td>0.85 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

L* indicates lightness, a* is the red/green coordinate, b* is the yellow/blue coordinate, ΔE is the total color difference.
3.3.6. Shelf-life Study

The results of effects of UV light treatment on natural micro-flora and pathogenic organisms such, as *E. coli* P36 and *Listeria innocua* ATCC 51742 of UV light treated tiger nut drink samples at 18.4mJ/cm² during 5days’ storage at refrigerator temperature 4ºC summarized in (Table 3.16).

Initial count of *total aerobic count*, *E. coli* P36 and *Listeria innocua* at the beginning of experiments (day 0) in average 5.20 ± 0.04 7.15 ± 0.1 and 7.02 ± 0.03-log_{10} CFU mL⁻¹, respectively. The UV treatment only decreased *TAC*, *E. coli* and *Listeria* level by 3.5 ± 0.2, 5.06 ± 0.1 and 5.43 ± 0.05-log reduction, respectively. *E. coli* and *Listeria* levels decreased to 2.60 ± 0.4 and 2.77 ± 0.3-log reduction during the 5 days’ storage period. In similar, UV treatment with 1.5% H₂O₂ decreased *TAC*, *E. coli* and *Listeria* level by 5.06 ± 0.5, 6.01 ± 0.03 and 6.36 ± 0.1-log reduction, respectively.

![Image](75x452 to 550x720)

**Figure 3.15 - UV-C Light Treatment Effects on The Color Properties of Tiger Nut Milk**
"E. coli" and "Listeria" levels decreased to $4.54 \pm 0.7$ and $3.66 \pm 0.2$-log reduction during the 5 days’ storage period. In inoculated but non-UV treated controls there was no significant difference in "E. coli" or "Listeria" levels following the 5 days’ storage period. Inclusion of H$_2$O$_2$ (1.5%) in tiger nut milk neither affected the efficacy of UV, or the levels of "E. coli" or "Listeria" at the end of storage at 4°C (Table 3.16). Finally, 5-log$_{10}$ reduction as recommended in pasteurized products could not be accomplished within the investigated ranges of UV treatment and the microbial inactivation achieved by UV light treatment was not an effective treatment to the preservation of freshness attributes tiger nuts milk.

Table 3.16 - Effect of UVC Light Treatment on Natural Micro-Flora and Surrogate Organisms of UV-C Treated Tiger Nut Milk Storage 5 Days

<table>
<thead>
<tr>
<th>Storage Day</th>
<th>TAC*</th>
<th>E. coli P36</th>
<th>Listeria innocua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TAC*</td>
<td>E. coli P36</td>
<td>Listeria innocua</td>
</tr>
<tr>
<td></td>
<td>LCR*</td>
<td>LCR*</td>
<td>LCR*</td>
</tr>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.20 ± 0.04</td>
<td>7.15 ± 0.1</td>
<td>7.02 ± 0.03</td>
</tr>
<tr>
<td>UV only</td>
<td>3.50 ± 0.2</td>
<td>5.06 ± 0.1*</td>
<td>5.43 ± 0.05*</td>
</tr>
<tr>
<td>UV + 0.5% H$_2$O$_2$*</td>
<td>4.34 ± 0.4</td>
<td>5.41 ± 0.01*</td>
<td>5.85 ± 0.02*</td>
</tr>
<tr>
<td>UV + 1% H$_2$O$_2$</td>
<td>4.86 ± 0.1</td>
<td>5.93 ± 0.04*</td>
<td>6.22 ± 0.03*</td>
</tr>
<tr>
<td>UV + 1.5% H$_2$O$_2$</td>
<td>5.06 ± 0.5*</td>
<td>6.01 ± 0.03*</td>
<td>6.36 ± 0.1*</td>
</tr>
<tr>
<td>Day 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.01 ± 0.03</td>
<td>6.64 ± 0.2</td>
<td>6.21 ± 0.01</td>
</tr>
<tr>
<td>UV only</td>
<td>3.12 ± 0.1</td>
<td>2.60 ± 0.4</td>
<td>2.77 ± 0.3</td>
</tr>
<tr>
<td>UV + 0.5% H$_2$O$_2$</td>
<td>2.97 ± 0.02</td>
<td>2.75 ± 0.2</td>
<td>2.45 ± 0.05</td>
</tr>
<tr>
<td>UV + 1% H$_2$O$_2$</td>
<td>2.53 ± 0.3</td>
<td>3.28 ± 0.8</td>
<td>2.78 ± 0.3</td>
</tr>
<tr>
<td>UV + 1.5% H$_2$O$_2$</td>
<td>1.88 ± 0.1</td>
<td>4.54 ± 0.7</td>
<td>3.66 ± 0.2</td>
</tr>
</tbody>
</table>

TAC Total aerobic count, LCR Log Count Reduction, * more than 5-log cycles reduction and _ less than 5-log cycles reduction
Chapter IV

4. Discussion

The objectives of the current study were to compare the effects of thermal treatment at 60ºC for 30 min, HHP treatment at 600 MPa for 180s and UV-C at 254 nm and at UV-C dose of 18.4 mJ/cm² dependent on achieving kinetic inactivation of pathogen and surrogate organisms, preservation of physicochemical properties, color characteristics, and shelf-life extension of the tiger nut milk beverage.

4.1. Effect of Treatments on Inactivation of Surrogate Organisms

Effectiveness of thermal, HHP and UV-C light treatments were evaluated with regards to inactivating microbes (*Listeria innocua* ATTC 51742, *E. coli* P36 and *Salmonella typhimurium* WG49) introduced into the tiger nut beverage (*Figure 4.1*).

Thermal treatment performed at 60ºC for 30 min was sufficient to achieve 5-log reduction. The D₆₀ values for all the test bacteria was 12 min. Gabriel and Arellano (2014), who studied the effect of thermal treatment on *E. coli* O157:H7, *S. spp.*, and *L. monocytogenes* in young coconut liquid endosperm. Their results indicated that *E. coli* O157:H7 was more sensitive to heat treatment, which resulted in less significant heat resistance than inactivation of *S. enterica* and *L. innocua* with a D₅₅-ºC value for 7 min. Both were more heat resistant and *L. monocytogenes* exhibited the greatest increase in D₅₅-ºC value from 7.47 to 18.90 min. Tarazona-Diaz, and Aguayo’s (2013), who conducted a thermal treatment at 87.7ºC for 20s on watermelon juice. Results showed that thermal treatment was an effective process to achieve 5-log reduction in *E. coli*, *Salmonella* spp. and *L. monocytogenes*.
Similarly, Gabriel, Cruz and Guzman (2009) conducted a study about thermal death time of *E. coli* (American Type Culture Collection 25922) in young coconut endosperm drink. Results showed that thermal treatment at 60, 70 and 80°C for different times resulted in a significantly (P <0.05) decreased *E. coli* population with increasing exposure time and temperature of the coconut beverage. D-values ranged from 0.26 - 0.01 to 0.56-0.08 min.

High Pressure Processing more than 500 MPa for more than 60s supported a >5-log CFU. The inactivation curves follow diphasic behaviour with an initial rapid decrease followed by tailing. Patterson, McKay, Connolly and Linton (2012) reported that HHP treatment at 500 MPa and 600 MPa (1 min/20°C) reduced the total counts by 4-log CFU/mL and there was very little growth of the survivors during storage for up to 22 days at refrigerator temperature. Results showed that the inoculated carrot juice with *L. monocytogenes* and pressure treated (500 MPa/1 min/20°C) achieved >6-log inactivation. Moreover, Lukas (2013), who conducted a study with HPP (400, 500, or 600 MPa) for 120s to evaluate its effect on inactivation of three bacterial species (*E. coli* O157:H7, *Salmonella enterica* serovars Typhimurium, and *Listeria monocytogenes*). Results indicated that both high pressure treatment at 500 MPa and 600 MPa for 120s in coconut water achieved at least 6-log$_{10}$ CFU/mL reductions for all tested bacteria (*E. coli* O157:H7, *Salmonella enterica* serovars Typhimurium, and *Listeria monocytogenes*). Tailing effect could be decreased by inclusion of LEA. Tola and Ramaswamy (2014b) investigated effects of high pressure treatment 400-600 MPa with heat treatment at 40 - 60°C for 0 – 40 min and LAE on *Bacillus licheniformis* in carrot juice. The result reported that the pH reduction of low-acid foods showed a significant enhancement of rate of destruction of *B. licheniformis* spores with using LAE.
The D values for the UV inactivation of all test bacteria in tiger nut milk varied between 0.4 – 0.44 mJ/cm². The D values obtained for the various microbes in saline were used to determine the inactivation kinetics in the more complex tiger nut milk matrix. It was assumed that the D values (UV dose to support a 1-logreduction) for the inactivation of microbes in tiger nut milk were more than D value in saline. Consequently, the tiger nut milk promotes the protection for the microbial cell against the UV penetration. On the other hand, Matak et al. (2005) demonstrated that the lethal impact of UV photon would be affected by the milk matrix.

![Microbial inactivation](image)

**Figure 4.1 - Effect of Treatments on Microbial Content of Tiger Nut Milk**

### 4.2. Effect of Treatments on Physicochemical Properties of Tiger Nut Milk

There are slight deviations between the values established in this study and previously reported values, which can be attributed to the fact that the composition of tiger nuts varies according to the environment in which they are cultivated. The study was conducted to determine the effects of thermal, HHP, and UV-C light treatments on physicochemical properties of tiger nut milk, as expressed in Figure (4.2).
Thermal treatment significantly decreased the viscosity of the tiger nut milk. On the other hand, thermal treatment increased titratable acidity and conductivity of tiger nut milk. These results are in agreement with Fontan et al. (2009) who reported that increased temperature caused a significant decrease in the viscosity values of coconut milk and significant increase in the conductivity. Vandresen, Quadri, Souza and Hotza (2009) showed that pasteurization of carrot juice at 80°C caused significant decrease in the viscosity. Moreover, Rivas et al. (2006) studied changes following pasteurization and pulsed electric field (PEF) processing of orange and carrot juice mixtures. An increase in the acidity and soluble solids was observed. Jaskari et al. (1995) reported that the viscosity of oat bran changed after thermal treatment when starch is completely degraded to water-soluble oligosaccharides and with increasing α amylase activity.

In the current study, the viscosity of tiger nut milk increased during pressure treatment and stabilized the beverage from separating. This finding is in agreement with that of Zhou et al. (2009) who found a significant increase in the viscosity of carrot juice after high pressure treatment. Indeed, a positive correlation was found between viscosity and HPP for many of the fruit and vegetable products studied. This result contrasted that of Riahi and Ramaswamy (2003), who determined that high pressure inactivation of pectin methylestrase (PME) impacted the viscosity and texture of fruit products. These researchers reported that decreased PME activity in apple juice via high pressure treatment at 400 MPa for 25 min produced a lower viscosity. Thus, the viscosity of juices increases as the high pressure treatment increases.

In the current study, UV treatment resulted in an increase in viscosity of tiger nut milk. This result is in agreement with Zhang et al. (2011), who stated that UV treatment at UV dose of 9685 J/L increased viscosity of watermelon juice in comparison to the untreated samples.
Their results reported that the UV-C treatments not only inactivate PME, but also inactivate the polygalacturonase, cellulase, xylanase, b-D-galactosidase, and protease that respond to the dynamic viscosity of the juice. Koutchma et al. (2016) stated that viscosity or absorption coefficient values play an important role in UV dose delivery for applied and absorbed UV doses. However, Koutchma et al. (2016) also mentioned that many studies did not determine such properties of juices; the current study sought to fill this gap by determining the viscosity of tiger nut milk after UV treatment.

![Physicochemical properties](image)

**Figure 4.2 - Effect of Treatments on Physicochemical Properties of Tiger Nut Milk**

4.3. Effect of Treatment on Nutrients Quality and Enzymes Activity

Effects of thermal and non-thermal treatment on the nutrient quality and enzyme activity of tiger nut milk are shown in **Figure (4.3)**. The following section discusses the effect of the three experimental treatments on the protein, vitamin C, total phenolic content and antioxidant of tiger nut milk.
Thermal and HHP treatment did reduce the protein, vitamin C, and anti-oxidants content of tiger nut milk. Additionally, thermal treatment caused significant decrease in the TPC, but HHP did not impact on it. These results are similar of the results by Tangsuphoom and Coupland (2009) studied the effect of thermal treatments at 90°C and 120°C for 1 h on the properties of coconut milk emulsions prepared with surface-active stabilizers. They concluded that TP at 90°C or 120°C for 1 h caused denaturation and subsequent aggregation of coconut protein. Bártová and Bártá (2008), who determined the effect of heat treatment on the re-solubility of potato proteins isolated from industrial potato fruit juice and exposed to temperatures ranging from 25°C to 70°C. Results indicated that protein was thermal-sensitive at temperatures greater than 30°C, and that temperatures greater than 45°C caused denaturation and insolubility in the protease inhibitors. Moreover, Zhang, Zhang, Sheng, Wang and Fu (2016) reported that high pressure treatment at ≥500 MPa causes denaturation in the almond protein content and caused damage to lysine, arginine, and cysteine residues. It can also change protein digestibility to some degree. Tola and Ramaswamy (2014a), who studied the impact of TP and HPP on the stability of vitamin C and lycopene in watermelon juice. Juice was exposed to various thermal (70 to 90°C) and high pressure (400 to 600 MPa) treatments; results showed that the degradation rates of both components were higher when exposed to the thermal vs. the high pressure treatment due to its instability to heat, light, metal catalysts, oxygen, and its relatively high water solubility. Wolbang, Fitos and Treeby (2008), who demonstrated the effect of high pressure treatment at 600 MPa for 10 min on watermelon juice. Result showed that there was a significant loss in vitamin C content.

The antioxidant activity and total phenols in carrot juice were significantly decreased after the thermal treatment. Additionally, phenolic content was not significantly affected after high pressure treatment of carrot juice (Zhang et al., 2016).
Patras et al. (2009) reported that high pressure treatment of carrot puree at (400-600 MPa, 15 min, 10-30°C) had no impact on phenolic content and led to significant reductions in antioxidant capacity.

In the present study, there was no change in the vitamin C. This result was not expected because Koutchma et al. (2016) stated that water-soluble vitamins are light-sensitive, which was considered a UV light absorbance at the maximum germicidal wave length 260 nm, but it did not absorb light above 300 nm. Likewise, there was a significant increase in the antioxidant treatment by UV. This result was expected and in agreement with Alothman, Bhat and Karim (2009) reported that UV treatment of fresh cut tropical fruits caused significant increase in the antioxidant activity. This result could be attributed due to the oxidation that might have occurred during treatment of some antioxidant groups that contribute greatly to the antioxidant capacity of fruits.

In the current study, enzyme activities after thermal, HHP and UV-C treatment of tiger nut milk decreased significantly. Limited and controversial information are available in the literature regarding the effect of non-thermal technology on the enzyme activity associated with fresh juices, including peroxidase and polyphenol oxidase (Koutchma et al., 2016). POD and PPO are the key enzymes involved in the enzymatic browning of fruits and vegetables (Park et al., 2002). These results fall within the range of data reported by Tan, Cheng, Bhat, Rusul and Easa (2014), who observed the effect of thermal treatment of POD and PPO in coconut water (80-95°C, 15 min). Their results showed that thermal treatment achieved a significant inactivation in PPO, and reported remaining enzyme activities of 45% and a complete inactivation of POD activity at 90°C and holding time 2.5 min.

Park et al. (2002) found that the activity of PPO decreased with increasing pressure values in carrot juice; however, at pressure 400 MPa or lower, a full inactivation of PPO was not observed.
Corrales et al. (2012), who found that peroxidase activity decreased according to an increase in shortwave UV-C dose and that 14% residual activity was recovered after UV treatment of tiger nut milk at a flow rate of 2.35mWcm$^{-2}$.

**Figure 4.3 - Effect of Treatments on Nutrient Contents of Tiger Nut Milk**

**Figure 4.4 - Effect of Treatments on Enzymes Activity of Tiger Nut Milk**
4.4. Effect of Treatment on Color Characteristics

Thermal treatment resulted in a darkening/browning of tiger nut milk. Ames and Hofmann (2001) studied the effect of thermal treatment at (109.3, 115.6 and 121.1°C for 5 min) on coconut milk, result showed that thermal treatment had significant impact on it color toward browning. Therefore, there was longer period of time to permit the browning reaction to occur. This resulted in the significant reduction in L* and b*values. HHP and UV-C treatments had no significant effect on the color compared to thermal. Zhang et al. (2011) evaluated the effect of thermal treatment (60°C for 5, 20, 40, and 60 min) and HP (300, 600, and 900 MPa/60°C/5, 20, 40, and 60 min) on the color of watermelon juice. They found that the browning degree of the watermelon juice subjected to HP treatment was lower than that of the juice subjected to thermal treatment.

These results contradict the results of (Hu et al., 2012; patars et al., 2009), who showed that high pressure treatment had significant change in the color of watermelon and carrot juices. Corrales et al. (2012), who demonstrated that UV-C treatment of tiger nut milk had no significant impact on its color characteristics. These researchers found that there was a slight increase in a* and a slight decrease in L* values; whereas, b* values did not change. Consequently, there was not considerable changing. The study by with Ochoa-Velasco, Cruz-González and Guerrero-Beltrán (2014) found slight increases in the color parameters of coconut milk with increasing UV-C dose; specifically, the b* value increased (yellow tendency) after UV-C light treatment). Koutchma et al. (2016) reported a correlation between color lightness and the absorption coefficient in UV-treated juice. Those results point out the high stability of food matrices of vegetable origin during HHP and UV-C treatments.
4.5. Effect of Treatment on Shelf-life Extension

Despite HHP decreasing levels of target bacteria, there was regrowth during storage. There was no shelf life extension by applying HHP or inclusion of LEA. High pressure treatment (500–600 MPa) of carrot juice reduced the total counts by 4-log cycles and only slight growth of the survivors was observed during storage at 4°C up to 22 days. The total counts increased during storage of the product at 8°C and 12°C, but it took significantly longer to reach maximum levels as compared to the untreated juice (Patterson et al., 2012). These samples had better firmness, texture, and histological structure of frozen carrots than the ordinary frozen samples.

Hydrogen peroxide and UV enhanced the lethal effects through an Advanced Oxidative Process by producing hydroxyl radicals, which are stronger oxidizing agents (Nederhoff, 2000; Runia & Boonstra, 2004). Waites et al. (1988) reported that combination of UV irradiation and H₂O₂ increased the inactivation efficacies of microorganism.
Similarly, Mc Donald et al. (2000) mentioned that combination of 1% hydrogen peroxide and UV treatment of 40 J/m² achieved 4-log reduction of spores, and 0.1% hydrogen peroxide with 270 J/m² achieved 7.5 log reduction of *Bacillus megaterium* spores.

**Table 4.1 - Effects of thermal, HHP and UV-C light treatments on microbiological stability, quality attributes and shelf-life of tiger nut milk**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Thermal Conditions</th>
<th>HHP Conditions</th>
<th>UV-C Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(60°C/30 min)</td>
<td>(600 MPa/180s)</td>
<td>(18.4 mJ/cm²)</td>
</tr>
<tr>
<td>Most resistant organism</td>
<td>Listeria innocua</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.5 ± 0.01</td>
<td>6.5 ± 0.01</td>
<td>6.5 ± 0.01</td>
<td>6.7 ± 0.01</td>
</tr>
<tr>
<td>Total Soluble Solids</td>
<td>4.5 ± 0.04</td>
<td>4.5 ± 0</td>
<td>4.5 ± 0.04</td>
<td>4.5 ± 0</td>
</tr>
<tr>
<td>Conductivity</td>
<td>3.2 ± 0.03</td>
<td>4.1 ± 0.01*</td>
<td>2.3 ± 0.01*</td>
<td>3.3 ± 0.01</td>
</tr>
<tr>
<td>Titratable acidity</td>
<td>22.5 ± 0.01</td>
<td>25 ± 1*</td>
<td>22.5 ± 0.01</td>
<td>17.6 ± 0.1*</td>
</tr>
<tr>
<td>Viscosity</td>
<td>10.4 ± 0.01</td>
<td>9.3 ± 0.1</td>
<td>12.2 ± 0.1*</td>
<td>12.1 ± 0.2*</td>
</tr>
<tr>
<td>Protein</td>
<td>5.53 ± 0.2</td>
<td>3.6 ± 0.02*</td>
<td>4.06 ± 0.1*</td>
<td>5.28 ± 0.03</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>8.22 ± 0.02</td>
<td>5.99 ± 0.4*</td>
<td>5.93 ± 0.02*</td>
<td>7.84 ± 0.33</td>
</tr>
<tr>
<td>Total Phenolic</td>
<td>139.14 ± 2.0</td>
<td>99.17 ± 6.9*</td>
<td>120.89 ± 6.1</td>
<td>137.4 ± 1.52</td>
</tr>
<tr>
<td>Antioxidants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>533.7 ± 2.4</td>
<td>361.4 ± 0.1*</td>
<td>471.65 ± 2.7</td>
<td>626.8 ± 29.3*</td>
</tr>
<tr>
<td>ORAC</td>
<td>1.21 ± 0.1</td>
<td>0.76 ± 0.4*</td>
<td>0.89 ± 0.3*</td>
<td>1.25 ± 1.06</td>
</tr>
<tr>
<td>Enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPO</td>
<td>0.2 ± 0.01</td>
<td>0.08 ± 0.01*</td>
<td>0.1 ± 0.05*</td>
<td>0.1 ± 0.01*</td>
</tr>
<tr>
<td>PPO</td>
<td>0.4 ± 0.05</td>
<td>0.07 ± 0.05*</td>
<td>0.1 ± 0.01*</td>
<td>0.1 ± 0.01*</td>
</tr>
<tr>
<td>Color (AE)</td>
<td>6.5*</td>
<td>2.7</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Shelf-life (Days)</td>
<td>3-2</td>
<td>ND*</td>
<td>5</td>
<td>3-2</td>
</tr>
</tbody>
</table>

* Significant differences (P<0.05) in compare with the control samples.
Chapter V

5. Conclusion

There is growing consumer demand for plant derived beverages because they are considered as high nutritive value beverages. Consumer perception of food quality depends not only on microbial safety, but also on other internal characteristics of food, such as nutrients content, biochemical and enzymatic reactions and structural changes.

In this research study, tiger nut milk was subjected to thermal and non-thermal technologies, such as high hydrostatic pressure and ultraviolet light processing to demonstrate the effectiveness of these methods at improving microbial decontamination and achieving a 5-log reduction of pathogenic organisms, assess and preserve quality attributes, and shelf-life extension of tiger nut milk. The study concluded that short UV-C light treatment at a dose of 18.4 mJ/cm² could ensure the commercialization of a sanitized product with quality attributes similar to fresh tiger nut milk. Our results suggest that UV-C light treatment is an effective method in terms of microbial inactivation, having inactivated ≥ 5-log_{10} CFU of vegetative bacteria in a short exposure time compared with thermal and HHP treatments. This microbial inactivation was primarily attributed to the effect of UV-C light on tiger nut milk.

Furthermore, UV-C treatment is the effective non-thermal technology in term of physicochemical properties and quality attributes preservation. UV-V light treatment had slightly effects on physicochemical properties, which decreased titratable acidity at residual content 77.7%, increased conductivity and viscosity at residual contents 103.1% and 116.3%, respectively, preserved vitamin C content and total phenolic compounds at residual contents 95.3% and 98.7%, respectively which were closed to fresh tiger nut milk. Likewise, UV-C light treatment had significant effects on peroxidase and polyphenol oxidase inactivation at residual content of 50% and 25%, respectively.
In terms of shelf-life extension, HHP treatment was an effective method to extend shelf life of tiger nut milk for 5 days; likewise, UV-C light treatment was not able to extend tiger nut milk shelf-life. Based on the previous findings, the specific conclusions that can be drawn from the study are:

1- Thermal treatment is an effective method to provide a highly microbial stable beverage and enzymes inactivation by 60% and 82.5% in peroxidase and polyphenol oxidase, respectively. However, it has significant effects on the quality attributes of tiger nut milk, which decreased protein, vitamin C and antioxidant by 35%, 30% and 37%, respectively, and increased titratable acidity and conductivity by 11.1% and 28.1%, respectively.

2- HHP treatment is an effective technology to achieve $5\log_{10}$ CFU for vegetative bacteria at 500 and 600 MPa for 180s. However, it has adverse effects on the nutrients content, which decreased protein, vitamin C and antioxidants at residual contents 73%, 72% and 73.5%, respectively.

3- HHP treatment at 600 MPa/180s is not an effective method in shelf-life extension of tiger nut milk; however, it extends the microbial stability for 5 days; moreover, inclusion of LAE in tiger nut milk neither affected the efficacy of HHP or the levels of microbes at the end of storage at 4°C.

4- UV-C treatment is confirmed as technology to reduce microbial loads in a natural tiger nuts' milk. We proved that UV-C is a suitable and a promising minimal processing technology for tiger nut milk, inactivating efficiently the main contaminant microorganisms (E. coli P36, Salmonella WG49 and Listeria innocua), and thus preserving significantly the quality attributes.
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Appendix A

A.1. D values of thermal treatment

\[ y = -0.084x + 7.33 \]
\[ R^2 = 1 \]
\[ D = \frac{1}{-K} = \frac{1}{-0.084} = 11.9 \]

A.1.1- D value of *Listeria innocua* after thermal treatment
A.1.2- D value of *E. coli* P36 after thermal treatment

\[ y = -0.083x + 7.34 \]

\[ R^2 = 1 \]

\[ D = -\frac{1}{K} = -\frac{1}{-0.083} = 12 \]

A.1.3- D value of *Salmonella Typhimurium* WG49 after thermal treatment

\[ y = -0.084x + 7.4 \]

\[ R^2 = 1 \]

\[ D = -\frac{1}{K} = -\frac{1}{-0.084} = 11.9 \]
A.2. D values of HHP treatments

**Listeria innocua at 400 MPa**

\[ y = -0.018x + 7.48 \]
\[ R^2 = 0.955 \]
\[ D = -1/K = -1/0.018 = 55.5 \]

**Listeria Innocua at 500 MPa**

\[ y = -0.046x + 7.48 \]
\[ R^2 = 0.958 \]
\[ D = -1/K = -1/-0.046 = 21.7 \]

A.2.1- D value of *Listeria innocua* after HHP treatment at 400 MPa

A.2.2- D value of *Listeria innocua* after HHP treatment at 500 MPa
A.2.3- D value of *Listeria innocua* after HHP treatment at 600 MPa

\[ y = -0.0485x + 7.48 \]
\[ R^2 = 0.9846 \]
\[ D = \frac{-1}{K} = \frac{-1}{-0.048} = 20.8 \]

A.2.4- D value of *E. coli P36* after HHP treatment at 400 MPa

\[ y = -0.018x + 7.46 \]
\[ R^2 = 0.9701 \]
\[ D = \frac{-1}{K} = \frac{-1}{-0.018} = 55.5 \]
A.2.5- D value of *E. coli* P36 after HHP treatment at 500 MPa

\[ y = -0.0459x + 7.46 \]
\[ R^2 = 0.961 \]
\[ D = \frac{-1}{-0.045} = 22.2 \]

A.2.6- D value of *E. coli* P36 after HHP treatment at 600 MPa

\[ y = -0.060x + 7.46 \]
\[ R^2 = 0.986 \]
\[ D = \frac{-1}{-0.060} = 16.6 \]
A.2.7- D value of *Salmonella Typhimurium* WG49 after HHP treatment at 400 MPa

\[ y = -0.016x + 7.06 \]
\[ R^2 = 0.987 \]
\[ D = \frac{1}{-K} = \frac{1}{-0.016} = 62.5 \]

A.2.8- D value of *Salmonella Typhimurium* WG49 after HHP treatment at 500 MPa

\[ y = -0.041x + 7.06 \]
\[ R^2 = 0.995 \]
\[ D = \frac{1}{-K} = \frac{1}{-0.041} = 24.3 \]
A.2.9- D value of *Salmonella Typhimurium* WG49 after HHP treatment at 600 MPa

A.3. D values of UV-C light treatment

- Collimated Beam

A.3.1- D value of *Listeria innocua* after collimated beam unit treatment
A.3.2- D value of *E. coli P36* after collimated beam unit treatment

\[
y = -2.548x + 7.9 \\
R^2 = 0.998 \\
D = -1/K = -1/-2.548 = 0.44 \text{ mJ/cm}^2
\]

A.3.3- D value of *Salmonella Typhimurium WG49* after collimated beam unit treatment

\[
y = -2.291x + 7.4 \\
R^2 = 0.995 \\
D = -1/K = -1/-2.291 = 0.43 \text{ mJ/cm}^2
\]
A.3.4- D value of *Listeria innocua* after Dean flow reactor treatment

\[
y = -0.331x + 7.08 \\
R^2 = 0.977 \\
D = \frac{1}{K} = \frac{1}{-0.331} = 3.02 \text{ mJ/cm}^2
\]

A.3.5- D value of *E. coli P36* after Dean flow reactor treatment

\[
y = -0.299x + 7.04 \\
R^2 = 0.984 \\
D = \frac{1}{K} = \frac{1}{-0.299} = 3.3 \text{ mJ/cm}^2
\]
A.3.6- D value of *Salmonella Typhimurium* WG49 after Dean flow reactor treatment

\[ y = -0.308x + 7.24 \]

\[ R^2 = 0.951 \]

\[ D = \frac{-1}{-0.308} = 3.2 \, \text{mJ/cm}^2 \]