



**QUALITY, SAFETY AND FEASIBILITY STUDY OF HIGH-PRESSURE
PROCESSING ON COW MILK**

By

LIM SHU HUEY

**Thesis Submitted to the School of Graduate Studies,
Universiti Putra Malaysia, in Fulfilment of the
Requirements for the Degree of Doctor of Philosophy**

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January 2024

Chairman : Prof. Ir. Chin Nyuk Ling, PhD

Faculty : Engineering

There is an increasing demand for innovative processing technologies due to the growing concern on food safety and quality. High-pressure processing (HPP) has emerged as a promising method for preserving the nutritional value and safety of perishable foods. However, its application to cow milk and feasibility within milk processing facilities remain underexplored. Current approaches to milk preservation often involve heat treatment which might not be the best preservation method as it can compromise the sensory and nutritional attribute of final product. This study investigated HPP as a viable method for preserving cow milk, given its potential to maintain nutritional value and safety. HPP treatment at 600 MPa for 10 mins was compared to ultra-pasteurisation (ESL) treatment at 125°C for 4s in terms of microbiological, physicochemical, nutritional and organoleptic properties of cow milk. The cost and environmental impact assessment were conducted to evaluate its sustainability. HPP milk exhibits a confidently extended shelf life beyond 60 days, meeting microbial safety standards, while ESL milk faced contamination during

filling. HPP milk demonstrated higher physicochemical stability, maintaining consistent pH and undetectable acidity, and better retention of vitamins A and B5 (75% and 65% respectively) as compared to ESL milk after 60 days storage. Both HPP and ESL have comparable results for mineral stability. Sensory evaluation revealed 89.5% of panellists were able to differentiate HPP milk from ESL milk with majority (61%) preferring HPP milk for its superior colour, milkiness, mouthfeel and aftertaste attributes, except for creaminess which required improvement. A feasibility study assessed the economic and environmental impacts of HPP technology in milk processing. The capital cost for industrial-scale HPP was estimated at RM 6,470,000, three times higher than ESL treatment, primarily due to high HPP equipment costs. Both systems have comparable operational cost with HPP inducing higher electricity, labour and facility-dependent cost, while the ESL has higher utilities cost such as steam, cooling water and chilled water. Economic feasibility study showed both treatments are regarded acceptable investment with $NPV > 0$ and $IRR > 10\%$ although HPP has higher break-even quantity (1.9 million bottles) than ESL heat treatment (0.6 million bottles). Total electricity consumption was higher by 77.4% for the HPP (874,500 kWh/yr) than the ESL (500,500 kWh/yr) The working principle of HPP that uses electricity contributed to lower carbon emission (0.018 kg CO₂/L) as compared to ESL treatment (205.488 kg CO₂/L) that uses steam as source of energy. In conclusion, the aforementioned results suggested HPP treatment at 600MPa for 10 min could be used as an alternative to replace heat treatment for milk processing with lower risk on post contamination, longer shelf life, higher stability on physicochemical, nutritional and organoleptic properties with potentially lower carbon emission except that of a 3-fold higher capital cost. Future research should focus on studying the effect of HPP on vitamin and mineral contents due to limited literatures found to support the

finding. Additionally, further investigation on customer acceptance and market viability is recommended to facilitate the widespread application of HPP in dairy industry.

Keywords: cow milk, feasibility, high-pressure processing, quality, safety

SDG: GOAL 9: Industry, Innovation, and Infrastructure



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**KUALITI, KESELAMATAN DAN KEMAMPUAN PEMROSESAN
TEKANAN TINGGI TERHADAP SUSU LEMBU**

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Terdapat permintaan yang meningkat untuk teknologi pemprosesan inovatif disebabkan oleh kebimbangan yang semakin meningkat tentang keselamatan makanan dan kualiti. Pemprosesan tekanan tinggi (HPP) telah muncul sebagai kaedah yang menjanjikan untuk mengekalkan nilai pemakanan dan keselamatan makanan mudah rosak. Walau bagaimanapun, aplikasinya kepada susu lembu dan kelayakan di dalam kemudahan pemprosesan susu masih belum diterokai sepenuhnya. Pendekatan semasa untuk penyimpanan susu sering melibatkan rawatan panas yang mungkin bukan kaedah penyimpanan terbaik kerana ia boleh mengompromi atribut sensori dan pemakanan produk akhir. Kajian ini menyiasat HPP sebagai kaedah yang sesuai untuk menyimpan susu lembu, mengambil kira potensinya untuk mengekalkan nilai pemakanan dan keselamatan. Rawatan HPP pada 600 MPa selama 10 minit dibandingkan dengan rawatan ultra-pasteurisasi (ESL) pada 125°C selama 4s dari segi sifat mikrobiologi, fizikokimia, pemakanan, dan organoleptik susu lembu. Penilaian kos dan impak alam sekitar dijalankan untuk menilai kelestarian. Susu HPP menunjukkan jangka hayat simpanan yang melebihi 60 hari dengan keyakinan,

memenuhi standard keselamatan mikrob, manakala susu ESL menghadapi pencemaran semasa proses pengisian. Susu HPP menunjukkan kestabilan fizikokimia yang lebih tinggi, mengekalkan pH yang konsisten dan keasidan yang tidak dapat dikesan, serta pengekal vitamin A dan B5 (masing-masing 75% dan 65%) berbanding dengan susu ESL selepas penyimpanan selama 60 hari. Kedua-dua HPP dan ESL memberikan keputusan yang sebanding untuk kestabilan mineral. Penilaian sensori mendedahkan bahawa 89.5% panelis dapat membezakan susu HPP dari susu ESL dengan majoriti (61%) lebih menyukai susu HPP kerana sifat warna, kekekalan susu, rasa mulut, dan selepas rasa, kecuali kemanisan yang memerlukan peningkatan. Satu kajian kelayakan menilai impak ekonomi dan alam sekitar teknologi HPP dalam pemprosesan susu. Kos modal bagi HPP berskala industri dianggarkan sebanyak RM 6,470,000, tiga kali ganda lebih tinggi daripada rawatan ESL, terutamanya disebabkan oleh kos peralatan HPP yang tinggi. Kedua-dua sistem mempunyai kos operasi yang sebanding, dengan HPP menyebabkan kos elektrik, buruh, dan fasiliti yang lebih tinggi, manakala ESL mempunyai kos utiliti yang lebih tinggi seperti stim, air penyejukan, dan air sejuk. Kajian kelayakan ekonomi menunjukkan kedua-dua rawatan adalah pelaburan yang diterima dengan nilai sekarang bersih (NPV) > 0 dan kadar pulangan dalaman (IRR) $> 10\%$, walaupun HPP memerlukan kuantiti titik impas yang lebih tinggi. Penggunaan tenaga elektrik HPP adalah 77.4% lebih tinggi daripada ESL, tetapi bergantung kepada elektrik menyebabkan pelepasan karbon yang jauh lebih rendah berbanding dengan penggunaan stim ESL. Secara kesimpulannya, keputusan yang disebutkan di atas mencadangkan rawatan HPP pada 600MPa selama 10 minit boleh digunakan sebagai alternatif untuk menggantikan rawatan panas untuk pemprosesan susu dengan risiko pencemaran selepas, jangka hayat rak yang lebih panjang, kestabilan yang lebih tinggi pada sifat fizikokimia, pemakanan dan

organoleptik dengan potensi pelepasan karbon yang lebih rendah kecuali kos modal yang tiga kali ganda lebih tinggi. Kajian masa depan seharusnya memberi tumpuan kepada kajian kesan HPP terhadap kandungan vitamin dan mineral kerana terhadnya literatur yang dapat menyokong dapatan tersebut. Selain itu, penyelidikan lanjut mengenai penerimaan pelanggan dan kebolegunaan pasaran disyorkan untuk memudahkan penggunaan meluas HPP dalam industri tenusu.

Kata Kunci: susu lembu, kebolehlaksanaan, pemprosesan tekanan tinggi, kualiti, keselamatan

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LIST OF ABBREVIATIONS

HPP	High pressure processing
ESL	Extended shelf life
TPC	Total plate count
CFU	Colony-forming unit
HTST	High temperature short time
LTLT	Low temperature long time pasteurisation
UHT	Ultra high temperature
TS	Total Solids
SNF	Solids Non-Fat
JAR	Just-about-right
DC	Direct cost
IC	Indirect cost
NPV	Net Present Value
IRR	Internal Rate of Return

CHAPTER 1

INTRODUCTION

1.1 Background

Malaysia's dairy industry has developed continuously with steady growth rates over the past few years due to the fast-growing demand for fresh milk and dairy products. While Malaysia has set a goal of becoming 100% self-sufficient in fresh milk by 2025, official statistics show that the country's annual milk consumption has been steadily outpacing domestic production in recent years (Kana, 2021). This is due to the health benefits of milk that always associated with its compositions such as protein and calcium contents. Milk protein has been proven to give much benefits such as promote growth, increasing muscle strength, promote weight loss, lowering blood pressure and blood sugar (Kawase et al., 2000; Kimball & Jefferson, 2006; Karlsson et al., 2006; Pal & Ellis, 2010) while calcium is important for the homeostasis of both infants and adults, bone and tooth health as well as muscular and cardiac (Cashman, 2006; Haug et al., 2007). Beside protein and calcium, other components in milk also provide great health benefits. For example, milk fat acts as good source of bioactive lipid and functional components as sphingolipids which are responsible for cancer inhibition, antimicrobial and immuno-modulatory activities and inhibition of cholesterol adsorption (Ebringer et al., 2008); milk unsaturated fatty acid, FAs such as oleic acid (C18:1) can lower plasma cholesterol and reduces risk of coronary artery disease (Ebringer et al., 2008); vitamin A in milk is important for maintaining healthy teeth, skeletal and soft tissue, mucus membranes and skin prevent night blindness (Pereira, 2014). Milk is also proven to improve anthropometric indices and cognitive function

in undernourished children; diminish the prevalence of biochemical and functional nutritional deficiencies, lowering morbidity and death (Daphna and Lindsay, 2011).

The Malaysia government, dairy farmers and other industry stakeholders have made every effort to increase the local fresh milk production to overcome the high demand. However, the nature of milk with high nutritional and water content and near neutral pH provide an ideal environment for growth of microorganisms (Quigley et al., 2013). Lack of hygienic practices in pre-milking udder preparation, sub-optimal hygiene of milk handlers, and poor sanitation practices associated with milking and storage equipment greatly facilitate growth of microorganisms in milk (Garedew et al., 2012) and cause early spoilage (Vahedi et al., 2013). The most common disease-causing bacteria in the milk are the *Salmonella* sp., *Mycobacterium bovis*, *Corynebacterium* sp., *Clostridium perfringens*, *Yersinia enterocolitica*, *Coxiella burnetii*, *Brucella*, *Staphylococcus* sp., *Campylobacter jejuni*, *Mycobacterium avium*, *Listeria* sp., *Escherichia coli*, and coliforms (Fadaei, 2014). Consumption of raw milk contaminated with these bacteria can cause illness and in severe case, death (Vellathurai, 2010). Microorganisms degrade milk quality and its shelf life; for example, psychrotolerant bacteria can multiply under refrigeration and cause spoilage by producing extracellular lipases and proteases (Quigley et al., 2013). The bacteria also cause the unpleasant taste, change in physical properties, reduction of nutritional quality and threaten consumers' health (Vahedi et al., 2013). These undesirable effects have raised public health concern on consumption of raw milk (Fadaei, 2014). Hence, for safety purpose, milk is commonly treated and undergoes processes that inhibit these pathogenic microorganisms before consumption and not consumed raw.

In Malaysia's dairy industries, thermal treatment is the most extensively used processing technology with the goal to suppress microbial growth in milk, ensure safe consumption and extend shelf life (Chye et al., 2004; Nordin et al., 2019; Frost & Sullivan, 2021). Those common heat treatments of milk include high temperature short time (HTST) pasteurisation, low temperature long time (LTLT) pasteurisation, extended shelf-life (ESL) processing, ultra-high temperature (UHT) and in-container sterilisation (Bezie, 2019). Most of these thermal treatments have been proven by studies with high effectiveness in inhibiting pathogenic microorganisms and achieved extended shelf life. These treatments however cause detrimental effects to milk as reported such as protein denaturation and change in milk functionality (Raikos, 2010); vitamin loss (Macdonald et al., 2011); generation of volatile component and cooked flavor which affect consumer acceptability (Gandy et al., 2008). The extent of nutrient losses and degradation caused by thermal treatments become a concern as consumers are increasingly demanding high quality and minimally processed foods. This scenario has largely driven the interest in developing and implementing new technologies to produce microbiologically safe and high-quality foods (Salazar et al., 2021). For milk and dairy products processing, various non-thermal processing technologies such as pulsed electric fields (PEF), high pressure processing (HPP), ultrasonication, cold plasma, and others have been tested (Özer & Akdemir-Evrendilek, 2014). Amongst these emerging technologies, the high pressure processing (HPP) provides a higher reliability and feasibility in preservation of milk (McClements et al., 2001; Dogan & Erkmen, 2004; Bull et al., 2005; Janahar et al., 2021).

HPP is a food processing technology that employs very high pressure at room temperature for a short duration to eliminate pathogenic microorganisms and extend

shelf life of the products with minimal alteration on nutritional and sensorial attributes (Hogan et al., 2005; Barba et al., 2017; Huang et al., 2018; Shabbir et al., 2021). Researchers have hypothesised this non-thermal technology could be applied to milk and several studies on the effect of HPP on milk' microbial inactivation and physicochemical properties in lab scale conduction are available (García-Risco et al., 1998, Huppertz et al., 2006; Liu et al., 2020). Generally, studies claimed HPP ensured milk safety and quality by effectively eliminate pathogenic and harmful microorganisms (Stratakis et al., 2019), exhibited high physico-chemical stability (Tan et al., 2020) and improved organoleptic properties (García-Risco et al., 2000).

HPP has been explored and adopted in various food and beverage but application of this technology on milk might not been widely commercialised anywhere else in the world including Malaysia. There are challenges in implementation of HPP as it requires specific infrastructure and equipment. Local manufacturing industries with limited resources or less exposed to technology innovation might face challenges in implementing and integrating HPP into their milk processing facilities. The subsequent operational and maintenance cost of HPP can be expensive which adding burden to the milk manufacturers. The regulatory support or acceptance of HPP technology in the milk manufacturing industry is also crucial for development as milk and dairy products should follow strict regulation. According to Malaysia Food Regulation 1985, all milk and dairy products shall be undergo specific treatment such as pasteurisation, sterilisation or ultra-high temperature treatment, but shall not distributed as milk, raw milk or fresh milk (Food Regulation, 1985). This non-conducive regulatory environment further restrict the local industry players to adopt

HPP in milk processing. Despite, HPP is the new revolution in the dairy industry worldwide without a doubt.

Most studies on the effects of HPP on milk focussed microbiological and physicochemical properties without investigation on the nutritional contents of milk such as vitamins and minerals, and also their stability during storage. Thus, the primary aim of this research was to determine the effectiveness of the HPP process with comparisons to the thermal treatment for processing of milk at the industrial scale through evaluations on microbiological properties, physicochemical properties and nutritional quality including vitamin and mineral contents for an extended shelf life storage. Sensory evaluation was also included in this research to identify the consumers' acceptance on HPP. Subsequently, economic and environmental assessments were conducted to determine the cost efficiency and sustainability of the HPP system for the milk industry. The findings of this study are expected to give a considerable commercial impact on our local milk industry and the dairy business.

1.2 Problem statement

Thermal treatments such as HSTS, ESL and UHT are commonly used in dairy processing industry Malaysia to inhibit the pathogenic microorganisms in milk and achieved longer shelf-life, but these methods resulted loss in nutrition including vitamins and natural antioxidants (Macdonald et al., 2011; Claeys et al., 2013); loss of texture and freshness, denaturation of proteins (Raikos, 2010; Claeys et al., 2013), generation of volatile components (Jo et al., 2019) and strong cooked flavour (Barraquio, 2014) which affect some cluster consumers' overall acceptability (Gandy et al., 2008). Pasteurisation produce acceptable milk taste, however, the capability of

pasteurisation in extending the shelf-life of milk which held under proper refrigeration is short with range from 14 to 21 days post processing (Sperber & Doyle, 2009).

In recent years, consumers' demand for minimally processed and natural foods are also increasing, whilst some consumers prefer consuming raw milk for its claims on healthiness, natural and refreshing taste (Shabbir et al., 2021). In actual, raw milk might contains high dense of bacterial or pathogenic microorganisms that can resulted serious illness if the consumers mis-consumed the contaminated milk. This is proven by researcher in which majority milk samples collected from Malaysia local dairy farm did not meet standard requirement, reflecting poor hygiene practices and presence of contaminations (Chye et al., 2004; Khairunnisak et al., 2019). Therefore, raw milk consumption is not encouraged and fresh milk should be treated sufficiently to eliminate all pathogenic microorganisms.

HPP application has been widely used in preservation of milk studies due to the increasing demand on fresh and less processed milk (McClements et al., 2001; Dogan & Erkmén, 2004; Bull et al., 2005; Janahar et al., 2021). However, most of the previous studies were carried out using small unit model or small volume of milk samples at pilot plant or lab high-pressure machines. Goyal et al. (2021) categorised HPP units' production with capacity of 0.1 – 2 L as lab-scale, 10 – 25 L as pilot scale, while up to 100 L as commercial or industrial scale. Some researchers even conducted comparative studies between HPP and thermal treatments on microbiological quality (Stratakis et al., 2019; Liu et al., 2020); physicochemical changes (Liu et al., 2020); and sensory quality of milk (Liepa et al., 2017; Liu et al., 2020) but all were using lab scale HPP unit. Scaling-up of HPP treatment for milk preservation from lab scale to

pilot or industrial scale continue to be a challenge especially for local researchers due to expensive cost, huge panel size and heavy weight of equipment. There is also no comprehensive comparative study between thermally treated milk with HPP milk at commercial scale that presents all the important properties including microbiological, physicochemical, vitamins and mineral, consumer acceptance and storage shelf life all at once.

The aforementioned problems bring us to this study in which a more relevant scenario in the application of HPP for the milk processing industry was conducted. This study compares the effectiveness of industrial scale HPP (600 MPa, 10 min) over ESL heat treatment (125 °C, 4 s) on quality and safety of cow milk in terms of its microbiological properties, physicochemical properties and nutritional value over 60 days of storage. Sensory evaluation is also conducted to evaluate consumers' acceptance and preference. Upon evaluating the product, i.e. milk, the study continued with feasibility study of using the HPP system in the milk processing industry through economic and environmental assessments in comparison to the existing heat treatment used.

1.3 Research objectives

The main objective of this research is to investigate the application of high-pressure processing (HPP) in the dairy industry. The specific objectives of this research are:

- 1) to study the effectiveness of industrial scale HPP treatment on safety and quality of fresh cow milk in terms of microbiological properties, physicochemical properties and nutritional values in comparison with heat-treated milk for the extended 60-days storage period.

- 2) to evaluate and compare the organoleptic properties of HPP treated milk using sensory analysis via the triangle and acceptance with preferences tests with heat-treated milk.
- 3) to conduct a feasibility study of HPP technology for milk processing in the Malaysian dairy industry scenario using cost and environmental impact assessments.

This study hypothesised HPP-treated milk exhibited lower microbial counts compared to conventionally heat-treated milk over long storage duration due to the high efficiency of HPP in microbial inactivation. HPP-treated milk is believed to have higher level of key nutrients retention compared to conventionally heat-treated milk due to the milder processing conditions of HPP. In addition, HPP-treated milk should be able to exhibits superior sensory qualities compared to conventionally heat-treated milk due to the preservation of flavour compounds and textural characteristics by HPP treatment. In term of feasibility, implementation of HPP technology for milk processing in the Malaysian dairy industry suppose can leads to a significant reduction in overall production costs as well as environmental impacts compared to conventional heat processing methods.

1.4 Thesis outline

This study is divided into six chapters beginning with an introduction on fresh cow milk, current issues and public concern related to milk in Malaysia as well as innovation of new technology in milk processing. Second chapter is a literature review devoted to milk processing. The literature review defines milk using thermal and non-

thermal treatment and their effects on microbiological properties, physicochemical properties, vitamin, mineral and organoleptic properties.

The third chapter was written based on the first research objective to evaluate the effect of industrial scale HPP treatment on fresh cow milk quality including microbiological properties, physicochemical properties and nutritional value in comparison with heat-treated milk throughout 60 days of storage. This chapter covers the introduction, methodology, results and discussion for the fulfilment of first research objective focusing on the milk microbiological properties, physicochemical properties and nutritional value including vitamin and mineral contents following treatments and the changes throughout refrigerated storage.

In chapter four, the sensory analysis of HPP and heat-treated milk were demonstrated to study the effect of industrial HPP treatment on organoleptic properties of fresh cow milk. The results and discussion of this chapter were divided into two subsections, i.e. the triangle test to determine whether there is a perceived difference between two differently treated milk and the acceptance and preference test to identify the overall liking.

Chapter five is the economic and environment study aimed to compare the cost efficiency and environmental impact of the HPP system operation in Malaysia. In this chapter, the cost model was developed to estimate the cost required to run an industrial scale HPP milk plant in comparison to the conventional heat-treatment system operation in a milk factory. The environmental study has focused on energy consumption and carbon emission of both treatments.

The last chapter summarises the key contribution and finding of this work as well as various conclusion and recommendations based on the objective achieved in this work. This chapter also includes future research potential and speculates on how much of these areas might best be explored in local dairy industry.

1.5 Scope of Study

This research focuses on the application of commercial HPP on fresh milk processing compared to the typical conventional heat treatment. The fresh cow milk was obtained from Ladang 16, Universiti Putra Malaysia and examined for its microbiological properties, physicochemical properties and nutritional value including vitamins and minerals. The effect of HPP on milk sensory properties was also conducted and evaluated using the triangle test and acceptability and preference test. Cost estimation and environmental impact studies were carried out to assess its feasibility as a new processing technology to be adopted in the dairy industry for a Malaysian setting. In summary, this study provides a roadmap for the dairy industry, whether to apply the new technology (HPP) in milk processing plant. This study also provides important information about the current practice of the milk industry and it is also a great resource for businesses and market participants who are interested in non-thermal technology and development of healthier food products.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of milk industry in Malaysia

Malaysia's dairy industry has developed continuously with steady growth rates over the past few years due to the fast-growing demand for fresh milk and dairy products. Based on the statistics from Department of Statistics Malaysia (DOSM), the Import Dependency Ratio (IDR) of fresh milk has increased from 43.9% in 2019 to 53.5% in 2020. IDR explains a country's dependence on imports of agricultural commodities to meet domestic needs, in which higher IDR indicated more supply of agricultural commodities to be imported to overcome the insufficiency. A report from Frost and Sullivan (2021) stated that Malaysian milk and dairy product consumption per capita increased by 0.4 percent from 53.3 kg per person in 2015 to 54.3 kg per person in 2020. Similar report also reviewed that the local fresh raw milk production in Malaysia grew from 36.5 million litres in 2015 to 43.3 million litres in 2020 at a compound annual growth rate (CAGR) of 3.5% to meet the fast growth local consumption. This situation is driven by the more health-conscious Malaysian, who adopting a more balanced diet rich in nutrient and proteins such as fresh milk (Frost & Sullivan, 2021).

The milk sold on market is usually ready for consumption or known as ready-to-drink (RTD) milk. RTD milk is categorised into chilled and ambient milk type depending on the method of processing and storage condition. RTD milk are either produce from fresh milk, reconstituted milk or recombined milk. Reconstituted milk is the liquid milk obtained by adding water to skim milk powder, whole milk powder, or the mix

of both skim milk powder and whole milk powder (Novakovic & Robert, 1980) while a recombined milk product is the milk obtained from the combining of milk fat and milk solids-non-fat (SNF) in various forms, with or without water to achieve the desired fat and total solids content (Kneifel, 2003). In Malaysia, most companies use reconstituted, recombined milk or milk powder for the production of some of their own-branded products except for Farm Fresh that produces chilled RTD milk, ambient RTD milk, edible yoghurt, and drinking yoghurt from only fresh raw milk (Frost & Sullivan, 2021). The fresh milk products are the brand specialities which have also promoted their company to develop successfully and now become one of the leading dairy industry in Malaysia.

Malaysia government, dairy farmers and other industry stakeholders have made every effort to increase the local fresh milk production to overcome the high demand. New Straits Time reported local farm (FGV Dairy Farm) imported 122 dairy cows from Australia to meet the growing demand for fresh milk and dairy products (Adilla, 2020). Fraser & Neave Holdings Bhd (F&N) announced their plan to invest RM650 million to establish dairy farm in Chuping, Perlis, as part of its strategic goal of becoming a leading dairy producer in the Asean region and to import 4,000 milk cows with a potential production of 40 million litres of fresh milk annually (Esther, 2019; Shazni, 2019). Latest news by Frost and Sullivan (2021) reported Malaysia Agriculture and Food Industry Ministry is also committed in boosting the country's dairy sector to achieve a 100% self-sufficiency level (SSL) for fresh raw milk by 2025 through its National Dairy Industry Development Strategic Plan 2021 – 2025 launched in July 2021.

Besides boosting the local fresh raw milk production, maintain the good quality of milk and ensure safe consumption is another challenge in local milk processing industries. Milk is highly perishable with short shelf-life as it is a great substrate for bacterial development due to its unique composition and characteristics. The main microorganisms associated with fresh milk consumption include *Staphylococcus aureus*, *Salmonella spp.*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Campylobacter* (Claeys et al., 2013). These microorganisms can contaminate fresh milk through unhealthy cow's, poor farm management techniques and environmental hygiene or improper temperature control during storage (Berhe et al., 2020). In a milk microbiological safety study, Nordin et al. (2019) observed over 50% of raw cow milk from local farms exceeded the limit imposed by the Malaysia Food Act 1983 and Food Regulation 1985 ($5 \log_{10}$ CFU/mL), also nearly 50% of the milk samples were contaminated with coliform. Consumption of contaminated milk could pose a severe hazard to health, hence it created awareness among consumers to avoid buying and drinking of unpasteurised milk that has not been adequately processed. In Malaysia, thermal processing has been extensively used by the local dairy manufacturers to ensure safe consumption and to extend shelf life of milk. The principle and objectives of this processing in the dairy industry and the effect of high temperature on physicochemical properties, microbiological properties, nutritional value and organoleptic properties of milk are discussed in following section.

2.2 Thermal Processing of Milk

2.2.1 Overview of Thermal Processing Applied to Milk

Thermal processing is the most common operations in Malaysia's dairy industries. The concept of thermal processing in dairy industry is to heat the milk or its derivatives for a certain period at a certain temperature. The historical development of thermal processing was started from a famous French scientist, Louis Pasteur who discovered heating to roughly 60°C for several minutes could avoid spoilage and destroy disease-causing microorganisms of wine and beer in period 1864 – 1866; in honour of this monumental achievement, mild thermal techniques applied to milk are today known as pasteurisation (Sun, 2006; Watts, 2016).

Pasteurisation is a process where heat treatment is applied to food products with the objective to minimise possible health hazards arising from pathogenic microorganisms associated with that particular food with minimal chemical, physical and organoleptic changes in the products (Lewis, 2003). Most traditional pasteurisation was now known as low-temperature long-time (LTLT) pasteurisation that involved heating of milk to 62 – 65°C for at least 30 min followed by rapid cooling to temperature <10°C, (Sun, 2006; Sarkar, 2015; Watts, 2016). However, high-temperature short-time (HTST) pasteurisation at 72 – 74°C for ≥ 15 s has progressively become the standard industrial process for thermal processing of milk due to the lower detrimental impact by heat and flavour alternation when compared to the extended holding time LTLT pasteurisation (Sun, 2006; Liu et al., 2020). Pasteurised milk have been reported with an approximate shelf life of 9 days stored at 5°C (Al-Farsi et al., 2021), between 10 to 15 days at storage temperature of 8°C and 4°C respectively (Simon & Hansen, 2001) and can up

to 36 days when stored at 4°C (Petrus et al., 2010). HSTS frequently operated in continuous processes using plate heat exchanger with higher efficiency and capable to produce higher quality products in shorter time using less energy when compared to batch process (Lewis, 2010). A typical plate heat exchanger with continuous pasteurisation system comprised of a heating zone, regeneration zone, holding tube and cooling zone as shown in Figure 2.1.

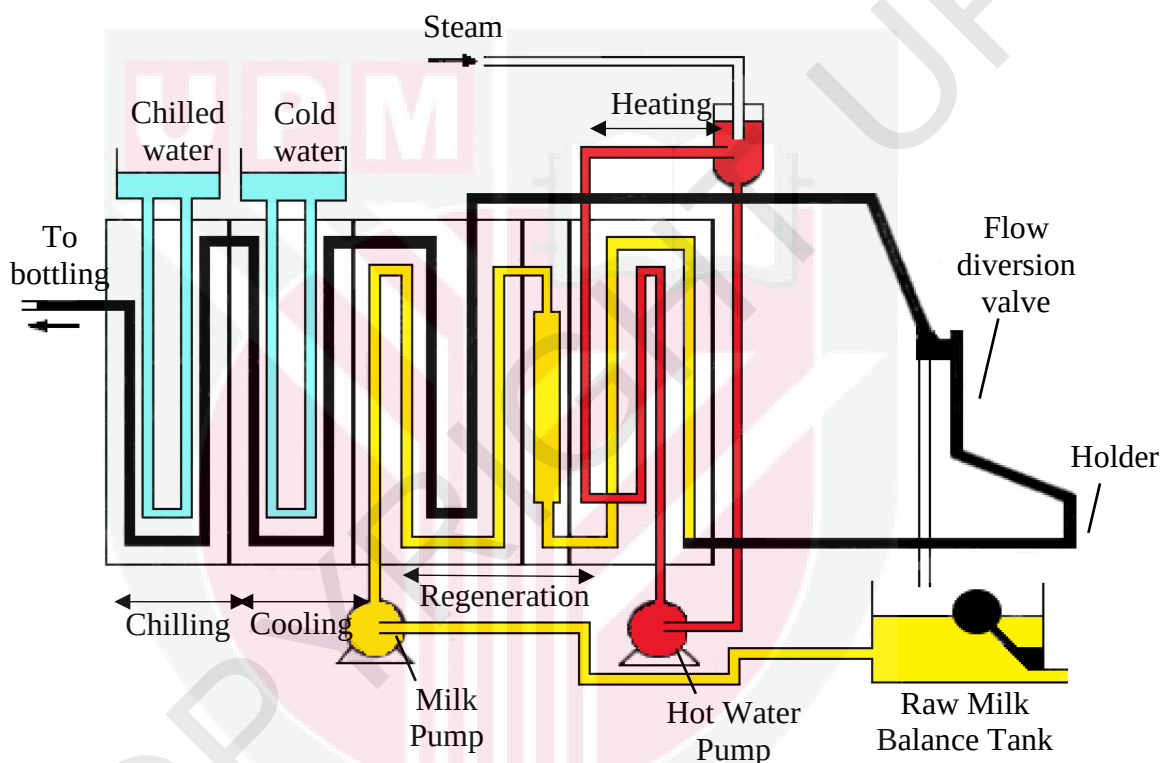


Figure 2.1: Plate heat exchanger for thermal processing of milk.
(Ibrahim, 2020)

Ultra-high-temperature (UHT) processes were introduced in 1940s, in which continuous flow milk is heated to temperatures of 135 – 140°C for 2 – 5 s (Sun, 2006). The resulting UHT products can have a long shelf life extending up to 6 or 9 months under ambient condition (Chavan et al., 2016). UHT process operated using 2 main heat treatment technologies, direct and indirect systems. Direct heating modes include steam injection, steam infusion, and scraped surface while indirect heating modes include plate or tubular heat exchangers (Chavan et al., 2011). The key factors enabling

long shelf life products is the parallel development of UHT with aseptic processing, where the sterile milk leaving the heat exchanger is directly filled into a sterile hermetically sealed packaging or container to prevent recontamination (Sun, 2006; Chavan et al., 2011). UHT is not an in-container sterilisation process which allows same bacterial destruction with less chemical damage while still inactivating thermophilic bacterial spores, hence the finished product must be packaged aseptically to ensure commercial sterility. In aseptic processing, packaging materials and equipment surfaces are sterilised by various methods such as heat, H₂O₂, irradiation, infrared light or through combinations methods (Ansari & Datta, 2003). The common causes of UHT milk quality degradation are generally associated with physicochemical alternation such as age gelation rather than microbiological or enzymatic processes (Sun, 2006). Study reported UHT milk stored at 4°C and 20°C having a shelf-life of 34 – 36 weeks with shelf-life limited by sediment formation, while storage at 30°C and 37 °C considerably decreased the shelf-life of UHT milk to 16–20 weeks with shelf-life limited by sediment formation, changed in taste and colour (Karlsson et al., 2019). UHT treatment is often resulting in cooked taste and brown colour which probably due to Maillard browning during high temperature treatment, while it also adversely affects nutrient components in milk including ascorbic acid, folacin, vitamin B12, vitamin B6, thiamine, and riboflavin up to an extent of 15–25%, 10–20%, 0–30%, 0–10%, <10–18%, 0–10%, respectively (Chavan et al., 2016)

Sterilisation is also one of the thermal processing methods in dairy industry used to sterilised milk. Sterilisation is aimed to inactivate heat-resistant spores, thereby producing a product which is commercially sterile with an extended shelf-life (Lewis, 2003). Traditional sterilisation process involves heating of milk in a sealed container

in the temperature range of 114 – 120°C for 20 – 30 min (Lewis, 2003), while Deeth (2021) defined sterilisation process as heat treatment at 110 – 120°C for 10 – 20 min. Sterilised milk is proven to have long shelf-life in which a study reported an unopened bottles of sterilised milk can stored at ambient temperature for about 6 months (Kontominas, 2019). Unlike UHT milk, sterilised milk is directly produced by in-container sterilisation through batch (autoclaves) or continuous retorting processes (vertical hydrostatic towers or horizontal sterilisers), and it can be done in prefilled and hermetically sealed cans or glass bottles (Chavan et al., 2016). This process does not require high cost aseptic packaging processing line, at the same time it is able to prevent recontamination after heat treatment. However, high temperature with a long holding time used in production of sterilised milk unavoidably results in burnt flavour and brown colour. Nutrient loss associated with sterilisation is also a substantial concern as some scientists reported major loss of vitamin C, thiamine and vitamin B12 after UHT treatment (Chavan et al., 2016).

Later development of thermal processing has given rise to extended-shelf-life (ESL) technology due to the requirement to further increase the shelf-life of pasteurised products, both for the convenience of the consumer and to provide additional protection against temperature abuse (Lewis, 2003). ESL thermal processing is a method to process milk using more severe conditions than pasteurisation but less severe than ultra-high-temperature (UHT) processing. According to Grabowski et al. (2013), ESL technology has been developed approximately 50 years ago but only introduced on an industrial basis and gain popularity in early 20s. Rysstad & Kolstad (2006) reported ESL milk has obtained significant market share in many countries due to the longer shelf life with acceptable flavour as like those pasteurised milk.

2.2.2 Extended Shelf Life (ESL) / Ultra-pasteurisation: Technology and Function

Extended shelf life (ESL) milk is not a well-defined milk category with no specific temperature-time requirements for this type of processing in most countries, but it has been manufactured and sold in the international dairy industry for many years (Rysstad & Kolstad, 2006). The most common type of ESL treatments used high-temperature to treat milk mostly in the range of 120 – 135°C for a short period of time in between <1 – 4 s (Sun, 2006; Grabowski et al., 2013; Deeth, 2017). The resulting product is known as ESL milk or ultra-pasteurised milk which have reduced microbial counts and a longer refrigerated shelf-life of 21 – 45 days when compared to the common LTLT and HTST pasteurisation, with some manufacturers claiming a shelf-life of up to 90 days (Deeth, 2017). Higher temperature can also be used for production of ESL milk. For example, U.S. regulations defined the ESL thermal processing as “ultra-pasteurisation” as heating milk at $\geq 138^{\circ}\text{C}$ for ≥ 2 s (Haumaru, 2020). While there is also country applying lower temperature for ESL process such as South Africa uses temperature between 94 – 100°C for ESL milk (Deeth, 2017).

The advantages of ESL thermal treatment over traditional thermal processing methods have been reported by several authors. ESL milk has lower microbiological counts and a substantially longer shelf life than regular pasteurised milk (Sun, 2006; Rysstad & Kolstad, 2006; Deeth, 2017). ESL milk is not regarded commercially sterile which packaged without a hermetic barrier under sanitary circumstances, giving them a longer shelf life under refrigeration. In a survey of the quality of ESL milk in relation to HTST and UHT milk conducted by Lorenzen et al. (2011) found that total viable counts of directly or indirectly heated ESL milk were significantly lower than those in

HTST milk, providing a better microbiological quality and extended shelf life. Ranjith (2000) demonstrated milks treated at temperatures of $\geq 120^{\circ}\text{C}$ had counts of $< 10^2$ CFU/mL after storage at 7°C for > 40 days. Rysstad and Kolstad (2006) reported ESL through direct heating at $130 - 145^{\circ}\text{C}$ for < 1 s with infusion heating and flash cooling times of < 0.2 s and < 0.3 s together with aseptic packaging resulting a shelf life of ≥ 45 days at refrigerated storage temperature of 10°C . ESL thermal treatment also provides economic advantages *i.e.* 1) less costly installation than UHT treatment, 2) cheaper packaging, storage and transportation costs, 3) having variety of options for packaging besides cartons (Kapadiya et al., 2001). Sensory evaluation also revealed that ESL milk has similar organoleptic qualities to pasteurised milk (Kapadiya et al., 2001; Haumaru, 2020). Unlike UHT milk, ESL milk does not have a pronounced cooked flavour, a stale or oxidised flavour or bitter taste due to peptides generated by heat-stable proteases, or a rancid flavour resulted from residual bacterial enzyme activity, making it is more pleasing for consumption.

As similar to normal pasteurisation, thermal processing ESL milk could be operated through indirect heat treatment (plate or tubular heat exchanger) and also direct heat treatment using injection or infusion (Deeth, 2017). Indirect heating is most common method uses to produce ESL milk which employs either plate or tubular heat exchangers for all stages of heating and cooling. In this regard, a significant amount of heat (up to 90%) can be recovered in regeneration part by heating the incoming cold milk with the heat from the heated milk after it passes through the holding tube. The heat recovery rate is about 50% higher than that of direct systems which offer an economic benefit of indirect methods. For direct heating techniques, the milk is first heated to $70 - 80^{\circ}\text{C}$ via indirect heating in a plate or tubular heat exchanger, and then

directly heated to the requisite high temperature via direct heating with steam. The milk is then passing through a holding tube and kept at the necessary temperature for few seconds depending on the required amount of time. The heated milk is next sent through a vacuum chamber for cooling. This process separates the water from the condensed steam and cools the milk to about the same temperature as before the steam heating stage. The milk is then chilled to 4°C in an indirect manner and ready for filling. In comparison, direct heating is better compared to indirect heating because this technology enables combination of high temperature heating with a very short and controllable heating time, resulting in good kill rate of pathogenic microorganisms and more better sensory properties of the product (Rysstad & Kolstad, 2006). However, in economic advantage wise, indirect heating will be more ideal as compared to direct heating with lower energy consumption and operating cost. For this reason, indirect heating become the selection of much manufacturers in producing ESL dairy products.

Although thermal processing technology served as an effective method in producing milk free of the most heat resistant pathogenic bacteria but it can also result in undesired effects such as change in milk constituents, nutrients deterioration and present of strong off-flavour. Therefore, it is important to study the effect of thermal processing on quality of milk when decide the type of treatment to be applied on milk.

2.3 Effect of Thermal Processing on Milk Quality

Quality of milk can be evaluated through its composition, stability and properties (e.g. microbiological, physicochemical, and organoleptic properties). Milk is a complex raw material with all nutrients and constituents before processing. The major composition of fresh milk comprises of water (87%), lactose (4 – 5%), protein (3%),

fat (3 – 4%), small amount of minerals (0.8%), and vitamins (0.1%) (Lindmark-Månsson et al., 2003; Pereira, 2014). Fresh cow milk also having near neutral pH (Marouf & Elmhal, 2017); pleasant aroma, mouth feel and slightly sweet taste (Chugh et al., 2014). However, these properties could be affected by type of thermal treatments with different operation temperature and holding time.

2.3.1 Inactivation of Microorganisms

Milk from a healthy cow is free of photogenic microorganisms but it becomes contaminated easily during direct contact with contamination sources including the feed, environment (air, water, barn, and pasture), cow's udder and milking equipment (Fusco et al., 2020). The most common microorganisms that induced souring of milk is the thermophilic lactic acid bacteria (LAB) which ferment lactose to lactic acid under conducive environment (Quigley et al., 2013). Other microorganisms that promote spoilage of milk included *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Enterococcus*, *Pseudomonas*, *Clostridium* and *Bacillus*. *Listeria*, *Salmonella*, *Campylobacter* and mycotoxin-producing fungi are the examples of pathogenic microorganisms which cause food poisoning and disease (Quigley et al., 2013; Poghosian et al., 2019). Thermal treatments can considerably minimise or eliminate the microbiological hazards associated with the consumption of raw milk (Owusu-Kwarteng et al., 2020). Combination of time and temperature management are critical control points to minimise overgrowth of surviving pathogens that contaminate the milk during harvest or inadvertent recontaminations processing and to prevent foodborne pathogens associated with milk (WHO/FAO, 2008). Each type of thermal treatment such as thermisation (57 – 68°C, 15 s), LTLT pasteurisation (63°C, 30 min),

HTST pasteurisation (72–74°C, 15 – 30 s), ultra-pasteurisation (120 – 130°C, < 1 – 5 s), indirect or direct UHT (135 – 140°C for 3 – 5 s) and in-container sterilisation (115 – 120°C for 10 – 20 min) having different microbiological effect and quality resulting in different shelf-life of milk (Sun, 2006).

Thermisation is a sub-pasteurisation heat treatment of raw milk commonly used as pre-pasteurisation to safeguard milk quality during prolonged storage in insulated silos (Swart et al., 2003). Thermisation reduces the counts of vegetative commensal microorganisms such as *Aeromonas spp.*, *coliform bacteria*, *Enterobacter spp.*, *Micrococcus spp.*, and *Pseudomonas spp.* in milk by 3 – 4 logs but does not completely inactivate all vegetative pathogens (Claeys et al., 2013); while Sun (2006) reported thermisation lead to 3 – 4 logs reduction in psychrotrophs. Thermised milk can be stored for up to 3 days longer at 8°C (Swart et al., 2003).

Pasteurisation is able to kill all vegetative microorganisms including *E. coli*, *Salmonella spp.*, *L. monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, enterotoxin-producing *S. aureus* and *Clostridium botulinum* (Owusu-Kwarteng et al., 2020). Both HTST and LTLT pasteurisation cause 1.02 and 0.71 logs reduction on total plate count (TPC) to 3.65 and 3.96 logs respectively after treatment (Mc Auley et al., 2016). According to Price Incentive Programme by the Department of Veterinary Services Malaysia, the basic standard of total plate count for raw milk of high quality should be less than 6 logs (Chye et al., 2004). However, after pasteurisation, TPC should be less than 5 logs in according to the Malaysian Food Act (Khairunnisak et al., 2017). Pasteurisation proven to reduce > 83.8% of TBC count, 99.9% of psychrotrophic bacteria count, 71.4% of mesophilic aerobic spore count,

41.1% of somatic cell count, 100% of total coliform count as well as 100% of total yeast and mold count in milk, providing a storage life up to 22 days (Pestana et al., 2015; Tan et al., 2020). However, pasteurisation is insufficient to destroy the heat-resistant spores of *C. botulinum* and *B. cereus*, meanwhile it may induces the germination of these spores, which subsequently cause development and production of toxins during the storage (Dumalisile et al., 2005; Claeys et al., 2013a).

The presence of aerobic and facultative anaerobic spore-forming bacteria of *Bacillus* spp. has been found to cause a major problem in fluid milk (Janštová & Lukášová, 2001). *Bacillus* is a gram-positive spore-forming microorganism in which the spores produced are extremely heat-stable and capable to survive over heat treatment, germinate and outgrow during storage resulting in food spoilage and production of toxins (Grutsch et al., 2018). Janštová & Lukášová (2001) reported some *Bacillus* spores can survive in milk even after high temperatures pasteurisation e.g. *B. licheniformis* spores had the highest heat resistance behaviour with surviving temperature of 135 °C, follow by *B. subtilis*, *B. cereus*, *B. sphaericus*, *B. megaterium*, *B. alvei* and *B. amylolyticus* which were able to geminate after heat treatment up to 120°C. Both *B. coagulans* and *B. pumilus* spores were less heat stable but also capable to germinate after heat treatment up to 115°C. The effect of heat treatment in eliminating spore of *Bacillus* spp had also been demonstrated by Hanson et al. (2005), who found that both pasteurisation at 72°C and 76°C for 15 s significantly increased the amount of *Bacillus* spore in milk over LTLT pasteurisation at 63°C for 30 min. *Bacillus* was not presented in the initial milk samples which pasteurised at 82°C for 30 min, but present in samples after 14 days of storage which indicating that injury and recovery time preceded growth.

Insufficiency of pasteurisation in destroying spores of most pathogens can be overcome by UHT treatment and sterilisation. UHT treatments and sterilisation are able to destroy all bacteria including spores, providing typical microbial quality of less than 2 logs TPC after 15 days of storage at 30°C (Sun, 2006). However, some spores are still capable to survive under UHT treatment as reported by Lindsay et al. (2021) that UHT (135 – 150°C, 1 – 10 s) are able to inactivate all non-spore-forming bacteria and all spores except highly heat-resistant spores of *G. stearothermophilus* and *B. sporothermodurans*, produces ‘commercially sterile’ and ambient drinking milk with shelf life up 6 – 9 months. *Geobacillus* (formerly *Bacillus*) *stearothermophilus* is a gram-positive, thermophilic, spore-forming bacterium often associated with plant food products, with the spore produced is extremely heat resistant which can survive canning and causes flat sour spoilage and sweet curdling defects (Hassan & Frank, 2011). These thermally resistant bacterial spores might be eliminated effectively using superheat steam treatment at 160 – 175°C with intermittent cooling periods, but this severe treatment is not suitable for thermally sensitive products as their properties and storage stability may be compromised (Head et al., 2008). Similarly, spores of *Bacillus sporothermodurans* is also highly heat resistant capable to survive under UHT treatment (D_{140} value 3.4 to 7.9 s) which is more heat-resistant than those of *B. stearothermophilus* (D_{140} values 0.9 s) (Huemer et al., 1998). Both these heat resistant bacterial spores normally only found in small numbers in fresh raw milk, in fact there are usually discriminated by manufacturing condition for UHT milk to prevent germination of spores during high temperatures processing (Burgess et al., 2010).

There are numbers of studies reported effectiveness of ESL treatment in microbial inactivation. Microorganisms was not detected in ESL milk processed at temperatures

at $\geq 134^{\circ}\text{C}$ during 30 days of storage (Blake et al., 1995). ESL treatment ($120 - 130^{\circ}\text{C}$, 1 – 5 s) using plate or tubular heat exchanger is able to destroy all vegetative bacteria and most spores (Sun, 2006). Lorenzen et al. (2011) observed no total viable count, bacilli, aerobic spore-formers detected in unopened ESL milk ($123-127^{\circ}\text{C}$, 1–5 s.) stored at 8°C for 26 days. However, psychrotrophic spore-formers are still a major concern in maintaining quality of ESL milk due to refrigeration storage (Deeth, 2017). Psychrotrophs are microorganisms that can proliferate at low temperatures with optimum and maximal growth temperatures of 15 and 20°C respectively (Oliveira et al., 2015).

2.3.2 Impact on Physicochemical Properties and Milk Constituents

Milk is a complex colloidal dispersion containing fat globules, casein micelles and whey proteins in an aqueous solution of lactose, minerals and a few other minor compounds (Singh et al., 1997). A number of compositional and processing parameters can influence its physical and chemical characteristics which further affect the overall quality of milk. Hence, it is important to know the impacts of conventional thermal treatments on these properties and to which extent if these treatments cause degradation.

pH of milk

The pH of milk commonly provides an indication of milk quality as well as milk freshness. Fresh cow milk normally has slightly acidic properties with pH $6.5 - 6.7$ (Marouf & Sara, 2018). Milk pH can change easily as it is sensitive to temperature, environment changes, handling and processing method.

Several studies reported heat treatments can cause milk pH alternation. According to Haq et al. (2013), heat treatments cause significant increase on pH values of skimmed milk, but it varied with type of heat treatments, i.e. thermisation (60°C for few s), pasteurisation (65°C for 30 min), and sterilisation (110°C for 10 min). The pH values of sterilised skimmed milk (pH 6.55) were remarkably higher ($P < 0.05$) compared to those pasteurised (pH 6.46) and thermised skimmed milk (pH 6.40) as well as to the un-treated skimmed milk sample (pH 6.38). Pestana et al. (2015) observed an increase in pH of milk from 6.47 to 6.77 after pasteurisation at 75°C for 15 s, probably due to the lower whey protein associated with the casein micelles. Whereas, Tan et al. (2020) reported drop in pH from 6.59 to 6.52 when milk undergo HSTS pasteurisation at 72°C for 15 s. Decrease in pH of milk could be due to increase in concentration of lactic acid produced from degradation of lactose content or transfer of calcium and phosphate to the colloidal phase during drastic heating (Raynal-Ljutovac et al., 2007; Haq et al., 2013).

Titrateable acidity of milk

Titrateable acidity is the amount of alkali required to bring the pH to neutrality. This parameter is useful for identifying the freshness of milk and controlling the manufacture of fermented dairy products (Abay & Kebede, 2018). The titrateable acidity of milk is usually determined by titration method between the milk (pH ~6.6) to the phenolphthalein end-point (pH ~8.3) and it is mainly affected by the composition of milk including casein, phosphates, proteins, citrate and carbon dioxide (Abay & Kebede, 2018).

Haq et al. (2013) found that heat treatments such as thermisation (60°C for few s), pasteurisation (65°C for 30 min), and sterilisation (110°C for 10 min) have different

impacts to titratable acidity of skimmed milk. The titratable acidity decreases with increasing severity of heat treatments. The titratable acidity of thermised skimmed milk ($0.17 \pm 0.0001\%$) was significantly ($P < 0.05$) higher than that pasteurised skimmed milk ($0.16 \pm 0.0002\%$) and sterilised skimmed milk ($0.14 \pm 0.0001\%$). However, different results were reported by Elhasan et al. (2017) in which acidity of milk samples were generally increased following heat treatments (Low pasteurisation at 68°C , Flash pasteurisation at 72°C , Boiling at 100°C and Sterilisation at 121°C). Whereas, Tan et al. (2020) reported non-significant changes of pasteurisation (72°C , $\geq 15\text{s}$) on titratable acidity of milk.

Specific gravity of milk

Specific gravity of milk is the ratio of density of milk to the density of normal water at a specific temperature. In milk and dairy industry, specific gravity has been used as an aid to detecting gross irregularity from the average composition of milk since specific gravity is depended on the protein and fat content. The specific gravity of normal milk is approximately 1.03 (Gemechu et al., 2015; Mahmood & Usman, 2010) typically determined by using pycnometer at 20°C . High specific gravity of milk indicated low fat content as the weight of fat is much lower than the water (Abay & Kebede, 2018). In contrast, low specific gravity suggests the water adulteration (water content higher than normal) in milk.

There are some researchers reported increase in specific gravity of milk after heat treatments in which Ibrahim et al. (2020) reported heat treatments ($72 - 85^\circ\text{C}$ for 5 – 10 s) cause slight increase in specific gravity, which is in accordant to Haq et al. (2013) who found the specific gravity in thermised, (60°C for few s), pasteurised (65°C for 30 min) and sterilised (110°C for 10 min) skimmed milk were relatively higher as

compared to untreated-skimmed milk. However, no significant changes observed by Tan et al. (2020) in specific gravity of milk after pasteurisation (72°C, ≥15 s).

Total protein in milk

Cow's milk protein makes up of approximately 82% of casein and 18% whey protein (Abay & Kebede, 2018). Casein is phosphate-conjugated which predominantly composed of calcium phosphate-micelle complexes with four primary components including alpha- (s1- and s2-casein), beta-, gamma-, and kappa-casein, whereas whey protein is a collection of globular proteins with a high level of α -helix structure and a fairly balanced distribution of acidic-basic and hydrophobic-hydrophilic amino acids and the most common whey proteins are alpha-lactoglobulin (-LA) and beta-lactoglobulin (-LG), which account for 70–80% of total whey proteins (Davoodi et al., 2016). Immunoglobulins (Igs), serum albumin, lactoferrin (LF), lactoperoxidase (LP), and protease-peptones are among the other forms of whey proteins. Whey protein is proven with much benefits such as promote growth, increasing muscle strength, promote weight loss, lowering blood pressure and blood sugar (Kimball & Jefferson, 2006a; Pal & Ellis, 2010; Kawase et al., 2000).

Whey protein have substantial levels of secondary, tertiary, and quaternary structures which are sensitive to heat. Under high temperature condition, the whey protein of milk will experience structural change (denaturation) accompany by protein unfolding and an exposure of hydrophobic groups (Raikos, 2010). For example, thermal denaturation of bovine Beta-Lactoglobulin (β -Lg) involves unfolding of the polypeptide chain and exposure of the sulfhydryl group, followed by self-association or with other proteins to form aggregates via sulfhydryl-disulfide interchange (Uniacke-Lowe et al., 2010). When the protein structure was changed, it leads to

change in functional properties of milk proteins such as emulsifying properties and solubility that could also affect their digestibility and nutritional properties (Claeys et al., 2013). Protein denaturation and aggregation also causes potentially thickening or gelation of the milk and dairy products. Previous study by Ibrahim et al. (2020) reported heat treatment causes reduction of milk protein due to concomitant decrease in moisture which causes increase in protein denaturation. Similarly, Hamodah et al. (2019) also reported pasteurisation resulted decrease in protein content of milk. However, several researchers reported no significant losses were observed on the milk protein after pasteurisation (Claeys et al., 2013a; Qi et al., 2015; Tan et al., 2020).

The effect of high temperature thermal treatment on milk protein can causes several consequences. For example, heat-induced release of k-casein from micelles that has the effect of removing a portion of the negative charge from the micelles, making them more vulnerable to coalescence, leading to milk sedimentation and age gelation (Anema, 2019). Heating of milk at $> 90^{\circ}\text{C}$ also results cooked flavour as volatile sulfur compounds produced by the degradation of whey proteins and the proteins of the milk fat globule membrane (Al-Attabi et al., 2014).

Total fat content in milk

The fat content in milk is essential as it will affects the cost, nutritional value, physical and sensory characteristics of dairy product (Abay & Kebede, 2018). Milk fat consists mainly of triglycerides (approximately 98%) which are composed of fatty acids of different length and saturation, while other milk lipids are diacylglycerol (about 2% of the lipid fraction), cholesterol (less than 0.5%), phospholipids (about 1%) and free fatty acids (FFA) (about 0.1) (Månsson, 2008). Although saturated fat may contains high fraction of saturated fatty acid which assumed to cause cardiovascular diseases

and obesity (Ebringer et al., 2008), however, some saturated fatty acids in milk are able to provide health benefit, for example, butyric acid (C4:0) is important for anticarcinogen, caprylic (C8:0) and capric acid (C10:0) may have antiviral activities (Claeys et al., 2013), while lauric acid (C12:0) may have antiviral and antibacterial functions (Thormar & Hilmarsson, 2007).

Studies revealed thermal processing has a minor or non-significant effect on the functional and nutritional properties of milk fat (Tan et al., 2020; Cappozzo et al., 2015; Elhasan et al., 2017; Haq et al., 2013). The fat content remained significantly higher in sterilised (110°C for 10 min) skimmed milk, while no significant difference observed between thermised (60°C, 5 s) and pasteurised (65°C, 30 min) skimmed milk over untreated skimmed milk (Haq et al., 2013). Heat treatments of low pasteurisation (68°C), flash pasteurisation (72°C), boiling (100°C) and sterilisation (121°C) were also not significantly affected the fat content of cow, sheep and goat milks (Elhasan et al., 2017). Thermal degradation of milk fat is not observed during pasteurisation could be due to the high temperature (> 200°C) required for non-oxidative decomposition of fatty acids (Claeys et al., 2014). However, José et al. (2015) reported decrease in total fat of milk from originally 3.58g/100g to 3.07g/100g after pasteurisation (75°C, 15 s), and to 3.15g/100g after ultra-high temperature process (140°C, 3 s). This is agreed by Ibrahim et al. (2020) who reported fat decrease after heat treatments (72 – 85°C, 5 – 10 s).

Total Solids (TS) and Solids Non-Fat (SNF) in milk

Total solids of milk are the non-water components of milk including fat, protein, lactose and mineral, while solid non-fat of milk is the total solid without fat but could contains protein, milk sugar and mineral. European Union established quality

standards for total solids and solids non-fat content of fresh cow milk should not be less than 12.5% and 8.5% respectively (Gemechu et al., 2015). According to Roberts (1987), milk with high solids non-fat is more valuable to the consumer for its flavor and nutritional value and also valuable to the dairy manufacturer especially in cheese production. Extra solid in fluid milk indicating higher protein and mineral content in the milk while providing a richer tasting sensation to consumers.

In the effect of heat treatments on total solid, Pestana et al. (2015) reported slight decrease in total solid of cow milk after pasteurisation (75°C, 15 s) and ultra-high temperature processes (140°C, 3 s). In contrast, both Tan et al. (2020) and Bermúdez-Aguirre et al. (2009) reported higher value of solid in the heat-treated milk due to the water evaporation during pasteurisation which increase the concentration of milk.

For solids non-fat content (SNF), Elhasan et al. (2017) observed SNF in milk increases after heat treatments (low pasteurisation at 68°C, flash pasteurisation at 72°C, boiling at 100°C and sterilisation at 121°C), which probably associated with the decrease in moisture by heating. This is in line with Ibrahim et al. (2020) who reported heat treatment (72 – 80°C, 5 – 10 s) have significantly increase the solids non-fat content of milk due to evaporation of water from milk at high temperature, which caused decrease in water content, and change in nature of carbohydrate in milk.

2.3.3 Impact on Vitamin and Mineral Content

Milk is a highly nutritious liquid containing all the vitamins and minerals that are important for humans' health. Vitamins and minerals are essential nutrients which perform hundreds of roles in the body such as shore up bones, heal wounds, bolster

immune system as well as convert food into energy and repair cellular damage. The vitamin in milk generally composed by liposoluble vitamins A, D, and E and also water-soluble B complex vitamins such as thiamine and riboflavin (Pereira, 2014). While in mineral fraction, milk has been naturally recognised as a privileged calcium source but at the same time it also contains several other elements such as phosphorus, magnesium, zinc, and selenium (Gaucheron, 2011). The range of vitamin and mineral composition of normal milks from South Africa, United States, United Kingdom, Denmark and Australia New Zealand were summarised in Table 2.1.

Table 2.1: Vitamin and mineral composition (mg or µg /100g) of whole bovine milk from South Africa, United States, United Kingdom, Denmark and Australia New Zealand.

Vitamin	Unit	Content (/100g)
Retinol	µg	28.00-52.00
Beta Carotene	µg	5.00-21.00
Vitamin B1	mg	0.02-0.04
Vitamin B2	mg	0.16-0.18
Vitamin B3	mg	0.08-0.70
Vitamin B6	µg	20.00-60.00
Folic Acid	µg	5.00-11.00
Vitamin B12	µg	0.40-0.45
Biotin	µg	1.40-3.90
Vitamin C	mg	1.00-1.20

Stability of vitamin

Vitamins are extremely sensitive nutrients that can be degraded easily through improper and severe milk processing. From most study, thermal processing of milk increases vitamin A concentrations (Macdonald et al., 2011), but reduces vitamin C, B12, B6, B2, folic acid and vitamin E across a range of thermal operational temperature (Kilshaw et al., 1982; Haddad & Loewenstein, 1983; Oamen et al., 1989; Asadullah et al., 2010; Macdonald et al., 2011). However, some review papers reported minimum or no significant changes on the effect of heat treatments on vitamin content

of milk (Claeys et al., 2013a; Bezie, 2019). This could be due to the nature of milk with relative low vitamin contents that causes the effect of thermal treatments on milk vitamin becomes negligible. According to Deeth (2021), fat-soluble vitamins are heat stable and are affected minorly by heat-treatment of milk, whereas water soluble vitamins are impacted with the degree of heating exacerbating the damage. For example, in UHT milk, Oamen et al. (1989) reported averaged losses of 32.5% vitamin C, 17.9% vitamin B12, 12.5% folic acid, 3.4% vitamin B6, and 2.6% vitamin B2 following UHT processing of milk at two different time-temperature combination of 149°C for 3.4 s and 138°C for 20.3 s. Vitamins deterioration after UHT processing are largely depend on the content of dissolved oxygen in the milk, such as loss of vitamin C in UHT milk is much associated with oxidation of ascorbic acid to dehydroascorbic acid, while vitamin B12 oxidised and converted to cyanocobalamin during heating (Oamen et al., 1989). Typically, the losses of vitamin C are also higher during storage as compared to other vitamins due to the higher oxidation rate by dissolved oxygen (Deeth, 2021). Several studies and reviews on the effects of heat treatments on vitamin stability in milk are summarised in Table 2.2.

Table 2.2: Studies and reviews on the effects of heat treatments on vitamin stability.

Authors	Vitamin	Heat Treatments	Finding
Kilshaw et al. (1982)	Vitamin B12, Thiamine (B1), B6	Heat treatment (121°C, 20 min)	- All vitamin B12 destroyed. 60% thiamin and vitamin B6, 70% ascorbic acid and 30% folate of skimmed milk destroyed.
Haddad & Loewenstein (1983)	Thiamine (B1), Vitamin C	HTST (72-80°C, 16 s), indirect UHT (110-140°C, 3.5 s)	- Loss of 7.92 to 11.83% of thiamine from milk. - Losses of vitamin C in milk varied from 16.55 to 29.69% with the absolute UHT-sterilization treatment (140°C, 3.5 s) contributed the greatest loss.
Oamen et al. (1989)	Vitamin C, B12, Folic Acid, B6, B2	UHT (149°C, 3.4 s / 138°C, 20.3 s)	Losses of 32.5% vitamin C, 17.9% vitamin B12, 12.5% folic acid, 3.4% vitamin B6, and 2.6% vitamin B2
Isabel Sierra & Vidal-Valverde, (2000)	Vitamin B1, B2	Microwave heating (90°C, 30 - 60 s)	- Vitamin B2 was not modified. - Vitamin B1 was lowered (3% and 5%, respectively).
Schöne et al. (2003)	Retinol (A), α -Tocopherol (E), Thiamine (B1), Riboflavin (B2)	Pasteurisation	Pasteurised milk had a diminished content of vitamin A (retinol), E (α-tocopherol), B1 (thiamine) and vitamin B2 (riboflavin) as compared to the untreated raw milk.
Asadullah et al. (2010)	Vitamins B1, B2, B3, B6 and folic acid	Conventional boiling for 5, 10 and 15 min	- Vitamin B1 in milk decreased from 0.037 mg/100 g to 0.027 mg/100 g after 15 min boiling. - Vitamin B2 decreased from 0.115 to 0.084 mg/100 g, vitamin B3 0.062 to 0.044 mg/100 g, vitamin B6 0.025 to 0.019 mg/100 g and folic acid 3.38 to 2.40 mg/100 g.
Macdonald et al. (2011)	Vitamin B12, A, B6, B1, C, folate (B9)	Pasteurisation	- Vitamins B12 and E decreased following pasteurisation, and vitamin A increased. - No significant effect of pasteurisation on vitamin B6 but a decrease in vitamins B1, C and folate.
Wendie L. Claeys et al. (2013a)	Vitamin A, E, D, B-complex	Pasteurisation and UHT	- The effect of a heat treatment on the availability of the nutritionally relevant vitamins in milk, particularly vitamin B2 (riboflavin) and vitamin B12 (cyanocobalamin) is very low. - Only small or no losses reported for B6 (pyridoxine), niacin (vitamin B3, nicotinic acid, nicotinamide), pantothenic acid (vitamin B5), biotin (vitamin B7) and the fat-soluble vitamins A, D and E, even during conventional sterilisation of milk.
(Lalić et al., 2014)	Vitamin B1	UHT	- No significant difference between vitamin B1 in raw or UHT milk. - No remarkable loss of vitamin B1 was observed for opened UHT milk stored under refrigeration conditions for 10 days.
Bezie (2019)	Vitamin A, E, D, B-complex	Pasteurisation, UHT treatments	- Pasteurisation alone cause no change in the vitamin A and carotene content of milk. - Evaporation (as manufacture of condensed milk), caused 20% loss on vitamin A. - Vitamin E content of milk is not influenced by pasteurisation or evaporation but a loss of 9 % occur after drying and reconstitution. - No loss in vitamin D during heat processing of milk. - No loss of vitamins of the B-complex group riboflavin, nicotinic acid, pyridoxine, pantothenic acid and biotin by heat treatment but the losses of B1 (thiamin), B12 and C increase from 10-20% in pasteurisation and UHT treatment, while 90 % loss of B12 in bottle sterilization and evaporation.

Changes in minerals

Heat also induces changes in the mineral content of milk (Kilic-Akyilmaz et al., 2022). The distribution of minerals especially calcium and phosphorus between the aqueous and colloidal phases will be alternated when the temperature changed. During heat treatments, milk temperature raised, causes increase in dissociation of inorganic phosphate (Pi) in the aqueous phase, further led to a shift in the H_2PO_4^- and HPO_4^{2-} equilibrium. The HPO_4^{2-} , which has a stronger affinity for calcium, rises in concentration and interacts with calcium to create calcium phosphate salt, further led to decrease in the concentrations of ionic calcium and Pi in the aqueous phase. Heat treatment causes precipitation of calcium phosphate as the solubility of calcium phosphate is reduced due to a shift to the colloidal form during heating (Raynal-Ljutovac et al., 2007). The modifications of salt balance or reversibility are depending on the intensity of the heat treatment. Most of these changes are reversible on cooling, except for high intense heat treatment ($>95^\circ\text{C}$) for few min (Gaucheron, 2005). For example, in UHT processing, minerals from the aqueous phase are transferred to the casein micelle which lowers ionic calcium levels by 10 to 20% (Sun, 2006). Pouliot et al. (1989) found that the Ca and P contents of the milk permeate decreased by 60 and 35 % respectively as the temperature increased to 90°C , together with smaller losses of Mg, while Na and K levels were not affected. In comparison to the macro-elements, there is significantly less evidence on the impact of heat treatment on the distribution and concentrations of trace minerals in milk such as zinc, manganese and selenium (Kilic-Akyilmaz et al., 2022). In fact, trace minerals in milk can be lost by forming complexes with or without proteins during heat treatment or other unit activities requiring heat, such as evaporation and drying in fouling deposits. The studies and

reviews on the effects of heat treatments on mineral content of milk were gathered in Table 2.3.

Table 2.3: Studies and reviews on the effects of heat treatments on milk mineral.

Authors	Mineral	Heat Treatments	Finding
Pouliot et al. (1989)	Ca, P, Mg, Na, K	Heating (80-90°C, 40 min)	- Ca and P in milk permeate decreased by 60 and 35 % respectively. - Smaller losses of Mg observed with increasing treatment temperature, while Na and K levels were not affected.
Foster et al. (1998)	Selenium	Pasteurisation (7 1.7°C, 15 s) and spray drying (outlet temperature 110°C, 19 min)	- Pasteurisation reduced intrinsic selenium level in milk by 7.9%. 49.2% selenium losses after pasteurisation followed by spray-drying.
On-Nom et al. (2010)	Ca	Dialysis at 20-80°C	Ionic calcium in milk decreases as the heat-treatment temperature rises.
Seiquer et al. (2010)	Ca	Sterilisation (116°C, 16 min), UHT (150°C, 6 s)	- Heat treatments affect calcium solubility. - The diet containing sterilised milk as the protein source having significantly lower values of apparent calcium absorption and retention than those UHT milk diet.
Yoo et al., (2013)	Ca	LTLT (63°C, 30min), HTST (72°C, 15 s), UHT (130°C, 2s)	- No significant difference was found in total calcium content of milk treated with various temperature. - Raw milk contained the highest total calcium, while UHT milk was the lowest.
Claeys et al. (2013a)	Na, K, Mg, Ca, P	Commercial UHT and sterilisation	No significant differences observed on milk minerals and trace element levels between raw bovine milk and commercially UHT-treated and sterilised milk
Šestan et al. (2016)	Ca	Pasteurisation (82°C, 15s)	- Pasteurisation of milk (82°C, 15s) lead to loss of calcium from 20 to 40%. - Heat treatment convert Ca^{2+} into the insoluble form that cannot be utilised by the human body.
O’Kane et al. (2018)	Iodine, Selenium	Pasteurisation	- No change in iodine of milk. - No change in selenium concentrations of milk.

2.3.4 Changes in Sensory Properties

Milk is a widely consumed beverage with pleasant aroma, mouth feel and slightly sweet taste (Chugh et al., 2014). Unfortunately, the common heat treatments used in dairy industry were known to change the flavour or organoleptic property of milk such as colour, flavour and appearance (Lewis & Deeth, 2009). Previous studies established that heat treatments (72 – 145°C) alter the sensory quality of milk with the

development of cooked or caramel flavour taste and smell (Walstra, Wouters, and Geurts, 2006; Gandy et al., 2008). Similarly, Lee et al. (2017) reported cooked or caramelised flavour, sweet, bitter and astringency as well as colour differences in ultra-pasteurised milk (138°C for 2 s). Strong flavour intensities of heat treated milk are undesirable and they will affect acceptance by consumers (Lee et al., 2017; Perkins & Deeth, 2001).

The cooked flavour of heat treated milk are attributed to the generation of aroma active compound during heat treatments. Jo et al. (2018) reported significant cooked and sulphur flavours were present in ultra-pasteurised milk (140°C, 2.3 s) with higher diversity in aroma active compounds when compared to HTST milk (78°C, 15 s). In their study, the high-impact aroma active compounds in direct steam injection ultra-pasteurised milk were included hydrogen sulphide, dimethyl trisulfide, and methional, while indirect ultra-pasteurised milk were included 2 and 3-methylbutanal, furfural, 2-heptanone, 2-acetyl-1-pyrroline, 2-aminoacetophenone, benzaldehyde and dimethyl sulfide. Similarly, Chugh et al. (2014) observed considerable changes in flavour associated milk composition including ketones, free fatty acids, hydrocarbon and sulfur compound after HTST (75 and 95 °C, 20 – 45 s), while Liu et al. (2020) found that the LTLT milks (63 °C, 30 min) were characterised by higher levels of ketones as compared to HTST and non-thermal treated milk.

The factor affecting flavour of milk during thermal processing are typically correlated to Maillard reactions (Colahan-Sederstrom & Peterson, 2005; Kokkinidou & Peterson, 2014), lipid degradation (Calvo, 1992) and thermal denaturation of whey protein and other proteins in the milk fat globule membrane (Bull et al., 2017). Under high

temperature condition, amino groups and reducing sugars (lactose) in milk proteins undergo Maillard reactions leading to formation of off-flavour compounds such as Strecker aldehydes, sulfur- and nitrogen-containing compounds, maltol, and diacetyl (Jo et al., 2018). Lipid degradation during heating of milk also produces methyl ketones, lactones and aldehydes that could affected the taste of milk (Calvo, 1992). The presence of sulphur containing compounds give rise to the “cooked” flavour, while the dissipation of sulphur volatiles and formation of methyl ketones and aliphatic aldehydes contribute to “stale” flavour in the heat treated milk products (Claeys et al., 2013). Denaturation of whey proteins contribute to astringency and drying sensation due to the hydrophobic interactions or disulphide bonds with the oral mucosae which increase muco-adhesion (Bull et al., 2017).

Although most thermal treatments have high efficiency in inactivating pathogenic or spoilage microorganisms, however these methods usually cause changes in nutritional, chemical or biochemical and sensorial properties of foods that reduce their acceptance by consumers. In addition, the environmental and wellness concerns served as key factors for the development of novel food preservation methods using non-thermal technologies. The new method or technology developed should be able to eliminate the detrimental effects by thermal treatments by producing products that are safe for consumption, with longer shelf life and high nutritional value, at the same time having taste or flavour nearest to fresh food.

2.4 Non-Thermal Processing Technologies for Milk

Non-thermal technologies such as pulsed electric fields, ultrasound, ultraviolet irradiation, membrane microfiltration and high-pressure processing are the recent

advances in assuring microbial safety of milk (Shabbir et al., 2021). Each of these processing methods have their own operational principle and system in milk preservation with the ability to inactivate both the pathogenic and spoilage microorganisms in milk and dairy products.

Pulsed electric field (PEF) is a mild technology for inactivation of microorganisms especially in liquid foods through the application of high electric fields in the form of short pulses at an intensity of 10-80 kV/cm for microbes (Shabbir et al., 2021). This approach is claimed to be superior compared to typical thermal processing in terms of nutritional profile, quality, sensorial, and physical attributes of food as it reduces detrimental effects in nutritional profile, quality, sensorial, and physical attributes of food (Abbas, 2017). This process preserve food by inducing the electroporation of the microbial membrane which causes alteration of membrane permeability. As the electric field is applied, the current flows into the liquid food sample and transferred to each point due to the presence of charged molecules. When the external electric field passes across the cellular membrane, a potential difference will occur. The cellular membrane will breaks down to achieve microbial inactivation when the external electric field exceeds the critical transmembrane potential (Barba et al., 2017). In dairy research, Guerrero-Beltrán et al. (2010) observed an application of 3 pulses (2 μ s pulse width) at 40 kV/cm for < 10 s caused a bacterial reduction of 4.3 logs in PEF-mild-thermal treated milk, while Šalaševičius et al. (2021) found that PEF at 24 kV/cm, pulse duration 25 μ s, 20 pulses resulting significant decrease in total bacterial viability of 2.43 logs and coliforms of 0.9 log. However, this process is claimed to be more efficient when combined with heat treatment. Fernández-Molina et al. (2005) reported combination of PEF system (36 kV/cm) and conventional heating (65°C, 21 s) of skim

milk produced a superior product in bacterial and shelf-life quality compared to either PEF or conventional heating alone. The combination of PEF and heating treatment prolonged the shelf life of skim milk to 30 days when stored at 4°C.

Ultrasound technology (US) is a non-thermal technology based on the mechanical waves with a frequency of above the threshold level (>16 kHz) of human hearing (Soria & Villamiel, 2010). The microbial inactivation of ultrasound technology is attributed to the intracellular cavitation, which promotes formation of shock waves when the bubbles generated during the ultrasound processing collapse, leading to structural and functional components damage (Barba et al., 2017). These shock waves promote the generation of high temperatures and pressures for microbial inactivation (Butz & Tauscher, 2002). Although several studies have reported the potential of US treatments in inactivating microorganisms of milk (Matak et al., 2005; Cameron et al., 2009; Herceg et al., 2012), however, in most of the cases when ultrasound is utilised alone, it is not sufficient in achieving more than 5 logs reduction of microorganisms (Butz & Tauscher, 2002). Hence, most application use combination of US treatments with other preservation methods such as temperature (thermosonication), pressure (manosonication), and pressure and temperature (manothermosonication) to create a synergistic effect in food preservation (Barba et al., 2017).

Ultraviolet irradiation (UV) is a technology corresponds to the component of the electromagnetic spectrum with wavelengths between 100 and 400 nm. The main cause of microorganism inactivation is the formation of lesions in the genomic DNA of the organisms caused by UV-B and UV-C radiation, which prevent DNA replication and resulting in the inactivation of microbes (Barba et al., 2017). For example, UV-C light

damages the DNA of pathogenic and spoilage-causing microbes (viruses and protozoa), preventing the transcription and replication processes and eventually leading to cell death (Choudhary & Bandla, 2012). Previous studies by Reinemann et al. (2013) showed that fresh cow milk treated with UV dose (1.5 J m/L using 1 and 2 pure version of reactors) resulted in a 3 logs reduction of natural microflora, while Bandla et al. (2012) found that UV irradiation using dean flow reactor (1.6 mm), μ v dose (0.05 J/mL) caused a 4 logs reduction of *E. coli* in fresh cow milk. However, milk turbidity is a major issue when UV light treatment is utilised for pasteurisation. This is because the lesser the UV light penetration in turbid milk causing microbial inactivation reduces. Milk is turbid due to the presence of suspended and colloidal particles in large concentrations, which causes milk to be opaque.

Membrane microfiltration (MF) is a separation technique that uses semi-permeable membranes to concentrate or fractionate liquids into two distinct compositions (Shabbir et al., 2021). It has been applied in dairy industry since 1960 for milk clarification, increase the concentration of the selected components as well as separation of the specific valuable components from milk or dairy by-products (Kumar et al., 2013). Initial reports have indicated that MF will reject fat and microbes while allowing other milk constituents to pass through the membrane, resulting in (theoretically) fat-free, bacteria-free milk (Kumar et al., 2013). For example, ceramic membranes with a pore size of 1.4 μ m can be used in a cross-flow setup to reduce the microbial content of skim milk while leaving the protein composition nearly unchanged (Gosch et al., 2014). Studies on microbial removal in milk using microfiltration were reported by Awad et al. (2010) who found membrane filtration of skim milk (MF-1.4 μ m, ceramic membrane, $T_p = 50^\circ\text{C}$) resulting 5.18 logs reduction

of total bacterial load while Gosch et al. (2014) reported more than 2.3 logs reduction of total bacterial load after microfiltration (MF-0.8 μm , tubular ceramic ISOFLUX® membrane). However, membrane fouling due to blockage of membrane pores, adsorption of particles on the pores, deposition of protein and minerals, cake formation and depth fouling as well as bacterial biofilms is the limitation in microfiltration technology and this remains the major concern in dairy industry despite all the efforts on optimisation (Kumar et al., 2013).

High-pressure processing (HPP) is a non-thermal technology that employ high pressure generally 100 – 600 MPa for duration up to 20 min to eliminate pathogenic microorganisms to increase the shelf life of food (Evelyn & Silva, 2015). HPP is an efficient preservative treatment normally applied to pre-packaged food products with minimal modifications of nutritional and organoleptic properties (Barba et al., 2012). The mechanisms of microbial inactivation by HPP are based on a combination of changes in the cell membranes (eg. structural changes in protein and membrane phospholipids which can alter membrane permeability and the function of membrane-bound proteins), cell walls, proteins and enzymes (Patterson, 2014). These changes are associated with the disruption of moderately weak bond such as ionic bonds, hydrogen bonds and hydrophobic bond during HPP (Shabbir et al., 2021). The HPP treated products are more likely to have higher nutritional value and natural taste compared to thermal treated foods as the small molecules such as simple-sugars, amino acids, vitamins and flavour components are mostly unaffected during treatment. Several studies have demonstrated that high-pressure treatments alone can achieve a microbial population reduction of 5 – 7 log cycles of most common microorganisms found in milk such as *E.coli*, *L. monocytogenes*, *Salmonella spp.* and others (Serna-Hernandez

et al., 2021). Stratakos et al. (2019) found that HPP (600 MPa for 3 min) significantly reduced total viable counts, *Enterobacteriaceae*, lactic acid bacteria and *Pseudomonas spp.* in milk, extending the microbiological shelf life by one week as compared to the pasteurised milk (72 °C, 5 min).

Among all novel non-thermal technologies that have been studied for milk and dairy products, HPP is believed to have better capability and more effective in milk preservation as it helps to eliminate microorganisms, pathogens and vegetative spores, preserves nutrients and improves sensory qualities. It is also a uniform and non-chemical treatment that produces ready to drink and long shelf-life products. Application of HPP on pre-packages milk also reduced the risk of post contamination. Studies also reported HPP treatments requires less energy per kg or L of food than the conventional thermal processes, although it still required significant amount of energy to build up pressure or maintain the pressure during holding (Tsevdou et al., 2019). To date, although there is no cost-effective evaluation compared both HPP and thermal treatment, but a recent study has contrasted the sustainability of HPP versus thermal processing of orange juice as a more particular illustration of this technology's environmental impact. HPP was found to be more environmentally friendly than thermal pasteurisation, as it has a reduced environmental impact in majority of the categories investigated, such as carbon emissions and ozone generation (Cacace et al., 2020). In this regard, deep investigation in operational system of HPP is conducted and further discussed.

2.4.1 Overview of High-Pressure Processing (HPP) Applied to Milk

High Pressure Processing has been generally recognised as a safe and effective food processing method worldwide with numerous potential application on dairy industry but it is estimated that only around two percent of the global HPP machines are working on dairy applications (González, 2021). The main factor is that milk and dairy products follow strict regulations, which sometimes restrict the innovation capacity of producers. The specific regulatory approvals for HPP milk can vary by countries.

In Malaysia, the approval of HPP milk would typically fall under the jurisdiction of Malaysian Food Act 1983 and the Food Regulation 1985. This regulation only defined the process parameter of thermal processing used in milk treatment including pasteurisation, sterilisation and ultra-high temperature (UHT) treatment. As there is no specific regulation amended for HPP treatment, milk manufacturers would require to obtain approval from the relevant regulatory authorities such as the Ministry of Health in Malaysia if they would like to use HPP treatment for milk processing.

According to Holtslander (2016), HPP is not considered a novel process in the United States of America., United States Department of Agriculture USDA Inspection Program Personnel (IPP) are required to verify the hazard analysis supports the use of HPP treatment in controlling pathogens in the product when a processor uses HPP as an antimicrobial treatment.

In the European Union, HPP is considered as a processing with detrimental effect on microorganisms equivalent to heat pasteurisation (González, 2021). This is aligned with the guidance for the implementation of Regulation (EC) No 853/2004 which

clearly indicates that “HPP with the purpose of decontamination should be considered as processing”.

In New Zealand, HPP is not considered a novel food processing technology (Holtslander, 2016). HPP foods are required to meet the requirements of the food standards in terms of microbiological limits (Standard 1.6.1) and schedule 27 of Food Standards Code 7 as stated in Australia New Zealand Food Standards Code.

In Canada, food products treated with HPP were no longer considered to be novel foods from December 2016 and all HPP products must comply with Food and Drugs Act and Regulations (Holtslander, 2016). In the Canadian Guidance on Food Products Treated with HPP, there is a requirement for consultation and validation with the Canadian Food Inspection Agency (CFIA) if HPP is used as a critical control process for pathogen reduction.

In summary, the regulatory support or acceptance of HPP in food industry is crucial. If the government or related parties could create a more conducive regulatory for HPP, it could encourage and attract more food manufacturer in implementing this technology.

2.4.2 High Pressure Processing (HPP): Principle and Operational System

High Pressure Processing (HPP) is a cold pasteurisation technique or non-thermal food preservation technology by which the pre-packaged products are introduced into a vessel and subjected to a high level of isostatic pressure (300–600MPa) transmitted by pressure transmitting fluid to eliminate pathogenic microorganisms (Abera, 2019). The

application of high pressure for food preservation come prior to the late nineteenth century on the inactivation of microorganisms in milk (Hite, 1899). This technology is now being adopted by a number of countries and it has been used to process a variety of food products including jams and fruit juice (tomato juice) (Mohácsi-Farkas et al., 2002), meat products, seafood and fish, vegetable products, fruits and beverages (Campus, 2010). The interest of HPP is derived from its ability to deliver foods with fresh-like taste with long shelf-life without the need of preservatives. The most appealing feature of this process is the ability to process uniformly regardless of mass or time (Chawla et al., 2011). HPP can also be used to process both liquid and solid (water-containing) meals, providing benefits such as (i) kills bacteria, (ii) extending shelf life, (iii) creating additive-free and fresh food, (iv) manipulating texture and (v) enhancing desirable attributes (digestibility) (Chawla et al., 2011).

Principles of HPP technology

The principles that justify the behaviour of foods under effects of high pressure include the Le Chatelier, isostatic pressing, and microscopic-ordering principles (Yordanov & Angelova, 2010; Elamin et al., 2015).

Le Chatelier's principle states that any system or phenomenon under equilibrium condition would experience a reaction change, accompanied by a reduction in volume when enhanced by pressure (Elamin et al., 2015). The isostatic pressure during HPP will only disrupt the non-covalent bonds such as hydrogen, ionic, and hydrophobic bonds that are very sensitive to pressure. In this regard, the small molecules with low molecular weight such as vitamins, alkaloids, saponins, flavonoids, peptides, fatty acids, and saccharides remain relatively undamaged, whereas the native structure and functionality of macromolecules with high molecular weight such as proteins,

enzymes, lipids, polysaccharides and nucleic acids can be disrupted by HPP (Chawla et al., 2011). The sensitivity of different bonds to pressure from high to low is in the order of hydrophobic > electrostatic bonds > hydrogen bonds > covalent bonds.

Isostatic pressing which also known as Pascal's Principle described that the pressure is transmitted in a uniform manner in every direction and the material returns to its original shape during decompression (Yordanov & Angelova, 2010; Elamin et al., 2015). This principle provides a justification to the nonporous foods with high-moisture content are not damaged macroscopically by pressure treatment (Balasubramaniam et al., 2015).

Microscopic ordering principle states that increases pressure at a constant temperature will increases the degree of ordering of the molecules of a given substance. This causes the pressure and temperature exert antagonistic forces on molecular structure and chemical reaction (Yordanov & Angelova, 2010; Elamin et al., 2015).

High Pressure Equipment

Despite the fact that the principles of HPP and their impact on microbial inactivation have been known since the late 1800s, the first commercial HPP equipment was installed in Japan in 1990 (Yordanov & Angelova, 2010). At present, HPP have been adopted worldwide and there are two most prominent manufacturers of HPP equipment which are JBT Avure and Hiperbaric (Elamin et al., 2015). Both manufacturers have been involved in the design, technological development, production and commercialisation of HPP equipment. Meanwhile, they also provide numerous solutions for dairy and dairy alternatives.

Typically, a commercial high-pressure processing unit consists of a high pressure vessel, closure for sealing the vessel, device for holding the closure in place (while the vessel is under high pressure), high pressure intensifier pump, pressure and temperature control device (optional) and material handling system (Yordanov & Angelova, 2010; Elamin et al., 2015). In high-hydrostatic-pressure equipment, the pressure vessel is the most critical component, typically made of forged steel or low-alloy steel of high tensile strength or reinforced with tensioned wire windings. This pressure vessel must be designed in a safe fail manner to be dimensionally stable. If there is some undesired issues or failure during operation, it should be failed with a leak rather than a fracture (Yordanov & Angelova, 2010). In the pressure vessel, pressure-transmitting fluids are employed to transmit the pressure to the products sample uniformly and instantaneously. The most commonly used pressure-transmitting fluids include water, glycol solutions, silicone oil, sodium benzoate solutions, ethanol solutions, inert gases, and castor oil (Yaldagard et al., 2008). The food products should be packaged in a sufficiently flexible packaging as during pressurisation in the pressure range 400 – 800 MPa, the pressure medium and pack contents are compressed to roughly 80 – 90% of their original volumes, but they reverse to their original volumes when the pressure is released (Yaldagard et al., 2008).

The high pressure during HPP can be generated either by direct compression or indirect compression (Figure 2.2). For direct piston-type compression, the pressure medium in the high-pressure vessel is directly pressurised by a piston, driven at its larger diameter end by a low-pressure pump (Yaldagard et al., 2008). This method allows very fast compression, but the limitations of the high-pressure dynamic seal between the piston and the vessel's internal surface restrict the use of this method to

small-diameter laboratory or pilot plant systems (Yordanov & Angelova, 2010). In contrast, the indirect compression method uses a high-pressure intensifier which pumps the pressure medium from the reservoir into the closed and deaerated high-pressure vessel, until the desired pressure is reached (Yaldagard et al., 2008). The schematic of indirect method is as shown in right side of Figure 2.2. and this is the method of the high-pressure process used in this study.

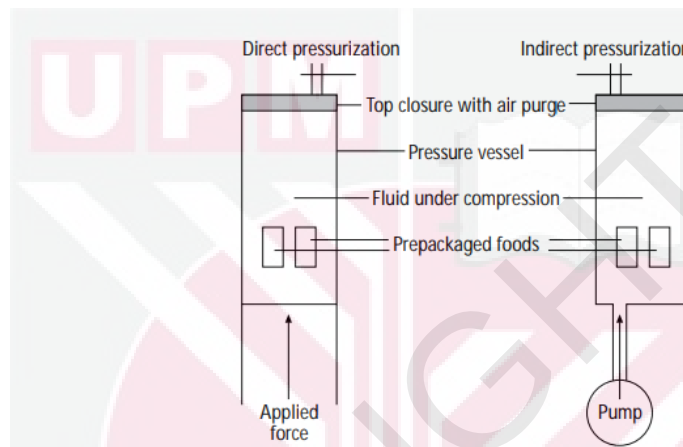


Figure 2.2: Schematics of direct and indirect high-pressure food processing techniques.

Batch processing system

HPP processing of food can be performed in batch, semi-continuous and continuous system. In batch processing system, food products packaged in a high-barrier, flexible pouch or a plastic container are placed in specific designed containers for HPP. The packages in the containers are then loaded into the high-pressure chamber (Figure 2.3). The vessel is sealed and it is then filled with pressure transmitting fluid, normally water to remove remaining air in the vessel. Once the high-pressure vessel is completely filled, the vessel is pressurised by a high-pressure pump which injects additional quantities of fluid to rise pressure inside. The food packages surrounded by the

pressure-transmitting fluid are subjected to the same pressure as present in the vessel itself. When the desired pressure is reached, the pump or piston is stopped, the valves are closed and the pressure can be maintained without further energy input. The products are held for the desired time between 1 and 9 min (depending on the food) at the target pressure. Typically, products are held for 3-5 min at 600 MPa (Yordanov & Angelova, 2010). After holding time, the vessel is decompressed by releasing the pressure-transmitting fluid. The high-pressure vessel is opened to remove the processed batch. Lastly, the processed product is removed from the vessel, stored at refrigerator and distributed in a conventional manner. The whole process flow to conduct batch processing system of HPP is summarised in Figure 2.4.

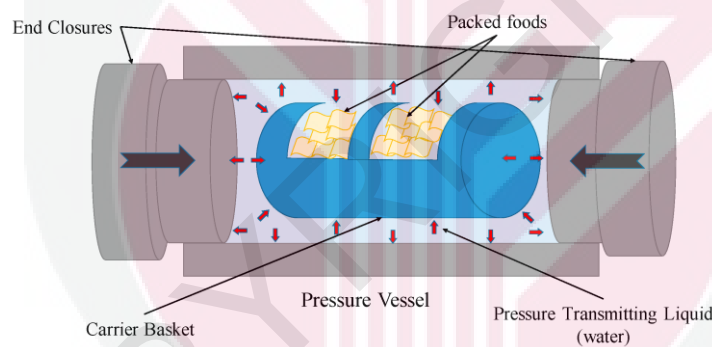


Figure 2.3: Representation of a high-pressure processing (HPP) vessel.
(Balakrishna et al., 2020)

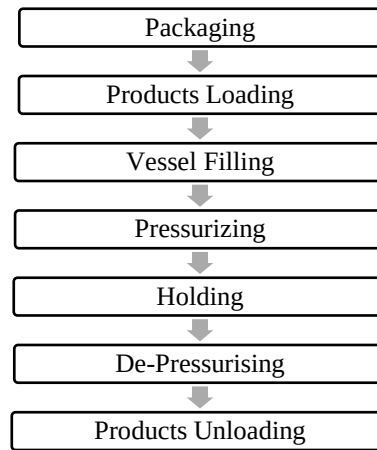


Figure 2.4: Process flow diagram for HPP of food.

Semi-continuous processing system

Semi-continuous processing system of HPP is used to pressurise liquid food products without packaging. Typically, semi-continuous high-pressure equipment use two or more pressure vessels, each containing a free-floating piston that allows each vessel to be divided into two chambers. According to Hogan et al. (2005), semi-continuous HPP machines were built as single vertical vessels (up to 210 L) or medium or small vessels (up to 3 x 20 L) connected in a series with a free or floating piston that could move up and down within the vessel. The pressurising fluid was pumped and compressed into the vessel on the opposite side of the free piston to displace and subsequently compress the product after the vessel had been filled with 85%–90% product. However, semi-continuous HPP systems had limited use and no commercial success due to corrosion and sanitation issues caused by direct contact of the product with the piston, vessel, plugs, and seals. Figure 2.5 demonstrates the semi-continuous process of HPP.

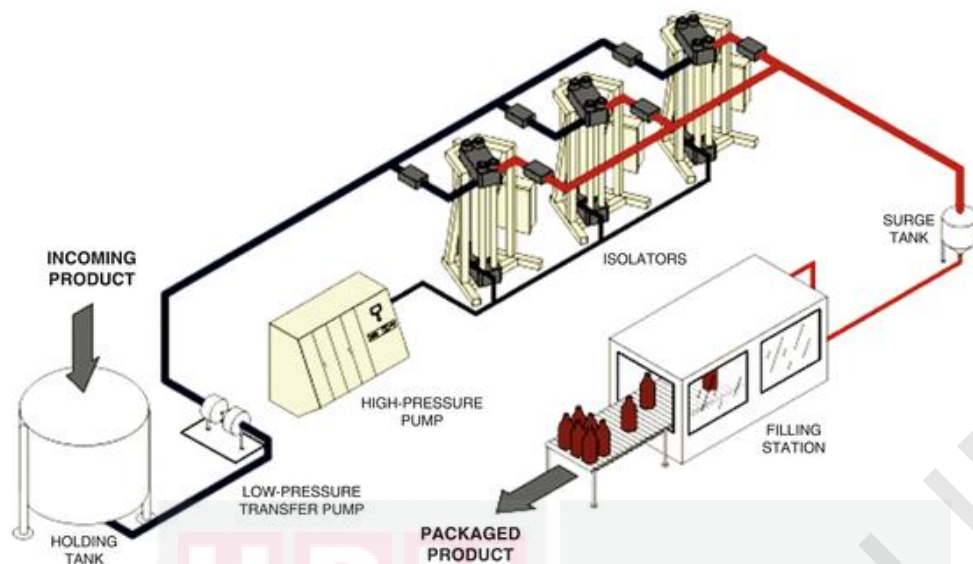


Figure 2.5: Semi-continuous process of HPP.
(Lelieveld & Hoogland, 2016)

Continuous processing system

Unlike batch processing method, continuous processing of high-pressure processing is only applicable for liquid food products and they are not pre-packaged before inserted into the pressure vessels. In this system, the liquid food products flow through the system that pressurises them at 100 MPa or more by means of high-pressure intensifiers. The products are then depressurised in such way that avoids extreme shear and heating. Finally, the processed liquid is transferred to a sterile tank for filling process to produce packed products (Elamin et al., 2015). Even though continuous HPP avoids the use of free piston and direct product contact with the vessel and plugs, and delivers filling efficiency of at least 90%, however this system involved complicated pre-operational cleaning and induced higher cost (20%) compared to batch processing HPP. In this regard, most studies on HPP used batch processing instead of continuous processing so as in this study.

2.5 Effect of High-Pressure Processing (HPP) on Milk Quality

There has been many studies and a lot of interest on how high-pressure processing affects milk quality. HPP is a non-thermal food processing method that uses high levels of hydrostatic pressure to eliminate harmful microorganisms and extend the shelf life of various food products, including milk. This innovative technology has shown promising outcomes in terms of enhancing the safety and preserving the nutritional value of milk, while also retaining its sensory characteristics and overall quality. By subjecting milk to intense pressure, HPP effectively inactivates pathogens without resorting to heat treatment, thereby reducing the risk of adverse impacts on taste, texture, and essential nutrients. HPP has emerged as a valuable method for ensuring milk safety and enhancing its quality, thereby contributing to advancements in the dairy industry and addressing consumer demands for safer and more nutritious dairy products. Further study on the effect of high-pressure processing (HPP) on milk quality were discussed in following section in term of microorganisms' inactivation, changes in physicochemical properties, vitamin and mineral retention and also changes in sensory properties.

2.5.1 Inactivation of Microorganisms

HPP inactivates most of spoilage and pathogenic bacteria present in milk but some microorganisms such as bacteria spores are pressure resistance. Bacteria spores can be extremely resistant to high pressure just like resistant to other severe treatments such as heat, irradiation and chemical agents (Patterson et al., 2007). Bacteria spores are more pressure resistant than vegetative cells because it contains calcium rich dipicolinic acid which protects it from excessive ionization (Naik et al., 2013).

Clostridium botulinum spores are one of the high pressure resistant spore that causes foodborne (Patterson et al., 2007).

Gram-positive bacteria are more pressure resistant than gram-negative bacteria, while cocci are more resistant than rod-shaped bacteria (Smelt, 1998). According to Li et al. (2020), the interferences of membrane permeability by high pressure are different in Gram-positive and Gram-negative bacteria due to the different cell wall structures. Gram-positive bacteria such as *Bacillus*, *Listeria*, *Staphylococcus*, and *Clostridium* have a thicker peptidoglycan layer, so having higher pressure-resistant properties than Gram-negative microorganisms (Georget et al., 2015). Yeasts and moulds are relatively HPP sensitive; however, ascospores of heat-resistant moulds like *Byssoschlamys*, *Neosartorya*, and *Talaromyces* were considered to be extremely HPP resistant (Considine et al., 2008). The sensitivity of microorganisms to pressure were summarised following order of increasing resistance: vegetative bacteria < yeasts and molds < viruses < bacterial spores (Patterson et al., 2007).

The lethal effect of HPP on bacterial or pathogenic microorganisms can be explained in numerous ways. In specific, damage of membranes, membrane proteins, and ribosomes are presumed to play a crucial role in inactivation of microorganisms by high pressure (Li et al., 2020). The cell membranes make up of a bilayer of phospholipids with a hydrophilic outer surface (composed of fatty acids) and an inner hydrophobic surface (composed of glycerol). During HPP, the high pressure and compression cause a reduction in the volume of the membrane bilayers and the cross-sectional area per phospholipid molecule. When the pressure release, the lipid bilayer experiences sudden expansion, leading to destruction and loses of cell membrane

integrity. Eventually, the damage cells are unable to control the transport of water and ions across the membranes and eventually death (Naik et al., 2013). According to Considine et al. (2008), variety of changes could occur throughout the bacterial cell under high pressure condition, including inhibition of key enzymes, protein synthesis inhibition, changes in cell morphology, cell membrane, and affects microorganism's genetic mechanisms such as disruption of transcription and translation, as well as cellular functions responsible for survival and reproduction. Cell morphological changes could include separation of the cell membrane from the cell wall, contraction of cell membrane, compression of gas vacuoles, cell lengthening and release of intracellular material (Considine et al., 2008). Consequently, these bacteria loss function and dead, producing food products that are safe for consumption.

Numerous investigations have been conducted about the microbial inactivation and shelf-life extension of milk using HPP. In 1899, Hite first revealed the potential of HPP in extending the shelf life of milk and found 5 – 6 logs microbial reduction in milk treated at 680 MPa for 10 min under ambient conditions (Hite, 1899). Following, Mussa and Ramaswamy (1997) reported milk subjected to a pressure of 350 MPa having shelf life of 25, 18 and 12 days when stored at 0°C, 5 °C and 10 °C respectively. Andrés et al. (2016) reported HPP (450–650 MPa, 3 min, 20°C) has maintained microbial stability in milk up to 45 days of storage at 4°C. Liepa et al. (2018) reported HPP (550 MPa, 3 min) reduced the total plate count (TPC) by 99.7%, less than those presented in untreated skimmed milk. According to Stratakis et al. (2019), HPP (600 MPa, 3 min) has significantly reduced the total viable counts in milk by 5 logs and extended the microbiological shelf life of milk by 7 days longer than heat pasteurisation. The similar pressure was used by Liu et al. (2020) and found that HPP

(600 MPa, 5 min) is able to reduce the total bacterial and *E. coli* counts to approximately the same level as heat treated milks.

2.5.2 Changes in Physicochemical Properties

pH and acidity

In addition to microbial inactivation and destruction, there have been numerous studies found on the impact of high pressure on physicochemical properties and composition of milk. For example, the pH levels and acidity of milk could be changed depending on the degree of pressure applied, presence of microorganisms and the milk composition (Serna-Hernandez et al., 2021). Studies reported HPP causes significant increase in milk pH up to 0.04 – 0.08 units at pressure of 600 MPa (Zobrist et al., 2005; Liepa et al., 2017). The increase in milk pH has been associated with the disaggregation of casein micelle under high pressure condition, which alters the distribution of minerals and raises the concentration of ionic calcium in milk, resulting an increase in the concentration of phosphate in the milk serum and thus, increase pH of milk (Chopde et al., 2014). In opposite, decrease in pH and increase in acidity is likely due to the buffering capacity of milk concentrates with high micellar casein concentration such and also migration of calcium phosphate into the soluble phase (Iturmendi et al., 2020). Milk fat can also operate as a protective barrier for casein micelles, limiting their dissociation and thereby minimising the pH changes induced by HPP in milk (Yang et al., 2020).

Proteins

Proteins are made up of a specific sequence of amino acids that are linked together by covalent bonds, electrostatic interactions, hydrogen bonds, disulphide bonds, hydrophobic contacts, and other interactions and are arranged differently in four different sequential levels: primary, secondary, tertiary and quaternary structures. High pressure can cause protein denaturation and affects the native structure of proteins in milk by inducing unfolding and disruption of different bond and interaction which led to functional and property changes (Serna-Hernandez et al., 2021). According to Goyal et al. (2013), high pressure affects the secondary structure (through hydrogen and electrostatic interactions), tertiary structure (through hydrophobic and hydrogen bonding) and quaternary structure (through hydrophobic interactions), whereas primary structure of proteins remains intact during HPP treatment as the covalent bonds are almost unaffected by high pressure. The sensitivity of different bonds to pressure from high to low are summarised as following: hydrophobic > electrostatic bonds > hydrogen bonds > covalent bonds (Goyal et al., 2013)

Caseins and whey proteins which represent approximately 80% and 20% of the total protein content respectively are two of the most frequently studied components in milk due to their nutritional properties, functionalities, and characteristics, such as colour and turbidity, rheological properties, pH, and emulsion stability (Serna-Hernandez et al., 2021). These proteins can be altered by different processing methods, including high pressure, affecting the specific properties of milk and dairy products. During HPP, the hydrophobic bonding of casein micelles disrupted due to water compression. This process alter the light transmission properties of milk and solubilised minerals mainly

on micellar calcium phosphate. Mineral solubility and ionisation are favoured under high pressure condition. The compacted water molecules penetrate and hydrate the protein molecule causing a dissociation of ion pairs that leads to release of calcium phosphate and caused structural changes of casein micelles (Serna-Hernandez et al., 2021). The mechanisms of HPP on the denaturation of casein micellar were summarised in Figure 2.6.

According to Goyal et al. (2013), HPP treatment cause two important changes in milk: first, an increase in the negative charge of the casein due to the association of denatured β -lg, and second, the disruption of the micelle into four different caseins in which both of these changes increase the hydration and solubility. Bogahawaththa et al. (2018) reported HPP (600 MPa) caused protein aggregation and denaturation of whey proteins mostly in form of β -lactoglobulin (β -Lg) and Immunoglobulin G (IgG), and disturbed structure of the casein micelle. Similar results obtained by Liu et al. (2020) in which denaturation of β -lactoglobulin was significantly higher for HPP milks (600 MPa, 5 min). Ye et al. (2004) observed HPP treatment (600 MPa, 30 min) caused approximately 80% denaturation of β -Lg, but only 10% for α -Lactalbumin (La). β -Lg is more sensitive to HPP because it has only two disulphide linkages and one free sulfhydryl ($-SH$) group, thus it is less rigid as compared to α -La which has four disulphide bonds (Goyal et al., 2013).

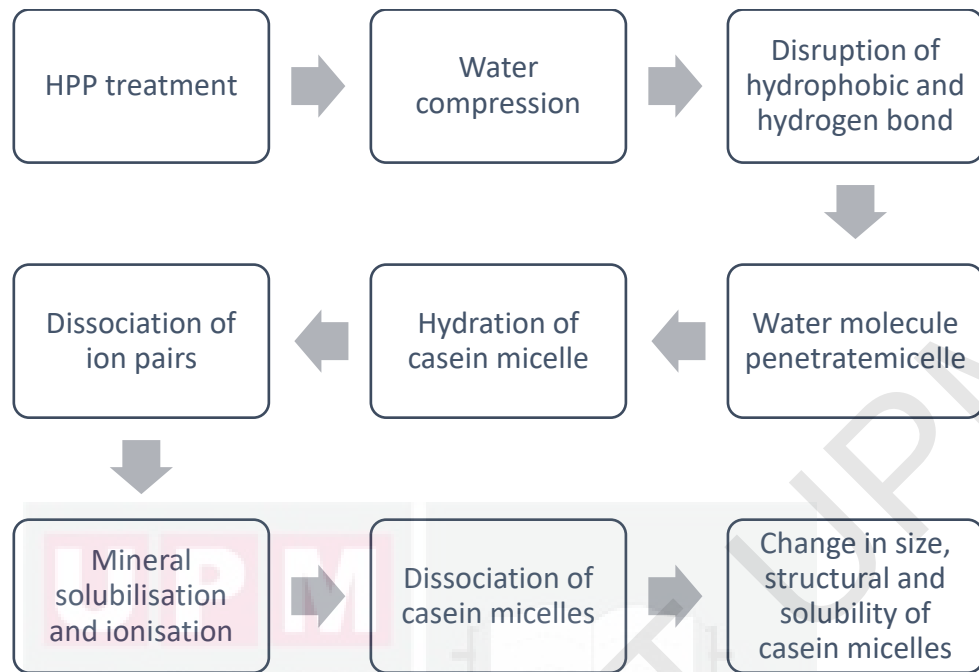


Figure 2.6: Mechanisms of HPP on the denaturation of casein micellar. (Serna-Hernandez et al., 2021)

Fat content

High pressure can cause modifications in size and distribution of milk fat (Chawla et al., 2011). According to Gervilla et al. (2001), HPP treatments at 25°C and 50°C tended to increase the number of tiny globules in the 1–2 μ m range, however the trend was reversed at 4°C and there is no damage on the milk fat globule membrane occurred. These changes in milk fat globule distribution could be attributed to fat globule membrane aggregation and disintegration during HPP treatment. The same study also showed that HPP treatments (100–500 MPa at 4, 25 and 50°C) did not increase FFA content, providing great benefit on avoiding off flavours, which otherwise develop because of lipolytic rancidity in milk. Similarly, Rodríguez-Alcalá et al. (2015) found HPP up to 900 MPa did not cause significant changes to the lipid classes or fatty acid composition of milk fat as the neutral and polar lipids remained stable in pressure range

250–900 MPa for 5 min. Meanwhile, Tan et al. (2020) reported the total fat in both cow and goat milk were not influenced by HPP (450 – 600 MPa, 5 – 7 mins). However, moderately high pressures (100–400 MPa) might induce the crystallisation of milk fat for production of dairy cream or other dairy products (Chopde et al., 2014).

As mention earlier, high pressure can alter the size of fat globules in milk in which it has a close relation to the milk homogeneity. Milk is homogenous when it has high consistency with uniform composition throughout and does not separate upon standing. According to Mustapa Kamal et al. (2021), HPP at 600 MPa contributes to the homogenisation of milk products through a reduction of fat globule size. The resulted smaller fat globules restrict formation of large cluster for creaming to occur. It also provides advantages such as to possess smoother and finer texture for the other milk products. Another study on fat globule size was conducted by Kielczewska et al. (2020), where caprine raw milk was treated at 200, 300, 400, and 500 MPa for 10 min at 20 °C and stored at 4 °C for 14 days. All treated samples had statistically similar fat globule size diameters when compared to their raw milk counterparts immediate after treatment. However, a dramatic increase in their size was observed after the 14-day storage for the untreated, 200, and 300 MPa samples while the diameters of 400 and 500 MPa samples remained statistically the same as for the samples observed immediately after treatment. The well maintain fat globule size of milk subjected to 400 MPa and above even after cold storage indicating high milk homogeneity and consistency.

HPP can also improve emulsion stability via the exposure to hydrophobic groups, leading to droplet size reduction and modifications of the protein molecular flexibility,

and causing a homogeneous distribution of polar and non-polar amino acid residues which further improving the emulsifying properties (Gharibzahedi et al., 2019). However, more intense and longer pressure treatments might negatively impact the emulsifying capacity as shown by Galazka et al. (2000). In their research, milk larger droplets were produced at 600 MPa, hence, samples that have been previously homogenised and later further subjecting them to these high pressures condition tend to increase droplet flocculation or aggregation.



Table 2.4: Studies and reviews on the effects of HPP on physicochemical properties.

Property	Authors	HPP Type	Finding
pH	Zobrist et al (2005)	600 MPa	Milk pH increase up to 0.04 – 0.08 units.
	Chopde et al., (2014)	-	- HPP cause milk pH increase due to disaggregation of casein micelle. - Alters the distribution of minerals and raises the concentration of ionic calcium in milk. - Increase in the concentration of phosphate in the milk serum and thus, increase milk Ph.
	Serna-Hernandez et al. (2021)	-	- Protein denaturation. - HPP affects the native structure of proteins in milk by inducing unfolding and disruption of different bond and interaction which led to functional and property changes.
	Goyal et al. (2013)	-	- High pressure affects the secondary, tertiary and quaternary structure. - Primary structure of proteins remains intact during HPP treatment as the covalent bonds are almost unaffected by high pressure.
	Bogahawaththa et al. (2018)	600 MPa	- Protein aggregation. - Denaturation of whey proteins mostly in form of β-lactoglobulin (β-Lg) and Immunoglobulin G (IgG). - Disturbed structure of the casein micelle.
	Liu et al. (2020)	600 MPa, 5 min	Denaturation of β-lactoglobulin was high.
	Ye et al. (2004)	600 MPa, 30 min	80% denaturation of β-Lg, but only 10% for α-Lactalbumin (La).
	Gervilla et al. (2001)	HPP at 25°C and 50°C	Increase the number of tiny globules in the 1–2 μm range.
	Gervilla et al. (2001)	100–500 MPa at 4, 25 and 50°C	No changes on free fatty acid (FFA).
	Rodríguez-Alcalá et al. (2015)	900 MPa	No significant changes to the lipid classes or fatty acid composition of milk fat.
	Tan et al. (2020)	450 – 600 MPa, 5 – 7 mins	Total fat in both cow and goat milk were not influenced.
	Chopde et al. (2014)	100–400 MPa	Induce crystallisation of milk fat.
	Mustapa Kamal et al. (2021)	600 MPa	Homogenisation of milk products increase through a reduction of fat globule size.
	Kielczewska et al. (2020)	200, 300, 400, and 500 MPa for 10 min at 20 °C	- All samples had statistically similar fat globule size diameters when compared to their raw milk counterparts immediate after treatment. - Increase in size was observed after the 14-day storage for the untreated, 200, and 300 MPa samples while the diameters of 400 and 500 MPa samples remained unchanged.
	Galazka et al. (2000)	600 MPa	Larger milk droplets were produced due to flocculation and aggregation.

2.5.3 Stability of Vitamin and Mineral Content

To date, the studies on the effect of high pressure on the vitamin contents of milk are still limited. In theoretically, vitamin content will not be affected during high pressure treatments as they are made of small molecules, linked together by covalent bond. In HPP research of human milk, Moltó-Puigmartí et al. (2011) found the total vitamin C and ascorbic acid levels were well maintained after HPP (400-600 MPa, 5 min, 22 – 27°C). Similarly, Jandhyala et al. (2002) found that the retention of ascorbic acid, thiamin or pyridoxal in sodium-1-pentane sulfonate (SPS) buffer solutions was significantly greater after HPP treatment at 689 MPa for 30 min (by 90.6%, 95.9% and 93.2% respectively) compared to those after thermal treatment at 121°C for 30 min (by 85.9%, 82.7%, and 88.8% respectively). The retention of ascorbic acid was smaller than the retention of thiamin or pyridoxal after high pressure treatment due to the fact that ascorbic acid is one of the least stable vitamins which can be easily affected by the treatments, pH, or exposure to oxygen (Jandhyala et al., 2002). However, Pitino et al. (2019) found that high pressure treatment (500 MPa, 8 min) appeared better retention of folate after pasteurisation (62.5°C, 30 min) but not vitamin C. They found that the folate content in human donor milk were retained by 93 % and 73% after high pressure treatment and heat pasteurisation respectively, while vitamin C content only retained by 25 % after high pressure treatments and by 36% after heat pasteurisation. Kim et al. (2008) also reported significant losses in ascorbic acid and riboflavin in milk after high pressure-low temperature treatment (200 MPa, - 4°C).

The changes in mineral content of milk are much associated with the changes in casein micelles of milk during high pressure treatment as casein micelles are stabilised and

structured due to the presence of colloidal calcium phosphate, as well as hydrophobic interactions and hydrogen bonding. Under high pressure condition, the hydrophobic and hydrogen bond undergo disruption, the compressed water penetrate the micelle causes disaggregation of casein micelles and dissociation of ion pairs which alter the mineral distribution of milk, further led to increase of ionic calcium concentration (Serna-Hernandez et al., 2021). The phosphorus and magnesium are also found in the casein micelles, which undergo dissociation and solubilisation under high pressure, similarly to the potassium and sodium which may also undergo minimum changes and can be found in the aqueous phase of the milk (Serna-Hernandez et al., 2021). A soluble mineral analysis was achieved by assessing the calcium-to-phosphorus ratio in a study using MCC and milk protein concentrates (MCPs), found that more intense pressure treated sample will have higher ratio than the untreated sample (Cadesky et al., 2017). Barba et al. (2012) in the review article reported HPP (200 to 400 MPa) increases the concentration of ionic calcium in milk as well as the level of total calcium and phosphate in the serum phase of milk, with most significant effect at 300 MPa. However, the increase in calcium phosphate solubility has been reported to be quickly reversible if pressure is released (Vandhana et al., 2020).

2.5.4 Changes in Sensory Properties

HPP can produce food with high sensory quality since less chemical modifications involved (Alcántara-Zavala et al., 2018; Liu et al., 2020). HPP milk (400 MPa, 15 min) has shown better score than the thermally pasteurised milk (78 °C, 15–20 s) in the hedonic evaluation of liking and sensory properties of milk, with better evaluation in colour, taste, and aftertaste, while no significant difference observed in aroma (Liepa

et al., 2017). Liu et al. (2020) through the results of volatile compound revealed both HTST (72°C, 15 s) and HPP (600 MPa, 5 min) treated milks exhibited very similar aroma profiles, except LTLT (63°C, 30 min) milks were characterised by higher levels of ketones. The same study also reported HPP samples had significantly lower boiled and sweet odours, boiled and cream flavours, intensity, and white colour compared to thermal pasteurised milk, causing the flavours close to those fresh untreated milk and making it more acceptable by consumer. This is in consistent to the finding of Andrés et al. (2016) who reported no significant differences in sensorial attributes between untreated and HPP (450 – 650 MPa, 3 min, 20°C) milk samples. In addition, HPP (400 MPa, 15 min, 25 – 60°C) is claimed to reduce the proteolytic activity and improved the organoleptic properties of milk by providing smoother and creamier sensory experiences (García-Risco et al., 2000).

Most studies reported HPP treatment affects the colour parameters and turbidity of milk products. The colour parameter L* value of whole milk decreased and appeared in darker colour after HPP treatment at 400 MPa for 15 min (Liepa et al., 2018). Similarly, Kim et al. (2008) observed a significantly lower L-value in HPP milk (200 MPa, 10 – 30 min) compared to fresh milk, while Stratakos et al., (2019) found that the HPP milk (400 – 600 MPa, 1 – 5 min) presented the lowest L* values and highest +b* value (yellowness) compared to untreated raw milk and pasteurised milk. In addition, Iturmendi et al. (2020) reported colour of reconstituted micellar casein concentrates was affected by HPP treatments (300 MPa, 450 MPa, and 600 MPa, 5 min) with a loss of lightness and increase of blue/grey tonalities. Under high pressure condition, the colloidal and emulsion components in milk undergo modification which led to colour changes due to a different light scattering effect (Goyal et al., 2013;

Stratakos et al., 2019; Kielczewska et al., 2020). During HPP treatment, casein submicelles disaggregated and casein fractions dissociated from micellar casein into soluble phase (Kielczewska et al., 2020). Disintegration of casein submicelles allows the compressed water molecules to penetrate and hydrate milk micelle, causing dissociation of ion pairs that leads to the release of soluble calcium and phosphorus from micellar casein (Serna-Hernandez et al., 2021). The disrupted micellar casein becomes smaller in size after releasing calcium and additional casein into milk serum (Orlien et al., 2006) which further reduces the light scattering effect and results in a darker or more yellowish colour appearance in milk (Liepa et al., 2017).

2.6 Feasibility of HPP via Cost and Environmental Assessment

The study on the effect of new processing technology on properties of milk are able to prove the great benefits and efficiency of treatment in producing milk of good quality. However, it is not sufficient to make a decision whether to implement this new process in industry. There are many concerns should take in consideration before implementing a new process in industry to ensure feasibility and sustainability of this new processing technology. In this extent, economic and environmental assessment are introduced to estimate the potential costs and assigns values to the anticipated benefits of the new processing technology. This method helps to understand the economic trade-offs and environmental impact between different alternatives to select the best and most appropriate technology in milk processing.

2.6.1 Cost Assessment

The cost incurred in a new project can be calculated using various methods depending on the parameters or scope of evaluation defined by the users. It is usually begin by calculating the capital investment cost. The capital investment cost is defined as the entire expenses required to complete a project and make it commercially viable. Total capital investment (TCI) is the representative metric of economic feasibility analysis that can be used in estimating the investment cost for the construction and operating stages (Kim et al., 2019). TCI is defined as follows:

$$TCI = FCI + OO = (DC + IC) + OO$$

where FCI is the fixed capital investment, OO is other outlays, DC is the direct costs, and IC is the indirect costs. Based on this equation, total capital investment is the sum of FCI and the other outlays, while FCI is composed of DC and IC. Direct costs included the cost of equipment purchasing, equipment installation, piping, building and others. Meanwhile, indirect cost is the expenses that cannot be allocated directly to a specific piece of equipment but it contributes a substantial part of the capital investment, it comprises engineering, supervision, construction, and contingency cost (Kim et al., 2019). The other outlays cost is the commissioning cost, working capital, licenses cost, research and development cost and Allowance for Funds Used during Construction (AFUDC).

According to Heinzle et al. (2007), capital investment can be calculated using multipliers to the purchased equipment cost. The multiplier values for the various cost categories in the estimating approach are obtained from empirical data (value vary

depending on the type of process). As this method involved direct multiplication of multiplier with the equipment cost, therefore accurate estimation of the equipment cost is crucial to improve reliability of the TCI estimation. Heinzle et al., (2007) reported vendor quotation is the most accurate source of equipment prices, at the same time, the price paid for the same or a similar piece of equipment in previous project is also a reliable source providing the old price is updated to latest price. If this information is unavailable, generalised values can be gleaned from the literature.

Operating Cost

Operating cost is also important in identify the sustainability of new product. Operating cost is defined as the total of all costs of operating the plant and recovering the capital investment, i.e. the annual amount of money necessary to produce the product and payback the investment cost (Heinzle et al., 2006). The operating cost can be subdivided into variable cost, fixed cost and plant overhead cost.

The variable costs are largely depending on the product output. These variable costs include the cost of raw materials; consumables or auxiliary materials; labour; operating supplies (*i.e.* clothing, tools, and protective devices for the workers and also everyday items needed to run the plant); laboratory, quality control, and quality assurance; utilities such as electricity, steam, and cooling water (determined in the process model and include the sum of the demand of all unit operations plus an additional amount for the general power load and unlisted equipment); waste treatment and disposal costs.

The fixed cost incurred in plant is also known as facility dependent cost composed of depreciation, equipment maintenance, insurance, local taxes, rent and other (Heinzle et al., 2006). The plant overhead cost are part of the operating cost but they are caused by the operation of facilities that are not directly related to the process such as medical

services, safety and protection, storage facilities, plant supervision, packaging, cafeterias, and others that can be estimated as 5% of the direct fixed cost (Heinzle et al., 2006).

Cost Analysis of HPP

The economic assessment and cost analysis discussed above were used by several researchers in HPP study even though it is not exactly similar. According to Cacace et al. (2020), the cost analysis of HPP can be easily divided into four cost macro-categories, namely initial investment, operating cost, maintenance cost and end-of-life cost. For initial investment of HPP, it involved purchasing cost and handling cost in which purchasing cost includes the cost of purchasing HPP plant and building, while handling cost covered the cost of a special machine needed to move the HPP plant within the company building. Operating cost refers to the operation and usage of the HPP plant, comprising cost of utilities, manpower, safety, cleaning and sanitation, packaging and other costs such as insurance and other annual fees. Maintenance costs are the cost incurred for all maintenance activities which can be categorised into preventive maintenance, scheduled maintenance and reactive maintenance. Meanwhile, end-of-life cost is the cost at the end of machine useful life or after a given lifetime, the plant and its components or materials can be sold to generate a profit.

Sampedro et al. (2014) reported the estimated cost of a proprietary HPP system (\$2,500,000) with desired throughput of 3,000 L/h (320-L and 420-L vessel) was higher than the capital cost for an industrial scale thermal pasteurisation system (\$66,000) based on the equipment such as plate heat exchangers, pumps and holding tube required to process juice at 88 °C with a holding time of 5 s at a production rate of 16,500,000 L/year (3,000 L/h). The total capital costs are estimated to be twice the

equipment costs when additional costs are incurred in equipment installation and commissioning, provide piping, electrical and process control systems, foundations and other necessary utility equipment for a complete operation. Meanwhile, Tonello (2011) reported the cost of high-pressure industrial units was found about \$770,000 for 55 litres, with the ability to provide pressures up to 600 MPa at room temperature and a maximum overall production of roughly 5000 kg/h. Although most research on cost evaluation showing higher cost incurred on HPP treatment, but the capital and operating costs will continue to decrease with increase implementation and production capacity. Table 2.5 provides the estimation cost for application of HPP with capacity of 55L and 300L, indicating a larger vessel HPP unit required lower operating cost.

Table 2.5: The estimation cost for application of HPP with capacity vessel size of 55L and 300L.

Description	Units	HPP (55L)	HPP (300L)
Intensity of use	h/year	2,000	6,000
Vessel volume	Litres	55	300
Hourly production	Ibs	582	3,175
	Kg	264	1,440
Yearly production	MIbs	1.2	19.1
	Tons	528	8640
Operator (s)	#/Shift	1	3
Maintenance personnel	#/Shift	0.1	0.3
Energy consumption	kWh/hr	17	90
Processing cost ^a			
Depreciation ^b	US\$/lbs	0.125	0.021
Wear of parts	US\$/lbs	0.028	0.027
Labour	US\$/lbs	0.039	0.022
Energy	US\$/lbs	0.002	0.002
Total	US\$/lbs	0.194	0.071

^a Processing conditions: 600 MPa, 3 min, 7.5 min total time per cycle, eight cycles/h 60% volumetric efficiency (vessel filling ratio).

^b 5-year depreciation period.
(Mújica-Paz et al., 2011)

Tonello (2011) reported the depreciation accounts for 65–75%, wear parts and maintenance for 22–33%, and utilities (process water and electricity) for 2–3%. HPP systems typically have a 5-year depreciation period. However, field experience indicates that well maintained high-pressure systems can have a functional operational life of more than 7 years. The depreciation of a 55L HPP unit (0.125 US\$/lbs) are about 6 times to those of 300L HPP unit (0.021 US\$/lbs) due to the higher capital cost per litre of the pressure vessel and the associated operating costs including labour and maintenance (Table 2.4). A 300L machine can process almost 5 times more products each cycle than a 55L machine, greatly reduce the cost per litre of the pressure vessel in the larger 300L HPP unit. The wear part costs for a 300L unit are slightly lower than those for a 55L due to the larger machine cycles less for a given yearly production rate. The mechanical parts of HPP unit wear out is associated with the high pressure increase and reduction, but not with the pressure holding duration (Tonello, 2011). Labour costs can be range from 10% to 40% of the overall processing cost, depending on the number of personnel necessary to operate the equipment each shift. According to Mújica-Paz et al. (2011), a 300L HPP unit required lower labour cost (by 44%) as compared to a 55L HPP unit.

2.6.2 Environment Impact Assessment

Issues on global climate change sourced by greenhouse gas (GHG) emissions have raise concern of public as they pose risk to the living environment, health, and human safety (Zhao et al., 2018). Many countries such as Switzerland, Denmark, Sweden, Norway and other are trying their best to protect the environment through multiple ways. Apart on that, Malaysia is also making progress towards sustainability and

managing climate change by resourcing efficient programs, environmental protection rule and regulation. The main legislation which governs the protection and conservation of the environment in Malaysia is Environmental Quality Act 1974 (“EQA 1974”). With this legislation, minister has the power to create regulations which specify the acceptable conditions for the emission of environmentally hazardous substances and pollutants as well as regulations prohibiting the emission into the environment of any gaseous matters under section 21 and 51 of the EQA 1974. To comply this legislation, environmental assessment become one of the important factors required to be taken into account before fully implementing new emerging technologies into an industrial setting. This assessment emphasises the critical point of each process that have the potential to harm the environment and assists companies in the decision-making process with respect to the implementation.

A number of environmental studies have been conducted for measuring carbon footprint of HPP products. According to Sampedro et al. (2014), the environmental impact of a given process can be expressed as the production of greenhouse gas level in terms of carbon dioxide equivalents (kg of CO₂ equivalents). It was estimated based on the electricity consumption (*i.e.* equipment, steam and cooling water) and fuel used in the systems by using conversion factors. On orange juice processing, HPP treatment was estimated having lower equivalent CO₂ emission as compared to thermal processing, even though it had high electricity consumption (Sampedro et al., 2014).

Life cycle assessment (LCA) is another method frequently used by researchers in environmental impact studies. LCA determines the potential energy and environmental loads at the various stages of the production and life cycle, using

appropriate impact indicators and returns as output related impact on the environment (Aganovic et al., 2017). Pardo & Zufía, (2012) used a cradle-to-grave LCA analysis and ReCiPe Midpoint method to assess the environmental impact of thermal and non-thermal technologies for fish and vegetables preservation. They found HPP and modified atmosphere packaging (MAP) had less environmental impact in terms of energy demand and CO₂ emissions (autoclaving) in comparison to conventional pasteurisation. Similar method was used by Aganovic et al. (2017) in environmental evaluation of thermal and alternative (pulsed electric fields (PEF) and high pressure processing (HPP)) technologies for preservation of tomato and watermelon juice, with results showed highest energy consumption on HPP (0.20 kWh/L of juice) while slightly less energy was required by PEF (0.12 kWh/L), followed by thermal treatment (0.04 kWh/L).

A complete set of environmental impact study could be complicated, high in complexities with many data acquisition, system boundary division etc., which is difficult for engineers to be implemented in real applications (Zhao et al., 2018). In this regard, all researches can modify or simplify the environmental assessment by setting the scope of study to be complied with their research objective.

2.6.3 Application of SuperPro Designer in Feasibility Study

SuperPro Designer[®] is a process simulator software used for analysing the techno-economic feasibility of any processes (Canizales et al., 2020). It facilitated modelling, evaluation, and optimisation of integrated processes as it has many advanced convenient features that can be used for the calculation of material and energy balances and extensive databases for chemical component and mixtures, as well as equipment

and resources, equipment sizing and costing thorough process economics (Ernst et al., 1997).

User can models and simulates complex processes including batch and continuous processing with the aid of this software. This software helps the user in understanding the dynamics of each unit operation within a production process. It allows generation of detailed material and energy balance that help researchers and engineers in understanding resource utilisation and process optimisation. In economic feasibility, SuperPro Designer aids in sizing equipment and estimating capital and operating cost related to the designed process, in which this is an important step for decision making especially in the early stages of a research project or process design. Meanwhile, in term of environmental impact assessment, user can assess the environmental impact of the designed process by considering factors such as energy consumption, waste generation and emission. These factors are important in determining whether the process was sustainable and environmentally friendly.

In this research, SuperPro Designer is highly recommend as this research involved process engineering. It was proven by Mokhtar (2019) who use this software in modelling and simulation of pasteurised milk production. Its ability to model and simulate different aspects of a production process allows researchers to explore various scenarios, optimise conditions, and make data-driven decisions. This is particularly important in the development of new process such as implementation of new HPP treatment, where understanding the economic feasibility, environmental impact and operational efficiency is crucial. Researchers can also streamline the decision-making

process, identify bottlenecks, and design more efficient and sustainable processes by the aid of this useful software.

In summary, this chapter provides a comprehensive overview of milk processing technologies in the Malaysian dairy industry. It discussed both the conventional thermal processing procedures used to preserve the safety and shelf life of milk and introduced newest non-thermal processing options, such as HPP. The literature reviews in this chapter also give an idea to the readers on the effect of milk processing technology on milk safety and quality in terms of microbiological, physicochemical, nutritional value and organoleptic properties. At last, this chapter also provided an overview on economic and environmental feasibility of implementing HPP in Malaysia.

CHAPTER 3

EFFECT OF INDUSTRIAL HIGH-PRESSURE PROCESSING (HPP) TREATMENT ON MICROBIOLOGICAL, PHYSICOCHEMICAL AND NUTRITIONAL PROPERTIES OF FRESH COW MILK DURING STORAGE WITH COMPARISONS WITH EXTENDED SHELF LIFE (ESL) TREATMENT

3.1 Introduction

Milk is a balance and nutritive food which presents an important part for human health. However, high moisture and nutrition contents of milk cause rapid microorganism's proliferation and results in spoilage. Thermal treatments including low temperature long time pasteurisation (LTLT, 63°C/ 30 min), high temperature short time pasteurisation (HTST, 72 – 75°C/15 – 20 s), ultra-pasteurisation/extended shelf-life treatment (ESL, 125 – 128°C/ 2 – 4 s) and ultra-high temperature treatment (UHT, 135 – 140°C/ 1 – 3 s) are common techniques used for extending shelf life of milk (Tamime, 2008). Among these thermal treatments, most manufacturers prefer ultra-pasteurisation process to produce extended shelf-life (ESL) milk of at least 60 days (Chapman et al., 2001) or >90 days (Deeth, 2017) instead of pasteurisation that normally provides a shelf life of 10 days (Lorenzen et al., 2011). However, thermal treatments can cause detrimental effects on milk quality such as whey protein denaturation, reduction of calcium, vitamin (thiamine, B12 and C) and change in organoleptic properties (Sun, 2006). In this regard, non-thermal technologies such as high-pressure processing (HPP), pulse electric field, ultra-sonication and irradiation are innovated, with aims to minimise loss of nutrients by thermal treatment while being still effective in destroying pathogenic and spoilage microorganisms in milk (Chavan et al., 2016). In comparative, the HPP offers promising potential in producing high

quality milk. This process normally employs pressure of 300 – 600 MPa at room temperature for 2 – 30 min to eliminate the pathogenic microorganisms and extends shelf life of milk with minimal alternation on nutritional and sensorial attributes (Mandal & Kant, 2017). Among the range of processing pressure, application of 600 MPa with holding time of 3 – 7 min is capable to extent the milk shelf life by 28 days (Stratakos et al., 2019; Tan et al., 2020). High isostatic pressure only disrupts the non-covalent bonds (hydrogen, ionic, and hydrophobic bonds), causing alternation in cell morphology and membrane that eventually leads to cell death and achieves microbial inactivation (Barba et al., 2017; Srinivas et al., 2018). Hence, the nutritional and sensory properties of the products are not affected. HPP also has other advantages such as low contamination risk and is declared as environmentally friendly processing technology (Srinivas et al., 2018).

Most HPP research was in lab-scale with García-Risco et al. (1998) reporting 45 days shelf life of milk when treated at 400 MPa for 30 min using 900 HPP apparatus (Eurotherm Automation, 69572 Dardilly Cedex, Lyon, France), while Mussa & Ramaswamy (1997) observed milk subjected to HPP at 350 MPa for 32 min using ABB Isostatic Press (ABB Autoclave System, Autoclave Engineers, Erie, PA) had a shelf-life of 12 – 25 days when stored at 0 – 10°C. At industrial scale, Stratakos et al., (2019) observed a storage life of 28 days at storage temperature 4°C when milk was treated at 600 MPa for 3 min using a 35 L commercial-scale high pressure press. Studies on industrial scale of HPP are still limited as scale-up of HPP treatment for milk preservation from lab-scale to pilot or industrial scale is a huge challenge due to high cost and equipment availability. To date, no study has been found reporting the effectiveness of HPP on milk over conventional heat technologies with complete data

on all properties, including the microbiological, physicochemical, nutritional contents of vitamins and minerals at industrial scale. Tan et al. (2020) conducted a study on physicochemical changes and microbiological properties of cow and goat milk up to storage duration of 22 days using industrial high pressure processing at different treatment combination of 450 MPa/7 min, 600 MPa/5 min, and 600 MPa/7 min, but their study did not include nutritional contents of vitamins and minerals which is important for human. Hence, this research was conducted to evaluate and compare the effectiveness of industrial HPP over ESL thermal treatment for fresh cow milk quality during extended storage of 60 days covering the most essential properties comprehensively, *i.e.* microbiological and physicochemical properties and nutritional quality which include vitamin and mineral content. The storage study on the quality and properties of HPP milk is important as certain impacts such as changes in structure and components of milk *e.g.* protein, fat or nutritional value may not be immediately impacted and can evolve over time especially during storage. In addition, the microbial activities in milk can change upon storage, which may indirectly affect the milk composition and stability. Comparison study between initial profile of post HPP with profiles at different storage time points helps in understanding how the combined effects of processing and storage contribute to changes in milk properties.

3.2 Materials and Methods

3.2.1 Processing of Milk

Raw cow milk was collected from the university farm, Ladang 16, Universiti Putra Malaysia. Milk was cooled to 4°C and stored in storage tank with slow speed rotating shaft. The milk then filled into polyethylene terephthalate (PET) bottles; 21 bottles of

350mL (labelled as fresh milk) for the used as control samples analysis, 53 bottles of 350mL (labelled as HPP milk) for HPP treatments and 13 bottles of 1.5L for ESL treatments. The 53 bottles of milk labelled HPP were treated in a high-pressure processing unit with capacity 55L (Hiperbaric 55; Hiperbaric High Pressure Technologies, Burgos, Spain) at 600 MPa for 10 min at 10 ± 2 °C using water as transmitting fluid. The transmitting fluid was reused as long there was no contamination. HPP treatment pressure time combination at 600MPa and 10 mins were selected based on the 'Review of High Pressure Processes (HPP) applied as an alternative to thermal pasteurisation' prepared by Horn et al. (2019), which consists huge data information regarding log reduction of microorganisms by which pressure and time combination. HPP at 600 MPa for 10 min is the 'safe harbour' strategy which can reduces most pathogens in milk by levels greater than 5 log₁₀ CFU/mL. The pressure and temperature profile of HPP treatment is plotted as shown in Figure 3.1a by referring to the reading on HPP unit by phases (pressure build up, pressure holding and pressure release).

The other 13 bottles of 1.5 L milk were subjected to ESL treatment at 125°C for 4 s using indirect heating method by pasteuriser (Powerpoint International Ltd., Saitama, Japan) which consists of a tubular heat exchanger system with 2 heating, 3 cooling, and a holding tube at capacity of 20 L/hr. The temperature profile of ESL heat treatment is showed in Figure 3.1b. ESL treated milk was filled into 53 bottles 350 mL PET bottles (labelled with ESL milk) at 6 °C. In this research, homogenization was not used on milk prior to its treatment with ESL to keep consistency with the HPP treatment.

All milk samples were stored at $6\pm 1^{\circ}\text{C}$ for storage analysis. Samples were analysed for microbiological and physicochemical properties, vitamin and mineral contents from Day 0. Fresh milk was kept for analysis until Day 6 while treated milk until Day 60. For fresh milk, microbiology analysis was conducted daily, while a 3 days interval was for physicochemical, vitamin and mineral analysis. The HPP and ESL milk samples were analysed at intervals of 5 days for microbiological, 6 days for physicochemical analysis, and 15 days interval for vitamin and mineral analysis during the 60 days' storage.

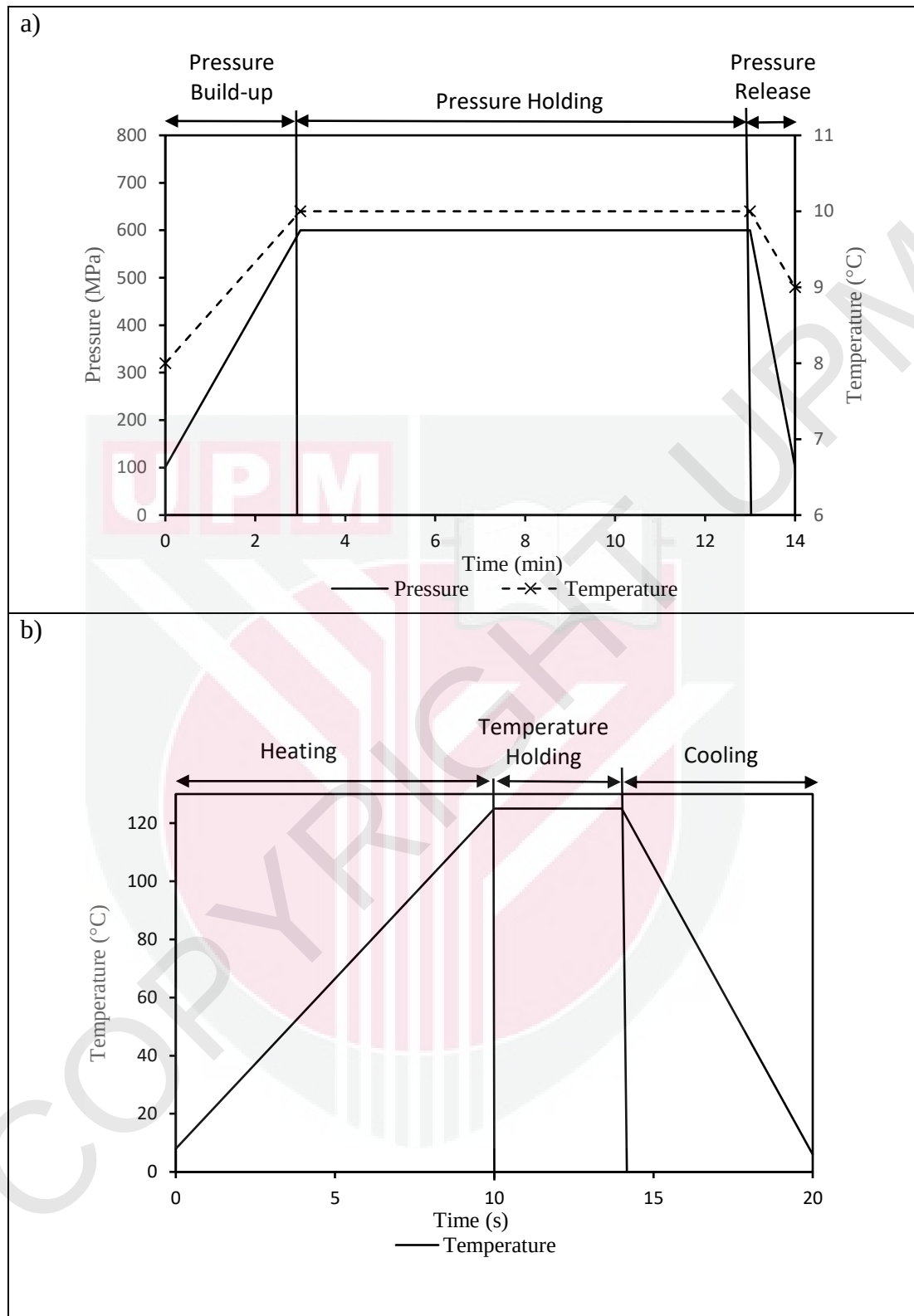


Figure 3.1: Pressure and temperature profile of a) HPP treatment and b) ESL treatment.

3.2.2 Microbiological Analysis

Total plate count (TPC) and total yeast and mold count in milk were determined using procedures described in Chapters 3 and 18 of the FDA Bacteriological Analytical Manual (BAM) (George et al., 2001). Total coliform count was calculated using AOAC Official Method 991.14 by incubating plate for 24 ± 2 h at $35 \pm 1^\circ\text{C}$. Mesophilic, thermophilic aerobic spore count and psychrotrophic bacteria count were determined following Chapter 23, 26, and 13, respectively in Compendium of Methods for the Microbiological Examination of Foods (CMMEF) with different incubating time and temperature, where mesophilic incubated anaerobically at 35°C for 3 to 5 days, thermophilic aerobic at $55 \pm 2^\circ\text{C}$ for 72 h while psychrotrophic bacteria at $17 \pm 1^\circ\text{C}$ for 16 h, followed by 3 more days at $7 \pm 1^\circ\text{C}$. *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* were determined following FDA BAM Chapter 14, 12, and 10, respectively. *Escherichia coli* and *Clostridium perfringens* were determined by AOAC Official Method 991.14 and 976.30 respectively. *Salmonella* spp. was determined using enzyme-linked immunosorbent assay (ELISA) method (Veling et al., 2001). Colonies were enumerated and results were expressed as the logarithm of colony forming units ($\log_{10}$ CFU /mL).

3.2.3 Physicochemical Analysis

The pH of milk samples was measured using pH meter (Mi805, Milwaukee, Hungary). Titratable acidity (TA) was determined according to Association of Official Analytical Chemists (AOAC) Official Method 947.05 and expressed as lactic acid %. Specific gravity was determined following method of AOAC Official Method 955.37 using pycnometer. Total protein was determined using formaldehyde titration described by

Pyne's method, in which oxalate was used as alkali for the titration, total protein was calculated using factor of 1.74 (Pyne, 1932). The total solid of milk was determined using oven drying method in accordance to AOAC Official Method 16.032 (William, 1980). Fat content was determined by Roesse-Gottlieb Method in AOAC Official Method 905.02. Solid-non-fat (SNF) was determined by subtracting the total solid with the total fat content.

3.2.4 Nutritional Analysis

Vitamin Content Analysis

Vitamins A (as Retinol) and E (as alpha-tocopherol) were determined using high performance liquid chromatographic methods (HPLC) after alkaline saponification. Vitamin B1, vitamin B2, vitamin B6 were analysed follow method as described by Agostini-costa et al. (2007). Vitamin B5 was determined based on Food Chemistry (2000), as similar to the method reported by Woollard et al. (2000). Vitamin B7, B9 and B12 were analysed based on AOAC Official Method 960.46 using microtiter assay (AOAC, 2006). Vitamin C (as ascorbic acid) was determined in according to AOAC 967.21 using 2,6-dichlorophenolindophenol (DCPIP) titration (AOAC, 1995). Vitamin K was determined based on AOAC Official Method 999.15 (AOAC, 2005). Vitamin D was determined based on AOAC Official Method 995.05 as described by Silva & Sanders, (1996).

Mineral Content Analysis

Milk mineral content including Calcium (Ca), Potassium (K), Magnesium (Mg), Phosphorus (P), Zinc (Zn) and Selenium (Se) were first undergo acid digestion as reported by Kira & Maihara (2007), following mineral content analysis based on AOAC Official Method 984.27 (AOAC, 1986), using inductively coupled plasma optical emission spectrometry, ICP-OES (Varian 720-ES, Varian Inc., Walnut Creek, CA, USA) with a solution of yttrium as internal standard.

3.2.5 Statistical Analysis

This experiment was subjected to one run with triplicated measurements of samples from the same batch of milk due to industrial scale except Vitamin K analysis. Mean values were compared by analysis of variance (ANOVA) using tool XLSTAT by Addinsoft and Microsoft Excel software. Tukey's test was performed to compare the differences among the mean values at a confidence level of 0.05.

3.3 Results and Discussion

3.3.1 Microbiological Analysis

Both HPP and ESL treatments successfully decreased TPC by 75% and 76% respectively, and other bacteria counts in milk by 100% meeting the food safety limit and local industrial standard (Table 3.1). Appendix A1 indicated a definite unsafe condition of fresh milk sample stored at $6\pm 1^{\circ}\text{C}$ with the continuous growth of all microbiological bacteria in the subsequent 6 days. However, the storage of treated milk for 60 days in Figure 3.2 using the HPP gave high microbiological stability as its

microbial activities was under control, while ESL milk exhibited an increase trend suggesting milk spoilage. ESL milk in this study was not safe for consumption after 5 days' storage despite meeting the safety standard on Day 0 as the total plate count, total yeast and molds and psychrotrophic counts exceeded permitted levels of <4, <1 and <4 logs between 5 – 10 days of storage. Premature spoilage of ESL milk could be due to contamination with heat-labile gram-negative bacteria that originate from the processing facility environment and recontamination after heat treatments (Doll et al., 2017; Martin et al., 2018).

Table 3.1: Effects of treatments on microbiological properties of fresh milk.

Bacteria count (log ₁₀ CFU/mL)	Fresh Milk	Treatment		Safety Limit
		HPP	ESL	
Total Plate Count	5.13 ± 0.06 ^b	1.26 ± 0.04 ^a	1.23 ± 0.09 ^a	< 5
Total Coliform Count	2.56 ± 0.02	< 1	< 1	< 1.7
Total <i>Escherichia coli</i> Count	1.53 ± 0.12	< 1	< 1	< 2
Total Yeast and Molds Count	1.61 ± 0.15	< 1	< 1	< 1*
Psychrotrophic Bacteria Count	6.81 ± 0.05	< 1	< 1	< 5
Psychrotrophic Spore Count	0.99 ± 0.15	< 1	< 1	< 1*
Mesophilic Aerobic Spore Count	0.72 ± 0.06	< 1	< 1	< 5
Thermophilic Aerobic Spore Count	< 1	< 1	< 1	< 1*
<i>Staphylococcus aureus</i>	2.96 ± 0.03	< 1	< 1	< 1.3
<i>Bacillus cereus</i>	< 1	< 1	< 1	< 5
<i>Clostridium perfringens</i>	< 1	< 1	< 1	< 4
<i>Listeria monocytogenes</i> per 25gm	Absent	Absent	Absent	Absent
<i>Salmonella</i> spp. per 25gm	Absent	Absent	Absent	Absent

^{ab} Mean ± standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$. Safety Limit values were compiled from Laws of Malaysia P.U.(A) 437 Of 1985 Food Act 1983 Food Regulations 1985 Arrangement of Regulations, Regasa et al. (2019), Council Directive 92/46/EEC (1990), Gilbert et al. (2000) and Food Standards Australia New Zealand (2018) except for * was obtained from industry (Fraser and Neave).

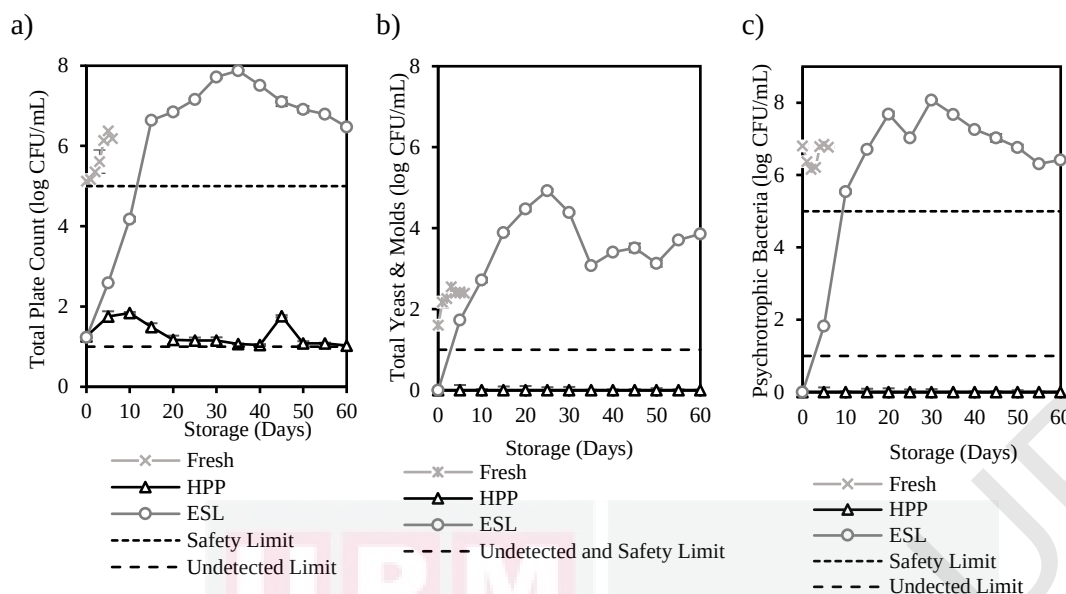


Figure 3.2: Changes in a) Total Plate Count, b) Total Yeast and Molds Count and c) Psychrotrophic Bacteria Count in log₁₀CFU/mL for HPP and ESL milk during 60 days' storage.
(Refer data from Appendix A2)

Total Plate Count (TPC) is a method of estimating the total number of microorganisms in products commonly used by dairy manufacturers in determining the microbiological quality of milk. In Malaysian Food Act (Food Regulations, 1985), the limit of TPC is less than 5 logs for safe consumption but the fresh milk in this study had an unsafe TPC count of 5.13 logs. It also increased significantly to 6.19 logs after 6 days' storage. The effect of HPP and ESL treatments on TPC were comparable as both have successfully reduced TPC of milk by approximately 4 logs, meeting industrial and safety limit (Table 3.1). Lab-scale HPP study by Liepa et al. (2018) reported more pronounced reduction of TPC by 99.7% using HPP (STANSTED fluid power LTD, Stansted, Harlow, UK) at 550 MPa for 3 min as compared to pasteurised skimmed milk (78°C, ≥15 s), while Razali et al. (2021) reported HPP (Avure 2L-700 HPP Laboratory Food Processing System, Avure, USA) at 400 MPa for 5 min reduces TPC to an undetectable level in milk. The commercial scale study by Tan et al. (2020)

reported higher TPC reduction at 600 MPa for 5 – 7 min (by 99.98%) as compared to 450 MPa for 7 min (by 96%) using commercial HPP (Hiperbaric 55, Hiperbaric, Spain). The effectiveness of HPP on TPC of milk could also be observed through the high microbial stability of HPP milk during storage. The TPC of HPP milk was within the safety limit and industrial standard after 60 days of storage. In contrast, the TPC of ESL milk surpassed the safety level after about 10 days of storage (Figure 3.2a). The ESL milk in this study had an unexpected short shelf life of 10 days instead of anticipated 21 – 45 days as documented in most other researches mostly due to post processing contamination during filling. The increase of TPC in ESL milk was mainly associated with the significant growth of psychrotrophic bacteria (Figure 3.2c) and yeast and molds (Figure 3.2b) during storage. This result was agreed by Stratakos et al. (2019) who showed that commercial scale HPP (Quintus 35 L, Avure Technologies, U.S.A.) at 600 MPa for 3 min successfully reduced TPC and prolonged the shelf life of milk to 28 days.

Yeasts and mold can cause development of off-flavours in milk due to generation of toxic metabolites by the mycotoxins which restrict shelf life of milk and pose a potential health risk (Godič Torkar & Golc Teger, 2008). Fresh milk used in this study had yeast and molds count above safety limit of 1 log (Godič Torkar & Golc Teger, 2008). Both HPP and ESL treatments have reduced the count to a safe and undetected levels. Similarly, Andrés et al. (2016) has reported non-detection of molds and yeasts in all milk either treated using HPP (450–650 MPa, 3 min at 20 °C) or pasteurisation (80 °C, 3 min). The effect of 60 days storage on yeast and molds counts of treated milk showed no changes for HPP milk, but significant changes were observed for ESL

treated milk. The yeast and molds count in ESL milk outstripped the industrial standard around Day 5 and reached highest average count of 4.93 logs on Day 25 (Figure 3.2b).

The spore-forming bacteria which include psychrotrophic, mesophilic and thermophilic spore formers are common in dairy products due to their ability to survive under different temperature (Martinez et al., 2017). The psychrotrophic microorganisms constitute a major cause of milk spoilage (Quigley et al., 2013) due to the ability to produce heat-resistant enzymes such as proteases, lipases and phospholipases under refrigeration (Xin et al., 2017). The psychrotrophic bacteria count of high-quality raw milk should be $<10^5$ CFU/mL based on Malaysian Food Act (Food regulations, 1985), but this study shows that the psychrotrophic bacteria count in fresh milk was higher at 6.81 ± 0.05 logs (Table 3.1). The high psychrotrophic bacteria reduced to undetected level after treatments, same as its spore and also both the mesophilic and thermophilic aerobic spores. Tan et al. (2020) also reported both psychrotrophic and mesophilic spore were not detected in HPP (600 MPa, 7 min) and pasteurised milk (72 °C, 15 s). Refrigerated storage of treated milk also show high effectiveness of HPP in controlling the psychrotrophic bacteria count in milk. In ESL milk however, proliferation of psychrotroph bacteria was detected and it exceeded the safety limit on Day 10 (Figure 3.2c), probably due to post-processing contamination that originated from various sources, including the atmosphere, milk piping, packaging and other food contact surfaces. The elevated temperature used in ESL processing have potential to contribute to premature spoilage with following explanations, *i.e.* (1) majority microorganisms in milk are killed during treatment, causing a suppressed influence of competitive microflora which results in rapid proliferation of heat-resistant microorganisms present at low levels after heat treatment. (2) the temperature

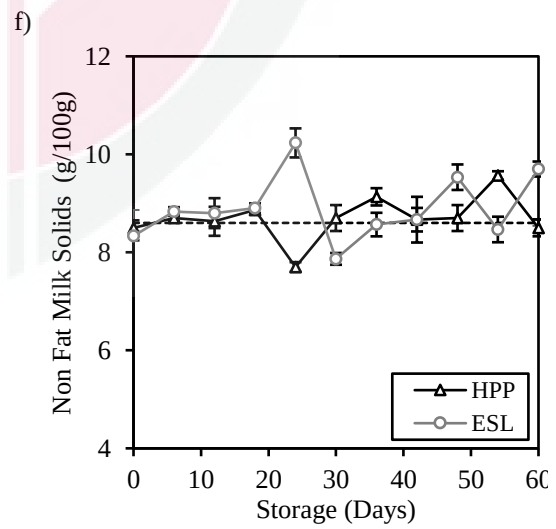
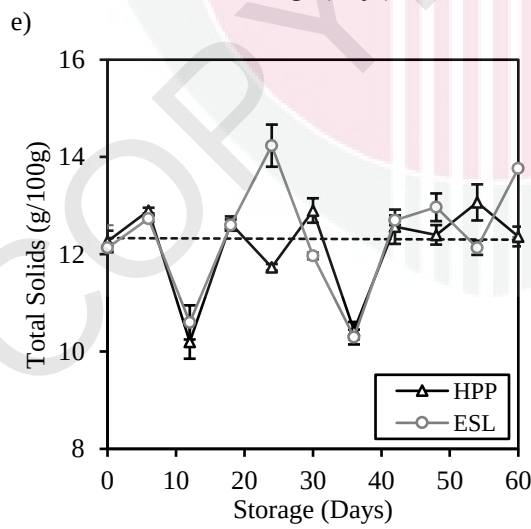
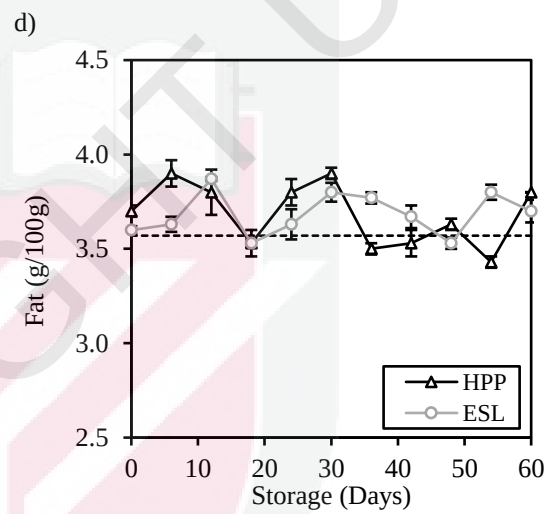
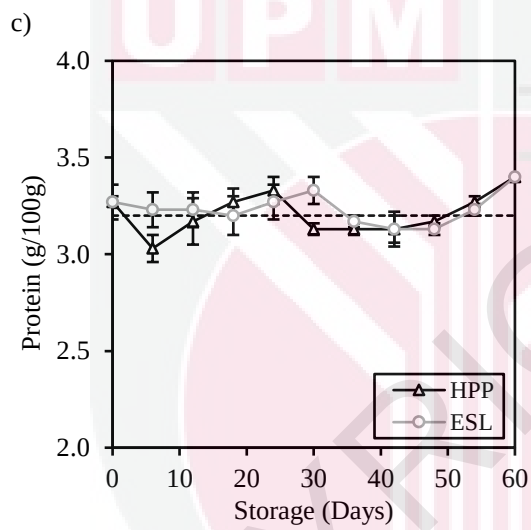
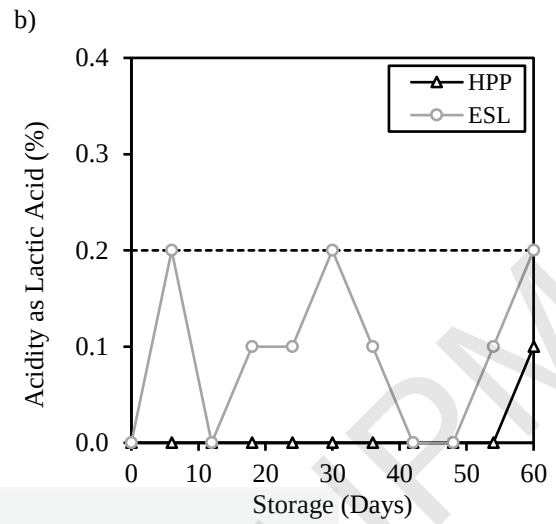
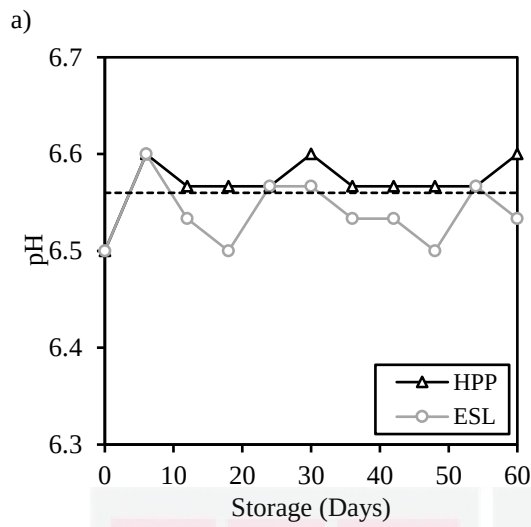
used in ESL processing may trigger activation of certain bacterial spores, and (3) milk's natural antibacterial mechanisms could be damaged (Rankin et al., 2011).

Other microbiological properties which counts were all well-kept below the safety limits during the 60 days' storage after both HPP and ESL treatments although they have counts exceeding safety levels before treatment are the coliform (1.7 logs) and *Staphylococcus aureus* (1.3 logs). This suggests that both treatments had high efficiency in ensuring treated milk is safe despite unavoidable conditions in milking process and farms causing microbial contaminations. Several researchers who have reported similar finding on effectiveness of HPP treatment in reducing these bacterial counts include Stratakis et al. (2019) on *E. coli* reduction by 5.6 and 6.8 logs at 600 MPa for 3 and 5 min respectively, Liu et al. (2020) on *E. coli* reduction by 2.9 logs at 600 MPa for 5 min and Tan et al. (2020) on total coliform reduction by 1.6 logs at 450 – 600 MPa for 5 – 7 min.

Pathogenic bacteria such as the *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes* and *Salmonella spp.* were all undetected in fresh milk and also throughout the storage for the HPP and ESL treated milk. For safety consumption, *Bacillus cereus* and *Clostridium perfringens* in food should be less than 5 logs (Official Journal of the European Communities, 1984) and 4 logs (Gilbert et al., 2000) respectively, while *Listeria monocytogenes* and *Salmonella spp.* should not be detected in 25g of sample (Food Standards Australia New Zealand, 2018).

3.3.2 Physicochemical Analysis

Figure 3.3 shows the non-significant changes ($p>0.05$) on all physicochemical properties of HPP and ESL treated milk during 60 days storage except for acidity which has reduced from initially 0.20% to $<0.1\%$ after treatments (Figure 3.3b). For HPP milk, several researchers have reported acidity reduction accompanied by pH value increase after HPP treatment at 600 MPa (Liepa et al., 2018; Tan et al., 2020; Zobrist et al., 2005). During HPP, the casein micelle disaggregation alters minerals distribution and raises the concentration of ionic calcium in milk, resulting increase in phosphate concentration of the milk serum and pH (Liepa et al., 2018). Contrarily, heat treatments resulted decrease in milk pH (Ma & Barbano, 2003; Raynal-Ljutovac et al., 2007). The milk pH decreased with increasing temperature due to the changes in dissociation of ionisable groups (Tamime, 2008). Heat treatments induced transfer of calcium and phosphate to the colloidal phase, causing thermolytic degradation of lactose which produces lactic acid that reduces pH (Raynal-Ljutovac et al., 2007).



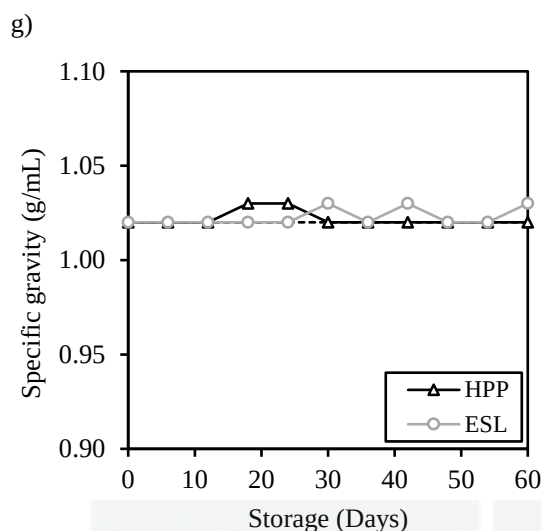


Figure 3.3: Changes in a) pH, b) Acidity as Lactic Acid, c) Protein, d) Fat, e) Total Solid, and f) Non-Fat Milk Solids and g) Specific gravity of HPP and ESL milk during 60 days' storage. Dotted lines represent the raw milk before treatment.

(Refer data from Appendix B3)

In comparing the pH of both treated milks, HPP had higher pH stability than ESL which had lower acidity starting from Day 12 to 60. This suggests that the ESL milk showed faster deterioration sign than HPP milk, probably due to the higher bacteria load that produces lactic acid and generation of free fatty acid through fat lipolysis (Michael et al., 2013). It is common to have acidity increase in spoilt milk as observed in the raw milk of 6 days' storage (Appendix B1). The changes in milk acidity is probably associated with an increase in free fatty acids during storage (Swartzel, 1983), age gelation (Andrew et al., 1977) or browning reactions (Vankatachalm & Macmahon, 1991). Similarly reported on pH decrease in HPP and heat-treated milk stored for 22 days by Tan et al. (2020) and increase in acidity by Hassan et al. (2009) for UHT milk of 12 weeks' storage.

The HPP and ESL treatments did not cause significant changes ($p>0.05$) to protein and fat contents of milk during its 60 days' storage (Figures 3.2c and 3.2d). This result was

consistent with Tan et al. (2020) who reported no significant changes in the total protein content of HPP and pasteurised milk samples during 22 days' storage. Although the amount of protein content seen unaffected, HPP can cause protein denaturation (Serna-Hernandez et al., 2021). Pressure implication causes denaturation of whey proteins which ultimately affect the structure of micelles and causes disruption of micelles, re-aggregation and removal of casein micelles. According to Abbas Syed et al. (2021), casein micelles denature and start to rupture at different levels of pressure. As the pressure level increases, casein micelles along with disintegration starts interacting with whey proteins. After partial denaturation at certain pressures, it starts interacting with casein micelles to form large globules of higher molecular weight. Consequently, the denatured proteins may form aggregates or clumps, influencing the texture and appearance of milk leading to loss of homogeneity. For fat contents, most studies have proven the effectiveness of HPP over heat treatments as high pressure do not damage milk fat globule membrane, thus lipolysis is prevented and fat content retained (Huppertz et al., 2002; Özer & Akdemir-Evrendilek, 2014). Heat treatment however has been reported to cause fats deterioration as severe temperature increased susceptibility of fat to oxidation, leading to release of free fatty acids by lipase activity and reduces fat (Hassan et al., 2009). Tan et al. (2020) observed that the total fat content HTST pasteurised cow milk reduced significantly starting from Day 11 but no significant changes for HPP samples throughout 22 days of storage. Heat treatment of several branded UHT milk was also reported to experience significant protein content reduction from 3.30 – 3.70 % to 2.35 – 3.48% after 12 weeks of storage (Hassan et al., 2009).

HPP treatment on milk was also found to give insignificant changes ($p>0.05$) to other properties of milk including total solids and non-fat milk solids. Only minimum increase in total solid in milk was observed after HPP and ESL treatment by 1.7 % and 1.3% respectively (Refer data from Appendix B2). For non-fat milk solids, HPP treatment slightly increased the non-fat milk solids to 8.50 g/100g but ESL heat treatment decreased minimally to 8.33 g/100g from originally 8.40 g/100g. In comparison, HPP treatment resulted in higher amount of total solids content and non-fat milk solids (when compared to ESL treatment - Figure 3.3e and 3.3f). The increase in total solids and non-fat solid of HPP treated milk could be due to precipitation fraction of large casein-casein and formation of casein-fat aggregates during HPP (Tran et al., 2018) or induced fat crystallisation due duration of pressure treatment and storage (Dumay et al., 1996). Tan et al. (2020) reported no significant difference in total solid and non-fat milk solids of HPP-treated samples. For heat treatment, Ajmal et al. (2018) found pasteurisation treatment (65°C, 30 min) did not cause significant changes in total solid of milk after 6 days of storage at 4°C while Aldubhany et al. (2014) reported gradual increase in total solids content in UHT milk treated at 143 – 145°C from 11.7% to 12.2 % during 180 days of storage at 4°C. In this work, ESL treatment decreased non-fat milk solids from 8.40 g/100g to 8.33 g/100g. Both HPP and ESL milk experienced inconsistent changes during 60 days storage (Figure 3.3e and 3.3f). Specific gravity was the only property that was not affected, retained at 1.02 – 1.03 g/mL throughout this study by any factors including the types of treatment and duration of storage (Figure 3.3g).

3.3.3 Nutritional Analysis

Vitamin Content

Most vitamin contents of milk in this study were found to be at a relatively low level due to the nature of milk where vitamins are not present as primary source of milk (Macdonald et al., 2011). The beta-carotene, vitamins A, B1, B2, B3, B6, C and E were below limit of reporting (LOR) which records the minimum concentration of a substance in a sample that can be reliably detected by a laboratory. Hence, levels below this limit may cause inaccuracies when reporting changes. Generally, some vitamins such as vitamins A, B6, B12 and C in fresh milk continued to degrade during the 6 days of refrigerated storage (Appendix C1).

Both HPP and ESL treatments did not cause significant changes ($p>0.05$) in milk vitamins except vitamin C which showed a slight increase after treatments (Table 3.2). This was unexpected and explained by variations due to detection level below LOR. In HPP studies on milk vitamin, Moltó-Puigmartí et al. (2011) reported the total vitamin C level in human milk was maintained after HPP (600 MPa, 5 min, 22 – 27 °C). Retention of vitamins in milk was probably due to the property of vitamin which consists of small molecules and covalent bond that was not affected by high pressure (Balci & Wilbey, 1999). However, Kim et al. (2008) observed a significant decrease in levels of vitamin C, niacin, and riboflavin in HP-treated samples at 200 MPa with increasing treatment duration from 10 to 30 min, while Martysiak-Zurowska et al. (2017) reported loss of vitamin C by approximately 6% after HPP treatment at 193 MPa. For heat treatment, a systematic review by Macdonald et al. (2011) reported vitamins B12 and E decreased following pasteurisation, whereas vitamin A increased.

Pasteurisation (62.5°C, 30 min) also caused vitamin C reduction in human milk by 35% (Martysiak-Zurowska et al., 2017) and 20% (Moltó-Puigmartí et al., 2011).

Table 3.2: Effects of treatments on vitamin content of fresh milk.

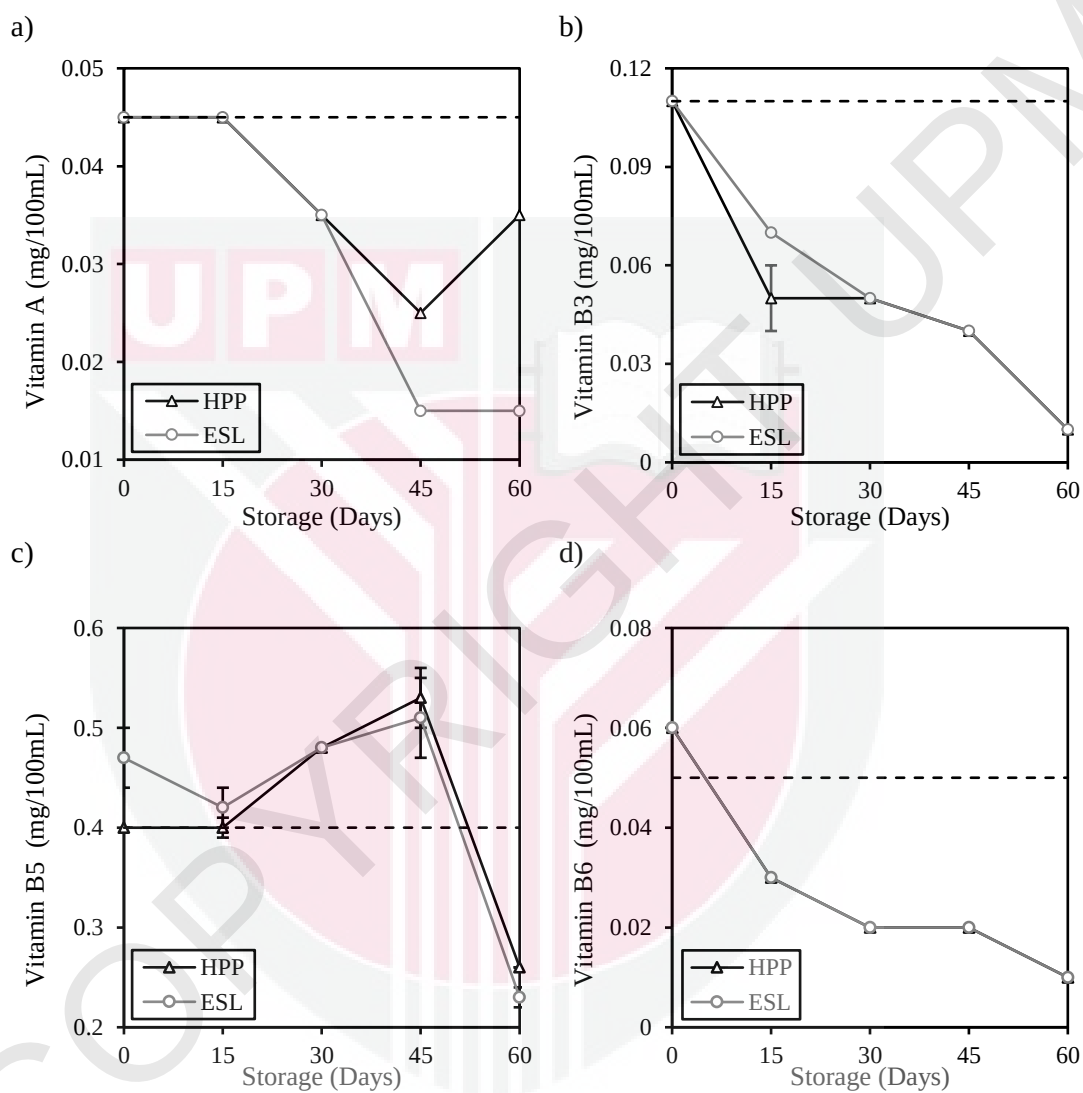
Vitamin Content	LOR Unit	Raw Milk	Treatments	
			HPP	ESL
Vitamin A (as Retinol) *	0.1 mg/100 mL	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a
Beta-carotene *	1 mg/100 mL	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a
Vitamin B1 *	0.25 mg/100 mL	0.04 ± 0.00 ^a	0.03 ± 0.00 ^a	0.04 ± 0.00 ^a
Vitamin B2 *	0.25 mg/100 mL	0.17 ± 0.00 ^a	0.18 ± 0.00 ^a	0.18 ± 0.00 ^a
Vitamin B3 (as Niacin) *	0.25 mg/100 mL	0.11 ± 0.00 ^a	0.11 ± 0.00 ^a	0.11 ± 0.00 ^a
Vitamin B5	0.25 mg/100 mL	0.40 ± 0.00 ^a	0.40 ± 0.00 ^a	0.47 ± 0.03 ^a
Vitamin B6 *	0.25 mg/100 mL	0.05 ± 0.01 ^a	0.06 ± 0.00 ^a	0.06 ± 0.00 ^a
Vitamin B7 (Biotin)	1 µg/100 mL	4.00 ± 0.00 ^a	4.00 ± 0.00 ^a	4.00 ± 0.00 ^a
Vitamin B9 (Folic Acid)	1 µg/100 mL	6.00 ± 0.00 ^a	6.33 ± 0.33 ^a	6.33 ± 0.33 ^a
Vitamin B12 (Cyanocobalamin)	1 µg/100 mL	1.67 ± 0.58 ^a	2.00 ± 0.00 ^a	2.00 ± 0.00 ^a
Vitamin C *	2 mg/100 mL	1.00 ± 0.00 ^a	1.67 ± 0.33 ^{ab}	2.00 ± 0.00 ^b
Vitamin D	0.1 µg/100 mL	2.50 ± 0.00 ^a	2.50 ± 0.00 ^a	2.50 ± 0.00 ^a
Vitamin E (Alpha-Tocopherol) *	0.1 mg/100 mL	0.07 ± 0.00 ^a	0.07 ± 0.00 ^a	0.06 ± 0.00 ^a
Vitamin K	0.1 µg/100 g	0.3	0.65	0.24

Mean ± standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$.

* Results showing vitamin content lower than LOR. LOR is the limit of reporting.

Most studies on the effect of high pressure on the stability of vitamins focused on fruit and vegetable juices instead of milk. Vitamins were well-retained in juices after high-pressure treatment but degraded during refrigeration storage (Cao et al., 2012; Terefe et al., 2013). Similarly, the vitamins in HPP and ESL milk in this study also reduced gradually over the 60 days' storage with some new observations (Figure 3.4). Most vitamins in treated milk include vitamins A, B3, B5, B6 and C fell below its original level at the end of storage except for vitamins B7, B9 and B12. From the wide range of vitamins studied, vitamins A and B5 were retained better in HPP milk by 75% and 65% respectively as compared with ESL milk by only 25% and 48.9% at the end of 60 days' storage (Figure 3.4a and 3.4c). The effect of heat treatment on the stability of vitamin in milk during storage were thoroughly studied with most researchers reported losses in vitamin content of milk during storage (Ajmal et al., 2018; Khan et al., 2017;

Löker et al., 2003). Surprisingly, vitamin C was retained better in ESL milk by 41.5% as compared to HPP milk with only 22.4% of vitamin C retained after 60 days' storage (Figure 3.4h).



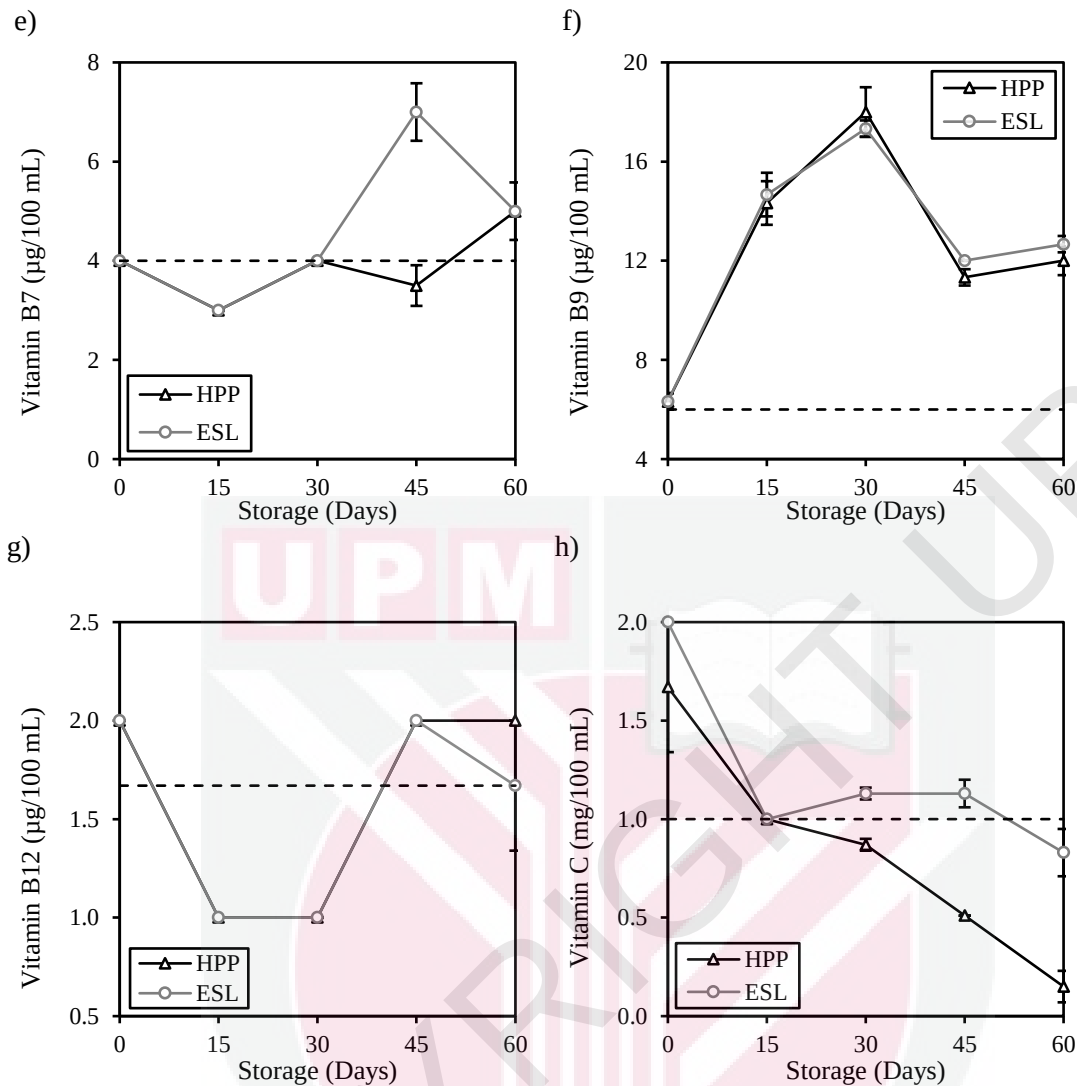


Figure 3.4: Changes in a) Vitamin A, b) Vitamin B3, c) Vitamin B5, d) Vitamin B6, e) Vitamin B7, f) Vitamin B9, g) Vitamin B12 and h) Vitamin C of HPP and ESL milk during 60 days' storage. Dotted lines represent the raw milk before treatment.

(Refer data from Appendix C2)

Mineral Content

The highest mineral contents in the milk samples used were potassium followed by calcium, phosphorus, magnesium and zinc. Selenium was not detected in the milk sample. All minerals in fresh milk showed inconsistent changes throughout the 6 days' storage except selenium which remained at an undetectable level (Appendix D1). The effects of HPP and ESL treatment on mineral contents of milk were not significant

($p>0.05$) except for zinc content in ESL milk (Figure 3.5). Despite no significance difference between mineral contents of HPP and ESL milk samples, the HPP treatment however gave slightly higher values for potassium, calcium, phosphorus and magnesium, suggesting better preservation than the ESL treatment. HPP milk also has higher calcium, phosphorus and magnesium contents retention by 99.3%, 99.4%, and 99.1% respectively, as compared to ESL treatment by only 95.8%, 96.5% and 97.2%.

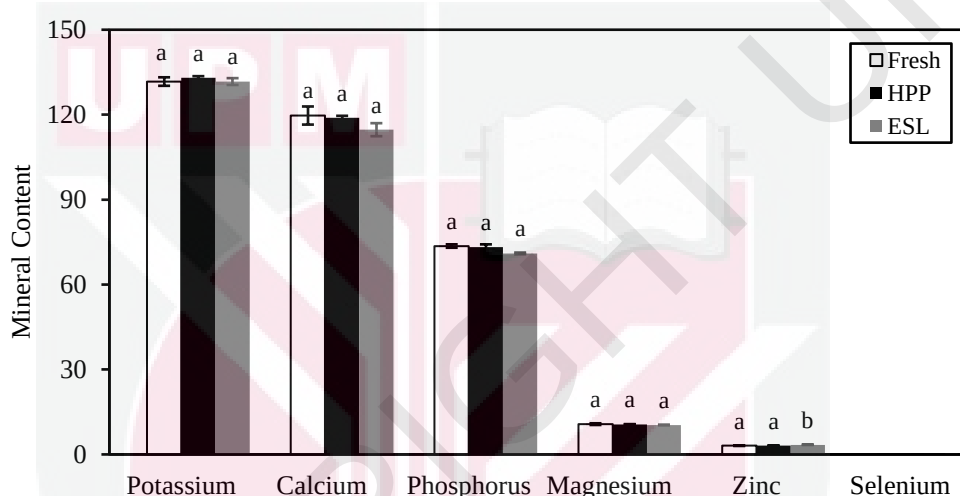


Figure 3.5: Changes in mineral content of raw milk after HPP and ESL treatment. Potassium, Calcium, Phosphorus and Magnesium in unit mg/100mL; Zinc and Selenium in unit mg/kg.
(Refer data from Appendix D2)

Several researchers also reported an increase in mineral content of milk after HPP treatment (Barba et al., 2015; Zobrist et al., 2005). The changes in mineral content of milk is associated with the change in mineral distribution and solubility due to disaggregation of micelles during HPP treatment (Serna-Hernandez et al., 2021). Under high pressure conditions, the compacted water molecules penetrated and hydrated the micelles, causing a dissociation of ion pairs that led to a release of calcium phosphate and physical alternations in the casein micelles (Goyal et al., 2013; Munir et al., 2019). Similarly, the phosphorus and magnesium found in casein micelles were

affected by disruption and destabilisation of micelles under high pressure conditions (Serna-Hernandez et al., 2021). Vandhana et al. (2020) reported HPP (100-1000 MPa) induced shifts in the mineral balance of milk, causing an increase in solubilisation of colloidal calcium phosphate (CCP), which led to the increase in concentration of ionic calcium in milk. Heat treatments, in opposite, caused conversion of mineral from liquid to colloidal form and reduce soluble salt content in milk (de la Fuente, 1998). This is supported by Šestan et al. (2016) who found heat treatment of milk (82°C, 15s) led to loss of calcium up to 40% due to transition of calcium ion into insoluble calcium salt such as calcium citrate and calcium oxalate that could not be utilised by the human body.

Significant changes were observed on the mineral contents of treated milk over 60 days of storage but in an inconsistent manner (Figure 3.6). In general, both HPP and ESL treatments have showed comparable results in mineral stability. There was no significance difference ($p > 0.05$) observed between mineral content of HPP and ESL milk stored for 60 days. As compared to fresh milk, significant loss ($p < 0.05$) was observed for both HPP and ESL milk in potassium and zinc, whereas calcium, phosphorus and magnesium contents increased after storage (Figure 3.6). The loss of potassium and zinc content were 6% and 19.2% respectively for HPP milk, while 4.8% and 20.6% for ESL milk. Selenium remained undetected at levels < 0.2 mg/kg.

The results obtained for mineral content were difficult to prove due to limited literatures and references found. However, the increase in mineral during storage could be related to dissociation of caseins and the release of free ionic mineral during storage. In the case for calcium, Guggisberg et al. (2022) believed that more free ionic calcium

was released by enzymatic/proteolytic degradation of caseins with undetermined microbes probably psychrotrophs as psychrotrophic proteases could partially degrade β - and κ -caseins. Guggisberg et al. (2022) stated that two-thirds of the calcium in raw milk is found in the micellar/colloidal phase and is linked to the micelles of the casein protein, while a one-third of the total calcium in the serum phase is soluble calcium, which is either ionic/free calcium Ca^{2+} (about 7% of total calcium), calcium citrate (about 23% of total calcium), or phosphates (about 2% of total calcium).



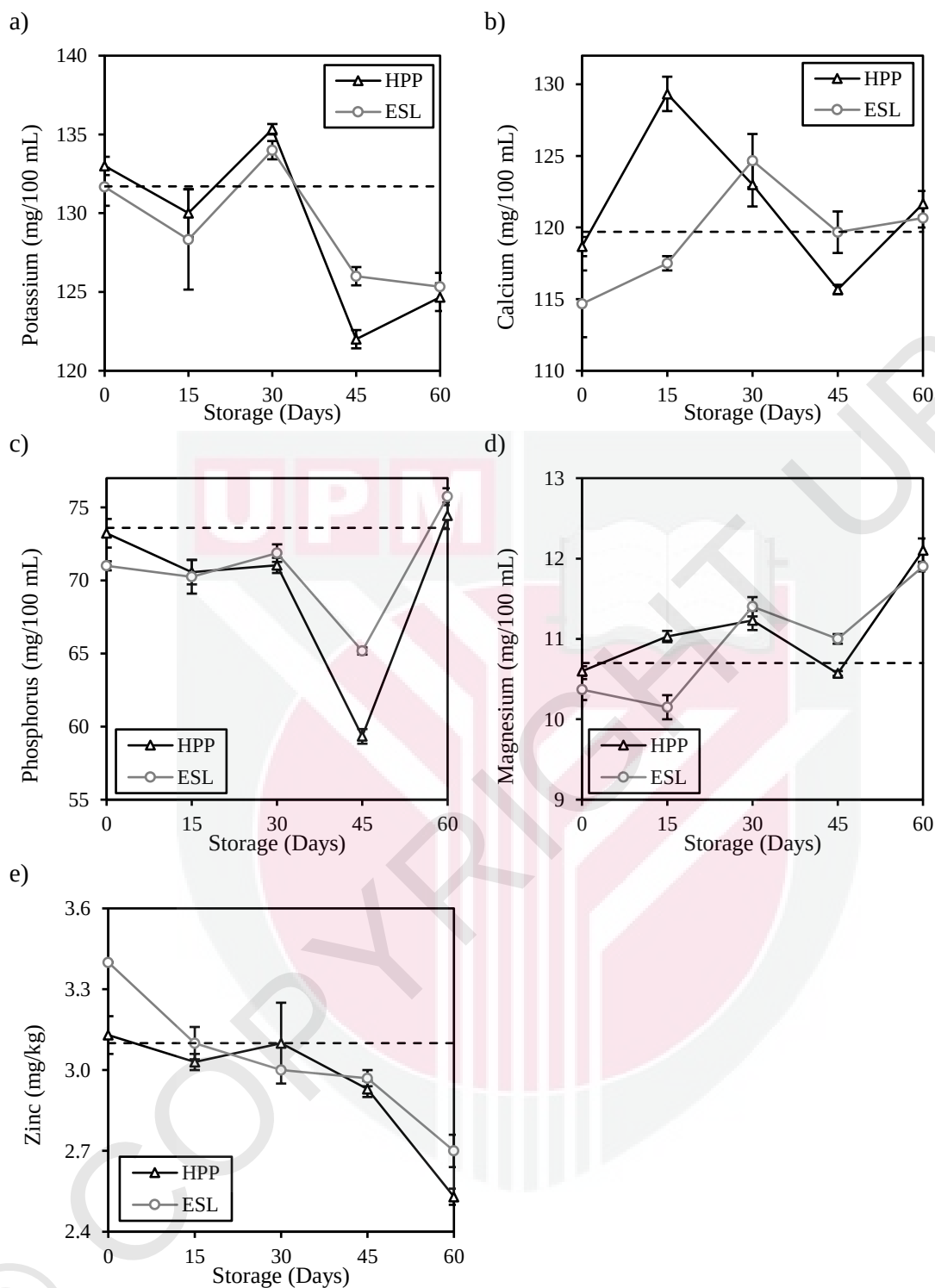


Figure 3.6: Changes in a) Potassium, b) Calcium, c) Phosphorus, d) Magnesium and e) Zinc contents of HPP and ESL milk during 60 days' storage. Dotted lines represent the raw milk before treatment. (Refer data from Appendix D3)

Andrés et al. (2016) observed different results with no significant changes in mineral profile of milk after treatments (i.e. pasteurisation at 80°C for 3 min and HPP at 450–650 MPa for 3 min at 20°C) and storage for 45 days at 4°C. In heat-treated milk, Khan et al. (2017) reported non-significant drop in selenium and zinc content of pasteurised milk (65°C, 30 min) stored at 4°C for 6 days, by only 10.6% and 0.9% respectively, while Ajmal et al. (2018) reported 0.8% dropped in zinc content of pasteurised milk (65 °C, 30 min) after 6 days of storage.

3.4 Conclusions

HPP milk showed promising results of storage shelf life beyond 60 days with all microbial analysis meeting permitted levels, while ESL milk has presented unsafe consumption status due to post contamination during filling process. This suggested that HPP has higher capability in reducing risk of contamination as the nature of HPP being an in-pack batch process where the product is first packaged and then processed as compared to ESL where product is processed and then aseptically filled in semi-continuous process. HPP milk also had superior vitamin A and B5 stability while ESL milk had better vitamin C retention (41.5%) as compared to HPP milk (22.4%). In terms of mineral stability, HPP and ESL milk were similar. The physicochemical, vitamin and mineral contents of HPP and ESL milk did not differ significantly, but there was a small degradation throughout the 60 days storage period.

CHAPTER 4

ORGANOLEPTIC PROPERTIES OF INDUSTRIAL HPP TREATED FRESH COW MILK VIA SENSORY ANALYSIS WITH COMPARISONS WITH THE ESL TREATMENT

4.1 Introduction

Sensory analysis is often used in food sectors to evaluate food products' quality and consumer acceptance via an attribute assessment of texture, flavour, taste, appearance, smell, etc. using the senses of sight, smell, taste, and touch (Hogan et al., 2005; Ruiz-Capillas & Herrero, 2021). The trend of using sensory analysis in new product development in the food industry is documented by the European Institute of Innovation and Technology (EIT) Regional Innovation Scheme (Świąder & Marczevska, 2021). Sensory analysis is also critical for every application of milk and it is widely used by dairy manufacturers to identify deviation in processing and handling (Schiano et al., 2017). With known positive effects of HPP treatment on milk as discussed in previous chapter, this chapter has focused on measuring the sensory attributes of HPP-treated milk in comparison with heat-treated milk in the effort of new product development.

In comparing two products that are virtually identical, the triangle test is most frequently applied in the dairy industry to determine whether two formulas are perceived to be different (Schiano et al., 2017). The outcome of this basic test can be analysed using binomial calculation where its p-value aids in determining whether a difference exists. The predefined statistical significance level for most difference tests is often set at $p = 0.05$. If the resulting p-value is less than 0.05, the null hypothesis is

rejected and the products are believed to be distinct. The triangle test has been employed by numerous researchers in milk studies. The triangle test was used by Lynch et al. (2005) to determine whether the taste of pasteurised milk samples with an elevated amount of conjugated linoleic acid and vaccenic acid were notably different. Hanson and Metzger (2010) conducted the triangle test to determine whether consumers could distinguish between samples fortified with increased amount of Vitamin D. Bandla et al. (2012a) used the triangle testing method to verify whether UV-C processing of raw cow milk treated in a continuous flow coiled tube ultraviolet reactor resulted in perceived changes to the taste of milk. Bottiroli et al. (2020) conducted the triangle test to examine if ultra-high temperature hydrolyse-lactose milk stored at various temperatures exhibits discernible sensory variations.

An acceptance and preference test are commonly included in a sensory test to get the consumer's judgment on the finished product. Manufacturers can use this evaluation to understand consumers' likes, dislikes, and preferences before spending a large amount of capital on the production of a new product or investing in a new technology. The acceptance test is a method of directly assessing each particular product, typically using a type of scale to quantify overall acceptability (Lawless & Heymann, 1999). Information obtained from an acceptance test can be analysed through parametric statistical analysis and data generation (Hein et al., 2008). The preference testing requires selection of the preferable product using ranking techniques or direct queries (Lawless & Heymann, 1999). This method enables identification of sample preference within the test set and from there the marketability of the new product can be assessed (Hein et al., 2008). In the acceptance and preference test, the just-about-right (JAR) scale and a hedonic scale are frequently employed to measure the suitability level of

an attribute and to test consumer preference and acceptability of foods (Zhi et al., 2016). JAR scales are bipolar labelled attribute scales with a midpoint anchored with “just-about-right (JAR)” or two semantically opposite anchors, e.g., “too strong” and “too weak” (Song et al., 2021). Tribst et al. (2020) used JAR and hedonic scales to indicate whether stirring and homogenisation have effects on the acceptance of milk products in terms of texture, flavour, acidity, and overall liking. Similarly, combination of JAR and hedonic scales were used by Dong et al. (2021) in their investigation on the effects of augmented reality (AR) environments on sensory responses of consumers towards different dairy products. For attributes which did not achieve the JAR level, the penalty analysis (PA), or mean drop analysis, is conducted to identify which attribute of the product deviated significantly from optimal levels. The PA allows the product developer to identify attributes that are most penalising to product performance, thus allowing improvement to be made on that attribute.

In general, the sensory quality of HPP milk has been reported to be better than that of heat-treated milk. The organoleptic properties of milk have improved with higher acceptability scores due to reduced proteolytic activity after undergoing high pressure treatments at 400 MPa for 15 min (García-Risco et al., 2000), higher aroma and acceptability of HPP milk treated at 450–650 MPa for 3 min over pasteurised milk (Andrés et al., 2016), and a different aroma profile in HPP milk at 600 MPa for 5 min compared to LTLT milk (63 °C for 30 min) due to higher levels of ketones (Liu et al., 2020). Andrés et al. (2016) added that HPP-treated milk was almost similar to untreated milk. By referring to the literature, this study was to evaluate the sensory properties of HPP treatment of milk at 600 MPa for 10 min in comparison with heat

treatment at 125 °C for 4 s for overall milk acceptance and in terms of colour, milkiness, creaminess, mouthfeel, and aftertaste attributes.

4.2 Materials and Methods

4.2.1 Processing of Milk

Fresh cow milk was collected from the university farm, Ladang 16, Universiti Putra Malaysia. After collection, the sample milk was mixed evenly and cooled to 4 °C. Milk subjected to high pressure processing was transferred into 350 mL food grade polyethylene terephthalate (PET) bottles. The milk samples were pressurised at 600 MPa for 10 min in a high-pressure processing equipment (Hiperbaric 55; Hiperbaric High Pressure Technologies, Burgos, Spain). The pressurisation process was completed at room temperature at 25 ± 2 °C. Milk subjected to heat treatment was ultra-pasteurised at 125 °C for 4 s referring to the indirect heating approach by Deeth (2017). The customised pasteuriser (Powerpoint International Ltd., Saitama, Japan) consists of a tubular heat exchanger system with 2 heating, 3 cooling, and 1 holding tube/s at a capacity of 20 LPH. Ultra-pasteurised milk was also transferred into 350 mL PET bottles at 6 °C. All milk samples were stored at 6 ± 1 °C before conducting the arranged sensory tests the next day after both treatments that were conducted on a same day. High pressure treated milk was labelled as HPP milk while ultra-pasteurised milk was labelled as ESL milk.

4.2.2 Sensory Analysis

Sensory profiling was conducted by internal panellists from a dairy manufacturing firm and personnel at Universiti Putra Malaysia with reference to the guidelines in ISO 8586:2014 which specify the selection, training, and monitoring of assessors and expert sensory assessors (ISO 8586, 2014). The panellists selected were non-smokers, capable to perform sensory tasks, and engaged in discussions of sensory attributes during a 10-min briefing session conducted to train the assessors to be familiar with the products, lexicon, and the prescribed sensory evaluation procedures. There were 38 panellists from different ethnic groups (68% Chinese, 5% Malay, 24% Indian, and 3% Siamese) participated in this evaluation. The sensory evaluation was divided into 2 different sessions, *i.e.* the triangle test and the acceptance and preference test. The samples of approximately 30 mL were served at a temperature of 17 °C. Plain water was provided for panellists to clean their palate between sample tastings. Standard fluorescent light was used during the evaluation.

Triangle Test

The triangle test was carried out in accordance with ISO 4120 (2004). Three samples with different 3-digit random codes were presented simultaneously to the panellists. The serving approach was relied on randomisation with an assured two samples from the same processing method and one from another (e.g., set of two HPP and one ESL milk samples, or two ESL and one HPP milk samples). Each panellist was required to taste all 3 milk samples and identify the odd sample. The panellists were instructed to rinse their mouth with plain water before evaluating each sample. After identifying the odd sample, each panellist was required to indicate the degree of difference between the odd and duplicate samples to the level of difference felt as “None”, “Slight”,

“Moderate”, “Much”, or “Extreme” in accordance with ISO 6685 (2017). In order to summarise the responses, the qualitative data were transformed to quantitative data as follows: None-0, Slight-1, Moderate-2, Much-3, and Extreme-4, and were analysed using descriptive statistics analysis. The binomial table was used to determine minimum numbers of correct judgements to establish significant difference at desired probability levels for the triangle test.

Acceptance and Preference Test

In the acceptance and preference test, the panellists were served with a new set of two samples consisting of one HPP and one ESL labelled with 3-digit random codes. Each panellist tasted and evaluated their overall liking using the 7-point hedonic scale followed by the acceptance and preference using the 5-point just-about-right (JAR) scale for five attributes: colour, milkiness, creaminess, mouthfeel, and aftertaste. For the 7-point hedonic scale evaluating overall liking, panellists rated each sample as 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = like very much. The hedonic data obtained were categorised into 3 groups, *i.e.* the T3B Overall Liking (Top 3 Box Rating), B3B Overall Liking (Bottom 3 Box Rating), and Neutral Overall Liking (Neutral) to determine the preferences of panellists. The attributes and rating description for JAR are displayed in Table 4.1. The JAR data obtained were analysed using penalty analysis (PA) consisting of 3 steps: (1) collapsing the 5-point JAR scale into 3 groups (too low, JAR, and too high), (2) calculating the percentage of consumers and the mean overall liking score for each group, and (3) calculating the mean drop by subtracting the mean overall liking score for the JAR group from the mean overall liking score of the “too high” or “too low” groups (Rothman, 2007; Zhi et al., 2016).

Penalty analysis was conducted to verify which of the attributes affected the acceptability of the product to a greater or lesser extent with the main objective being to improve the sensory quality of the product (Ortega-Heras et al., 2019).



Table 4.1: Attributes and 5-point just-about-right (JAR) scale used.

Sensory Attributes	Rating Description
Colour	1. Too Light 2. Slightly Light 3. Just Right 4. Slightly Dark 5. Too Dark
Milkiness	1. Not Milky Enough 2. Slightly Not Milky 3. Just Right 4. Slightly Milky 5. Too Milky
Creaminess	1. Not Creamy Enough 2. Slightly Not Creamy 3. Just Right 4. Slightly Creamy 5. Too Creamy
Mouthfeel	1. Too Thin 2. Slightly Thin 3. Just Right 4. Slightly Thick 5. Too Thick
Aftertaste	1. Too Weak 2. Slightly Weak 3. Just Right 4. Slightly Strong 5. Too Strong

4.2.3 Data Analysis

XLSTAT (Version 2021.4; Addinsoft, Paris, France) and Microsoft Excel software were employed to analyse the data obtained in sensory evaluation. For the triangle test, ANOVA analysis and binomial table for paired preference two-tailed test were used to identify whether there was any significant difference on the overall liking and preference between the different products. Results from the overall acceptance and preference test were analysed using analysis of variance (ANOVA) and Tukey's test to determine the significant differences between the products. Statistical differences with p-value less than 0.05 were considered significant.

4.3 Results and Discussion

4.3.1 Triangle Test

The triangle test showed significant difference ($p < 0.05$) between HPP and ESL milk at 95% confidence level with p -value < 0.0001 (Table 4.2). In a triangle test with three samples, the guessing chance was just $1/3$; yet, 89.5% (34 out of 38) of panellists correctly recognised the odd sample at ($p > 0.05$). According to Lawless & Heymann (2010), triangle test only required a minimum of 19 correct responses to imply a significant difference between the samples. Thus, this result demonstrates that the detection of difference between HPP and ESL milk samples was not accidental.

In order to express the sensory distance of the objects, the proportion of discriminators, p_d in the population of interest was calculated using formula $p_d = (p_c - p_g)/(1 - p_g)$, where p_c is the probability of a correct answer and p_g is the guessing probability, assuming panellists may comprise ignorant panellists who make guesses and discriminators who discriminate correctly and provide appropriate answers (Haubo & Christensen, 2020). In this test, the proportion of discriminators p_d , which was equal to 0.842, indicates that panellists had high discriminative ability.

Table 4.2: Summary of triangle test result.

Test	Triangle Test
Number of panellists	38
Probability of correct answers, p_c	0.895
Guessing probability, p_g	0.333
Proportion of discrimination, p_d	0.842
Statistic	33.0
p -value	< 0.0001
alpha	0.05

In previous study, Grabowski et al. (2013) revealed noticeable differences in the taste of HTST pasteurised milk (72.7 °C, 15–30 s) compared with ESL milk (125–127 °C, 2–4 s) where 80% of panellists were capable of distinguishing them clearly, as ESL milk had a noticeable cooked and sulphur flavour when compared with milk treated at a lower temperature. The key factor causing a change in flavour during the thermal processing of milk is correlated with Maillard reaction, lipid degradation, thermal denaturation of serum (whey) protein, and other proteins in the milk fat globule membrane (Jo et al., 2018). During thermal processing, sugar (lactose) and protein (amino group) in milk undergo a Maillard reaction leading to the formation of flavour components such as Strecker aldehydes, sulphur- and nitrogen-containing compounds, maltol, and diacetyl which affect the taste of milk (Boekel, 1998). Lipid degradation during thermal processing causes off-flavour due to the liberation of volatile fatty acids, such as butyric acid, and oxidation of free or glyceride bound unsaturated fatty acids with subsequent formation of volatile compounds (Andersson et al., 1981). Thermal denaturation of the whey proteins, primarily β -lactoglobulin, and the proteinaceous material associated with the fat globule membrane generates free sulphydryls and volatile sulphides resulting in cooked, cabbage, and sulphur flavours in milk (Calvo, 1992).

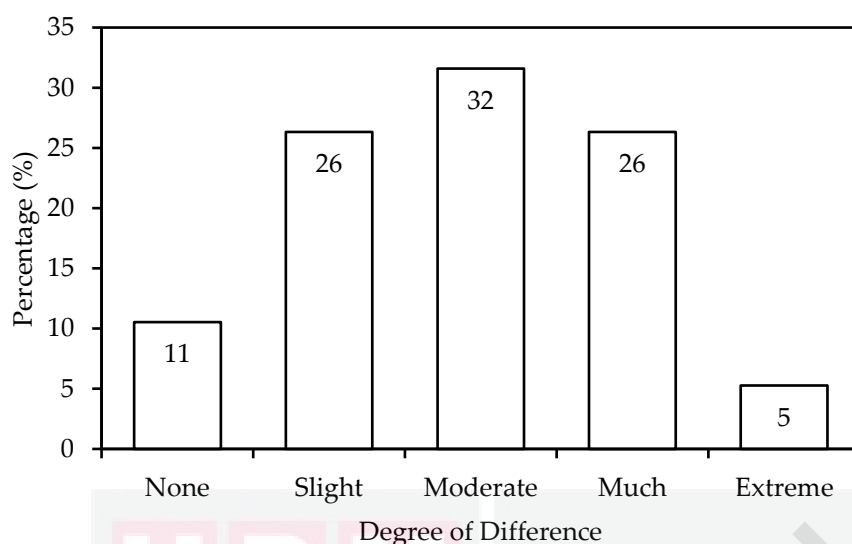


Figure 4.1: Selection of degree of difference “None”, “Slight”, “Moderate”, “Much”, and “Extreme” from the correct responses.

There were 89.5% (34 out of 38) panellists successfully identified odd samples indicated the degree of difference between the milk samples as shown in Figure 4.1. Twelve panellists (32%) selected “Moderate”, followed by 10 panellists (26%) equally selecting “Slight” and “Much”, and only 2 panellists (5%) indicating “Extreme”. The quantitative descriptive statistics analysis for the degree of difference at “Moderate” with a mean score of 1.895 were displayed in Table 4.3. The kurtosis value -0.689 indicates that this distribution is slightly less peaked than normal with a negative skewed distribution at the value of -0.049 . Further analysis in terms of specific attributes of odour, appearance, mouthfeel, taste, and aftertaste are used to describe these differences in milk samples.

Table 4.3: Descriptive statistics analysis for “Moderate” difference between milk samples.

Mean	1.895
Standard Error	0.176
Median	2
Mode	2
Standard Deviation	1.085
Sample Variance	1.178
Kurtosis	-0.689
Skewness	-0.049
Range	4
Sum	72
Count	38
Confidence Level (95.0%)	0.357

4.3.2 Acceptance and Preference Test

The overall liking evaluation on a 7-point hedonic scale is shown in Table 4.4 HPP milk scored higher on the T3B overall liking (Top 3 Box Rating) at 61%, while ESL milk scored slightly lower at 53%. The average mean score of overall liking of HPP milk was also slightly higher at 4.66 when compared with ESL milk at 4.34. This result revealed that the panellists slightly preferred HPP milk compared to ESL milk. However, statistical analysis showed no significant difference ($p > 0.05$) between HPP and ESL milk in the overall liking mean score and preference test.

Table 4.4: Summary for overall liking and preference test of HPP and ESL milk.

Overall Liking and Preference (7-Point Hedonic Scale)	Milk Samples	
	HPP	ESL
B3B Overall Liking (Bottom 3 Box Rating)	21%	26%
Neutral Overall Liking (Neutral)	18%	21%
T3B Overall Liking (Top 3 Box Rating)	61%	53%
Preference	47%	53%
Average Mean Score	4.66 ^a	4.34 ^a

^a Values marked with the same letters in the rows are not significantly different ($p > 0.05$).

Based on the average JAR score rated by 38 panellists for the 5 sensory attributes of colour, milkiness, creaminess, mouthfeel, and aftertaste (Figure 4.2), the aftertaste and milkiness of HPP milk were slightly lower at a rating value of 2.82 ± 0.7 and 2.89 ± 0.9 , respectively, as compared with ESL milk at a rating of 3.24 ± 1.1 and 3.08 ± 1.0 , respectively. The mouthfeel of ESL milk was slightly thinner based on panellist acceptance with a rating of 2.84 ± 0.8 . Panellists' perception toward creaminess of HPP and ESL milk were remarkably similar with a score of 2.95 ± 0.9 and 3.00 ± 1.0 , respectively. Except for colour ($p = 0.0003$), no significant differences ($p > 0.05$) observed in all attributes of milkiness ($p = 0.419$), creaminess ($p = 0.814$), mouthfeel ($p = 0.504$), and aftertaste ($p = 0.060$). More panellists rated HPP milk as being the just-about-right level for mouthfeel and aftertaste, while more panellists rated ESL milk as being the just-about-right level for colour, milkiness, and creaminess (Figure 4.3).

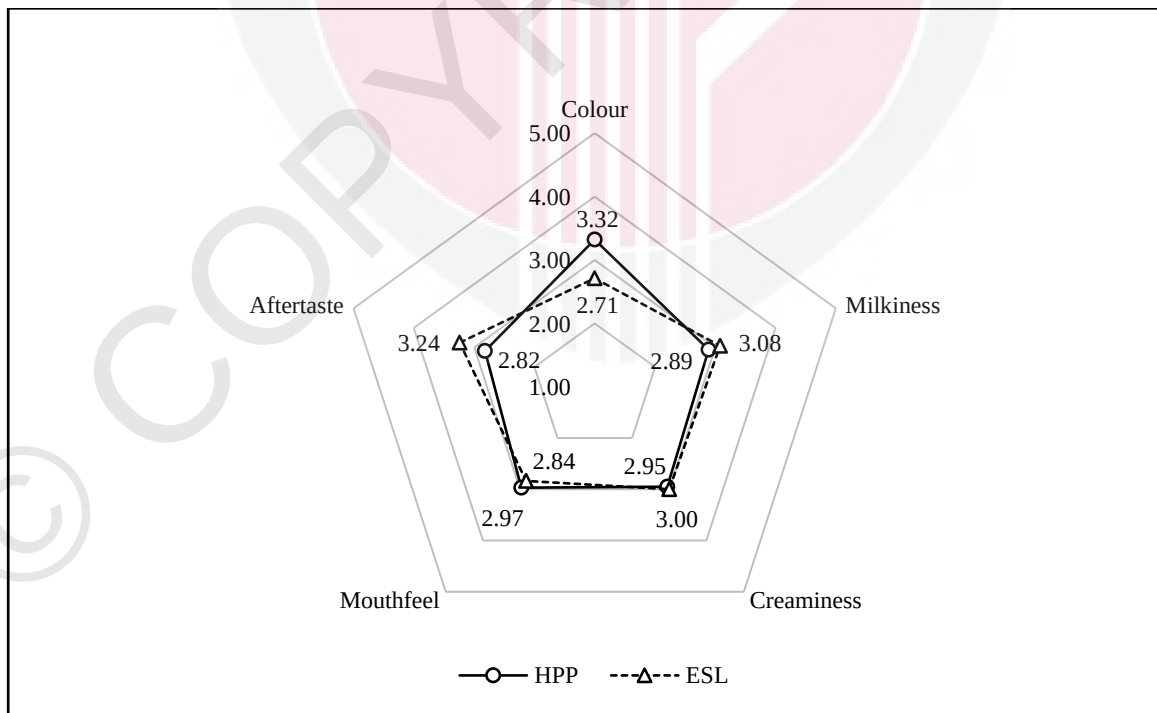


Figure 4.2: Comparison of average JAR scores between HPP and ESL milk.

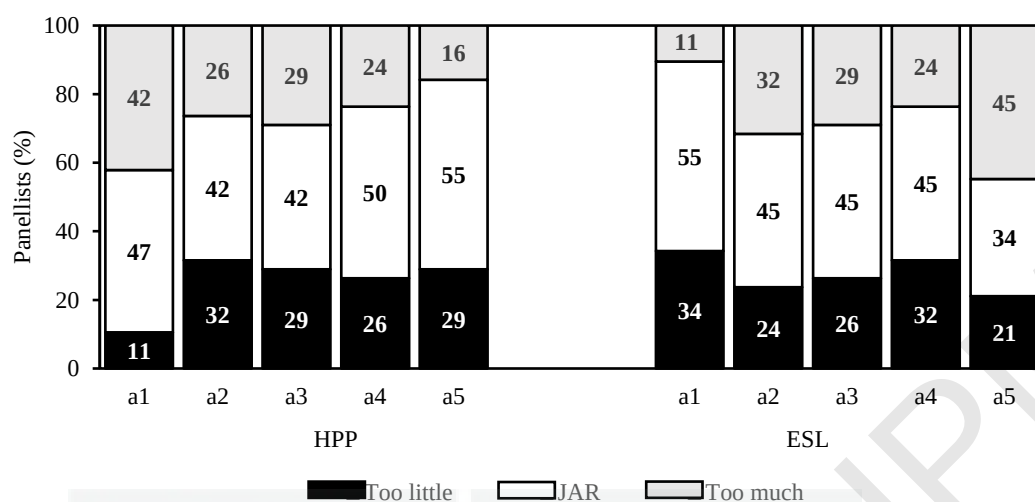


Figure 4.3: Percentage of panellists based on the collapsed JAR levels of the sensory attributes colour (a1), milkiness (a2), creaminess (a3), mouthfeel (a4), and aftertaste (a5) for HPP and ESL milk.

The noticeable change in colour in HPP milk was detected by panellists. This was verified by objective colour measurements instrumentally where the lightness, L-value, of HPP milk was lower at 90.8 as compared with ESL milk at 92.7 suggesting darker shades in HPP milk. The darker colour that appeared in HPP milk was in agreement with a previous study by Liepa et al. (2017) who found that there was a significant difference in colour ($p = 0.022$) of milk in the sensory properties acceptance evaluation of HPP milk (400 MPa) and pasteurised milk (78 °C, 15–20 s) by 80 panellists. The HPP milk was darker with significantly lower in the L* value (colour parameter) and had an increased b* value (yellowness). This could be a result of pressure-induced micellar fragmentation which reduces the micellar size, causing milk to lose its ability to scatter light, becoming translucent and dark (García-Risco et al., 2000). The disruptive effects of HPP on protein molecular forces such as hydrogen bonding, electrostatic, and hydrophobic lead to disaggregation of casein submicelles and dissociation of casein fractions from micellar casein to the soluble phase (Kielczewska et al., 2020). The compacted water molecules penetrate and hydrate milk

micelle, causing dissociation of ion pairs that leads to the release of soluble calcium and phosphorus from micellar casein (Serna-Hernandez et al., 2021). The disrupted micellar casein becomes smaller in size after releasing calcium and additional casein into milk serum (Orlien et al., 2006). The decrease in size of micellar casein further reduces the light scattering effect and results in a darker or more yellowish colour appearance in milk (Liepa et al., 2017). Although HPP milk was darker, the overall liking and preferences were not affected as the penalty was still lower by 0.712 at 0.439 when compared with ESL milk. The brighter appearance of ESL milk seems unfavourable to the panellists with a more significant penalty of 1.151 due to a wider range of scores in ESL than HPP milk despite ESL milk scoring well for selection and overall liking. This suggests that improvement is needed to reduce the brightness of ESL milk. ESL milk treated at a high temperature has a brighter colour due to serum protein denaturation and aggregation which increases the light scattering effect (Liepa et al., 2017).

The tactile sensations generated in the mouth during tasting can be used to evaluate the taste of dairy products. These attributes include milkiness, creaminess, and mouthfeel. A milky sample can be described as a product with a soft, slippery, and smooth mouthfeel; creaminess as a product which provides a greasy, miscible, and thick mouthfeel (Kirkmeyer & Tepper, 2003); while mouthfeel is a measure of the degree of thickness such as whether the sample provides a thin or thick sensation. The HPP milk scored lower in milkiness and creaminess but higher in mouthfeel properties when compared with ESL milk even though both were shown to be insignificantly different. The perceptions of milkiness, creaminess, and mouthfeel of differently treated milk samples presumably were associated with changes in particle size of fat

globules from different process methods rather than the difference in fat contents, as reported by Stratakos et al. (2019) who claimed the particle size of fat droplets present in dairy products is important in defining properties such as flavour release, mouthfeel, and emulsion. HPP milk had a lower creaminess sensation probably due to the HPP pressure applied was not sufficient to break down fat globules like those in the homogenisation process that break down fat globules into smaller and more uniformly dispersed particles. According to Galazka et al. (2000), high pressures condition at 600MPa tend to increase droplet flocculation or aggregation that cause formation of larger milk droplets. Larger milk droplets are often associated with a less creamy texture as larger fat globules can impart a grainy or gritty sensation, diminishing the perceived creaminess. Larger fat globules also more prone to creaming with formation of a layer of cream on top surface of milk leading to an uneven distribution of fat and less creamy sensation (Mulder & Walstra, 1974). However, Phillips et al. (1995) observed that the sensory scores for thickness, mouth coating, and residual mouth coating associated with the increase of fat content in milk.

Aftertaste is the residual mouthfeel remaining in the mouth after swallowing the sample from seconds up to a few minutes (Frøst et al., 2001). This study shows that HPP milk had a lower sensory score for aftertaste as compared with the ESL milk. Porubcan & Vickers (2005) reported that alternation of fat content, protein content, levels of defects such as oxidation, and other components of milk can change the sensory properties of milk, however, no researchers have discovered a connection between these variations with aftertaste of milk. The same author established that aftertaste was associated with the volatile compounds produced during the light-activated oxidation of milk and these presumably were associated with the increased

intensities of key attributes of “sour” and “dairy sour immediate”. This is consistent with the current findings where the majority of panellists voted ESL milk as giving a stronger aftertaste, probably due to a large number of volatile components generated during high temperature treatment. These volatile components resulting a cooked, sulphur, or eggy taste in milk.

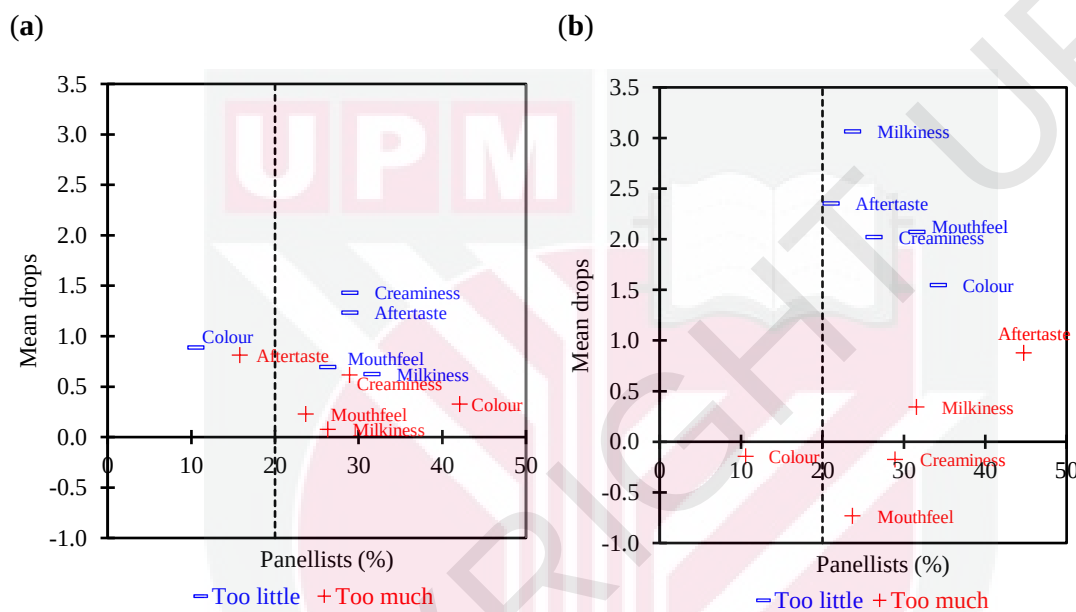


Figure 4.4: Mean drop plots for (a) HPP and (b) ESL milk. The dashed line represents the boundary of 20% of panellists.

The mean drop plot analysis in Figure 4.4 demonstrates the sensory attributes that have greater impacts on product acceptability. The mean drop is the difference between the mean overall liking score for the just-about-right group and the mean overall liking score of the “too much” or “too little” categories. The vertical dashed line at 20% of x-axis (Selection) denotes the boundary of respondents as a typical skew cut-off percentage used in the industry. If the number of responses is below the threshold of 20%, the penalisations are considered as the percentage of responses are too small and

this might not be reliable (Zhi et al., 2016). The sensory attributes which fall in the upper right quadrant (critical corner) of the chart are those that need most adjustment. Based on the mean drop plots in Figure 4.4, both creaminess and aftertaste fall at the critical corner indicating this product was perceived too little for creaminess and aftertaste. Hence, modification is required to improve the creaminess and aftertaste of HPP milk. ESL milk on the other hand was perceived as low intensity for colour, but high intensity for milkiness and aftertaste. Therefore, improvement is required to adjust the colour, milkiness and aftertaste of ESL milk. The attributes requiring improvement are depicted as “Penalty” in Table 4.5. The penalty is defined as the weighted difference between the means (mean of liking for JAR—mean of liking for the two other levels taken together) (Iserliyska et al., 2017).

Table 4.5: Penalty analysis table of HPP and ESL milk based on overall liking scores using the 7-point hedonic scale.

Milk Type	Variable	Level	Selection ^a (%)	Sum (Overall Liking) ^b	Mean (Overall Liking) ^c	Mean Drops ^d	Penalty ^e
HPP	Colour	Too light	10.53	16.0	4.000	0.889	0.439
		JAR	47.37	88.0	4.889		
		Too dark	42.11	73.0	4.563	0.326	
	Milkiness	Not milky	31.58	51.0	4.250	0.625	0.375
		JAR	42.11	78.0	4.875		
		Too milky	26.32	48.0	4.800	0.075	
	Creaminess	Not creamy	28.95	42.0	3.818	1.432	1.023
		JAR	42.11	84.0	5.250		
		Too creamy	28.95	51.0	4.636	0.614	
	Mouthfeel	Too thin	26.32	42.0	4.200	0.695	0.474
		JAR	50.00	93.0	4.895		
		Too thick	23.68	42.0	4.667	0.228	
ESL	Colour	Too weak	28.95	43.0	3.909	1.234	1.084
		JAR	55.26	108.0	5.143		
		Too strong	15.79	26.0	4.333	0.810	
	Milkiness	Too light	34.21	43.0	3.308	1.549	1.151
		JAR	55.26	102.0	4.857		
		Too dark	10.53	20.0	5.000	-0.143	
	Creaminess	Not milky	23.68	19.0	2.111	3.065	1.510
		JAR	44.74	88.0	5.176		
		Too milky	31.58	58.0	4.833	0.343	
	Mouthfeel	Not creamy	26.32	28.0	2.800	2.024	0.871
		JAR	44.74	82.0	4.824		
		Too creamy	28.95	55.0	5.000	-0.176	
ESL	Aftertaste	Too thin	31.58	33.0	2.750	2.074	0.871
		JAR	44.74	82.0	4.824		
		Too thick	23.68	50.0	5.556	-0.732	
	Aftertaste	Too weak	21.05	23.0	2.875	2.356	1.351
		JAR	34.21	68.0	5.231		
		Too strong	44.74	74.0	4.353	0.878	

^a Selection % is the percentage of panellists who rated the milk at levels of too low, JAR, or too high. ^b Sum (Overall Liking) is the total score of panellists who rated the milk at levels of too low, JAR, or too high. ^c Mean (Overall Liking) is the average score for each level of too low, JAR, or too high. ^d Mean drops is the decrease in liking compared to the mean liking of those who rated the attribute as JAR. ^e Penalty is a weighted difference between means (mean liking of JAR category minus the mean liking for the other two levels (too low and too high taken together). Refer to the appendix for calculations.

4.4 Conclusions

There were noticeable differences between HPP and ESL milk as up to 89.5% of panellists selected the correct sample in the triangle test. The hedonic evaluation of overall liking of HPP and ESL milk revealed that more panellists gave higher ratings on HPP milk. The JAR evaluation of acceptance and preference of the treated milk samples' sensory properties showed no significant differences ($p > 0.05$) in all attributes including milkiness, creaminess, mouthfeel, and aftertaste, with the exception of colour. Visible colour difference was observed between HPP milk (darker colour) and ESL milk (lighter colour) as proven by literature. The overall sensory properties of HPP-treated milk were evaluated better (lower penalty) for colour, milkiness, mouthfeel and aftertaste attributes than ESL milk with better score (lower penalty) for only creaminess. However, this penalty analysis suggested that HPP milk needs improvement for creaminess and aftertaste despite the later attribute still scoring better than ESL milk.

CHAPTER 5

FEASIBILITY OF HPP TREATMENT IN MILK PROCESSING INDUSTRY THROUGH COST AND ENVIRONMENTAL ASSESSMENT: MALAYSIAN CASE STUDY

5.1 Introduction

Feasibility study is a preliminary study to determine a project's viability and it should be conducted before implementing a new emerging technology into an industrial setting. It provides an overall view whether the project is technical and economically feasible using set of assumption such as the technology used, the facilities and equipment, the capital need and other financial aspects.

Cost estimation is included in feasibility study. The objective of cost estimation is to predict whether the volume, expenses, and cost of resources needed to execute a task were within the project's limits. The cost of HPP is normally influenced by the whole cycle time (pressure come up, holding, and loading/unloading durations), vessel filling ratio, energy costs, labour expenses, and capital costs (Mújica-Paz et al., 2011). Depending on the pressure holding period and packaging size, the cost for industrial HPP unit of 55 L and 420 L that can provide pressure up to 600 MPa at room temperature with a maximum overall production of 5000 kg/hr were estimated in US dollar \$770,000 and \$3,150,000 respectively (Tonello, 2011).

The environmental impact is also a significant factor that needs to be put into consideration before implementing a new technology in the industry. In the last decade, there has been a noteworthy concern about the rise in greenhouse gases and

their effects to the environment. The increase in public awareness on the connection between global warming and greenhouse gas emissions has led to a growing demand for green food product from the local manufacturers (Sampedro et al., 2014). Food processors are putting great emphasis attention to issues like wastewater, gas emissions and energy usage on each processing step (Pereira & Vicente, 2010). There has also great efforts in creating a sustainable industry. Many manufacturers are highly concerned on the matters of reducing usage of non-renewable energy sources, reducing production of air pollutants like CO₂, enhancing energy efficiency of equipment and processes utilising renewable energy (Pereira & Vicente, 2010).

In general, the traditional method of heat preservation is still reliant on temperature-time protocols, and this typically requires significant quantities of fossil fuels and water in an industry scale to generate steam as source of heat energy. Boilers are typically used in food processing plants to generate steam, where treated cold feed water is fed and heated by fuel combustion. However, use of boiler in industry requires scheduling and high standard of maintenance. Poor maintenance of boilers will reduce boiler efficiency by 20 – 30% which subsequently increase losses of energy and cause high emissions of CO₂ (Pereira & Vicente, 2010). The combustion process in boiler also releases a range of pollutants such as smoke, carbon dioxide, organic compounds *etc.* depending on the type of fuel used (Hroncová et al., 2016). Therefore, food processing systems powered only by electricity such as HPP may present an environmental advantage when compared with traditional processing technologies. HPP is considered to be environmentally friendly as it can fully eliminate or at least reduce the use of boilers or steam generation systems, which reduces wastewater production and increases water and energy savings (Pereira & Vicente, 2010).

Additionally, if the electricity is produced from a clean, renewable energy source such as hydroelectric power, then this process will significantly help to lower the pollution load and preserve the environment.

Irrespective of the fact that numerous of research have been published establishing the advantages of HPP on the safety and quality of foods, there are still no studies conducted on a thorough economic analysis and environmental impact of this technology for the fresh milk processing in comparison to conventional heat treatment. The objective of the present study was to determine the feasibility of treatments by analysing the cost and environmental impact of fresh milk processed using HPP in comparison to the heat treatment. While the main economic feasibility parameters were determined, three sensitivity analyses were made to evaluate how the raw milk unit cost, HPP unit selling price, as well as the HPP unit filling ratio could impact the economic feasibility of HPP milk production, such as impact on production cost, NPV and breakeven quantity. The methodology and findings of this study will provide a reference or guidance to the dairy manufacturers in decisions making on the implementation of this non-thermal technology (HPP) in milk processing.

5.2 Materials and Methods

Cost models for HPP and ESL heat treatment with heat recovery system were developed for a medium size facility pasteurising 27,500,000 L of cow milk per year with a throughput of 5,000 L/hr, 20 hr of operation per day (4 hr for equipment cleaning, sanitising and maintaining) and 5,500 hr of operation per yr. The models were developed using SuperPro Designer® Version 9, Build 2 software (Intelligent Inc., Scotch Plains, NJ, USA) and addressed sizing of unit operations, utility

consumption, capital and unit pasteurising costs. The models were constructed using information acquired from equipment suppliers and accessible scientific publications. The cost and energy consumption of all processing units were included into the model as inputs for the desired production scale. The final estimated cost is expressed in ringgit Malaysia (RM) per volume of milk produced.

5.2.1 SuperPro Designer Stimulation

The first step to start SuperPro Designer Stimulation is by specifying the mode of operation for the entire plant. For ESL treatment, the process operating mode is set to 'Continuous', all stream flows are displayed on a per-hour basis. Meanwhile, for HPP treatment, the process operating mode is set to 'Batch', all stream flows are displayed on a per-batch basis.

Registering Components

The raw material used to produce both HPP milk and ESL milk was raw milk from cow. Assuming the cow milk was made up of 87.3 % water, 4.0 % fat, 2.8 % casein, 4.6% lactose, 0.7 % ash and 0.6 whey protein (Meghwal et al., 2017), these components were added and registered.

The process model begun with storage of the raw milk in the plant silos after delivery from the farms and ended with cold storage of the bottled milk prior to shipment. In HPP treatment, the process included raw milk storage, filling, high pressure processing and cold storage. Meanwhile, the process included in ESL treatment were raw milk storage, homogenization, ultra-pasteurization (ESL), cooling, filling and packaging,

and refrigeration. Table 5.1 gives an overview of the key unit procedures and settings in the process model.



Table 5.1: Overview of key unit procedures in milk processing model for HPP and ESL treatment.

Unit Procedures	Equipment	Details
<u>HPP treatment</u>		
Raw milk storage	Storage Tank	- Capacity: 10000 L 5.5 kW operating power 20 h residence time - Milk stored at $\pm 6^{\circ}\text{C}$
Filling	Filing machine	- Capacity: 5000 bottles/ hr 3.5 kW operating power - Filling into 1 L PET bottle
High Pressure Processing (HPP)	HPP unit	- Capacity: 420 bottles/ cycle - Cycle: 4 cycles/ hr - Duration per cycle: 15 mins 40 kW operating power - Filling ratio: 80% - Water as transmitting medium 3 units to produce 5000 L/hr
Cold storage	Clod room	- Capacity: 50 ton 30 kW operating power - Milk stored at $\pm 6^{\circ}\text{C}$
<u>ESL treatment</u>		
Raw milk storage	Storage Tank	- Capacity: 10000 L 5.5 kW operating power 20 h residence time - Milk stored at $\pm 6^{\circ}\text{C}$
Homogenization	Homogeniser	- Capacity: 5000 L/hr 45 kW operating power 198 bar pressure drop 65°C exit temperature 52.7 kW power consumption
Ultra-pasteurisation (ESL treatment)	Indirect steam tubular-type pasteurizer	- Capacity: 5000 L/hr 7 kW operating power 125°C operating temperature 4 s holding time 75 % regenerative effect - Use steam as heat source $\pm 6^{\circ}\text{C}$ exit temperature of milk 2°C temperature of coolant
Filling	Aseptic bottle filling machine	- Capacity: 5000 bottles/ hr 3.5 kW Operating power - Filling into 1 L PET bottle
Cold storage	Cold room	- Capacity: 50 ton 30 kW Operating power - Milk stored at $\pm 6^{\circ}\text{C}$

(Source refer to Appendix F)

High Pressure Processing (HPP) Treatment

The 5000 bottles of milk were treated in three separated HPP units at 600 MPa with holding time 10 mins, using water as transmitting medium. In order to produce 5000 bottles of 1 liter milk per hour, 3 units of HPP units with 525L vessel capacity were required, assuming HPP units with 525L vessel capacity having maximum filling ratio of 80% for 1 liter PET bottle milk. When filling ratio of 80% was used, this indicated a total of 420 bottles of 1 liter milk loaded into baskets for each cycle of HPP treatment, while the remaining 20 % was occupied by transmitting fluid (~105 L). The duration of each HPP cycle was estimated to 15 mins (4 cycles/hr) included product loading and unloading, pressure rise time (to 600 MPa), pressure holding time (10 mins), pressure release and other additional operations. One HPP unit can produce 420 bottles of milk per cycle (Figure 5.1), hence three HPP units together estimated can produce in total 5040 bottles of milk per hour.

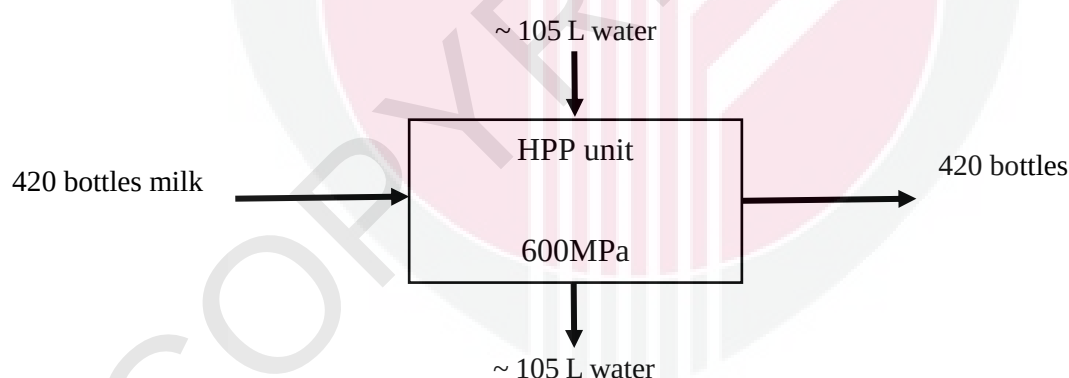


Figure 5.1: Mass and energy balance of HPP milk per cycle.

Ultra-pasteurisation (ESL) Treatment

After homogenization, milk transferred to the pasteuriser to undergo ultra-pasteurisation (ESL) treatment. Plate heat exchanger was used as it has both heating and cooling sections. Milk was first pump to a ‘regeneration’ section where it was pre-heated by milk that has already been ultra-pasteurised. The milk is then heated to the targeted ultra-pasteurisation temperature 125°C in the heating section and held for 4 s in a holding tube. The ultra-pasteurised milk was then cooled in the regeneration section which simultaneously pre-heats incoming milk. Further cooling took place in the cooling section by chilled water to $\pm 6^\circ\text{C}$. In this stimulation, raw milk entered plate heat exchanger at a rate of 5000 L/hr. Hot water was supplied at 7500 L/hr at 130 °C to heat the milk and chilled water has temperature 2°C. Assume the density of milk is 1030 kg/m³, density of water is 934.6 kg/m³ at 130 °C and 1000 kg/m³ at 2°C. This method saves heating and refrigeration energy with estimation of 75% of heat exchange take place in the regeneration section. Mass and energy balance of ultra-pasteurised (ESL) milk is shown in figure 5.2.

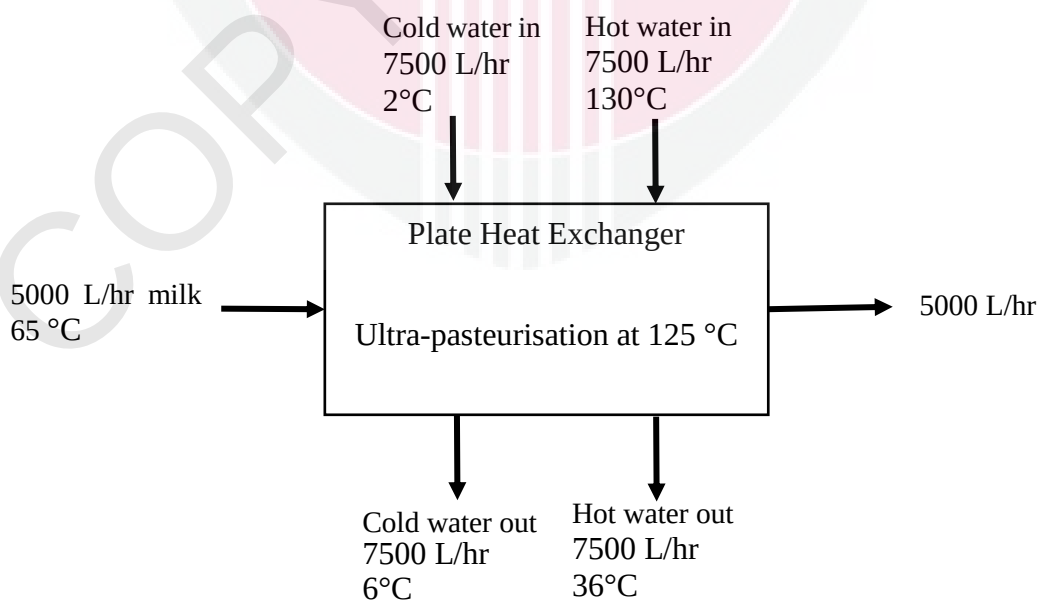


Figure 5.2: Mass and energy balance of ultra-pasteurised (ESL) milk.

5.2.2 Cost Analysis

The cost estimation in this study has accounted the entire process involved for milk production using HPP and ultra-pasteurisation (ESL). It is divided into two major cost including capital cost and operating cost accounted for labour cost and facility-dependent cost. The capital cost is fixed, one-time expenses incurred on the purchase of land, buildings, construction, and equipment used in the production of goods or in the rendering of services. In this cost model, the capital cost is subdivided into Direct Cost (DC) and Indirect Cost (IC). The direct cost covers equipment purchases cost (PC), installation, process piping, instrumentation, insulation, electrical, buildings, yard improvement and auxiliary facilities while indirect cost cover engineering and construction fees. For preliminary design purposes, these various items of capital cost were estimated based on the total equipment purchase cost (PC) using the average multiplier sometimes called “Lang Factors” as reported by Harrison et al. (2015). Table 5.2 provides the multipliers for calculations of capital cost.

Table 5.2: The multipliers for calculations of capital cost.

Cost Items	Multiplier
<u>Total plant direct cost (TPDC)</u>	
Equipment Purchase Cost (PC)	-
Installation	0.50 PC
Process Piping	0.40 PC
Instrumentation	0.35 PC
Insulation	0.03 PC
Electrical	0.15 PC
Buildings	0.45 PC
Yard Improvement	0.15 PC
Auxiliary Facilities	0.50 PC
<u>Total plant indirect cost (TPIC)</u>	
Engineering (Eng)	0.25 TPDC
Construction (Const.)	0.35 TPDC
Total plant cost (TPC)	TPDC + TPIC
Contractor's Fee	0.05 TPC
Contingency	0.01 TPC
Total Capital Investment (TIC)	TPC + Contractor's Fee + Contingency

(Harrison et al., 2015)

Operating cost is estimated based on electricity consumption, utilities, labour cost and other facility-dependant costs. The electricity prices used in this study were referring to the Tariff E1 - Medium Voltage General Industrial Tariff reported by the Malaysian multinational electricity company (Tenaga Nasional Berhad) with the minimum monthly charge of RM600.00, RM 29.60/kW for each kilowatt of maximum demand per month and 33.70 sen/kWh for all kWh (2022 prices). The unit price of steam for heat treatment was RM 0.01/kg, referring to the marginal cost of low-pressure steam provided by U.S. Department of Energy, Energy Efficiency and Renewable Energy (2003), while cooling water cost was RM 2.20 per m³ (RM 0.002/L) based on the Malaysia water tariff rate (adapted on July 2022, source from Suruhanjaya Perkhidmatan Air Negara, SPAN) and chilled water cost was RM1.28/MT as defined

by Mussatto et al. (2015). The energy estimation in the present study are specifically for the processing of fresh cow milk in Malaysia.

The total labour cost (TLC) was calculated by referring to the SuperPro Designer Manual User Guide (Intelligen, 2021). Each section was calculated as the sum of the labour costs of the different labour types (i.e., operator, supervisor) that may be required for that section. The labour cost of each labour type is calculated by multiplying the corresponding labour demand per type (LDT) by the corresponding labour rate (i.e., unit cost) per type (LRT). In this stimulation, itemised estimation (*i.e.* operating labour as defined in the process on a step-by-step basis) was selected as the labour demand per type (LDT) which was calculated as the sum of individual labour demands by all operations of that section. For the calculation of the labour rate type (LRT), a detailed labour rate is estimated by adjusting the basic rate (i.e., the basic unit labour cost) for the following additional costs:

- Fringe Benefits: Expenditures that are paid out by the company to cover various benefits which are not included in the basic labour rate.
- Supervision: The salaries of non-operational staff engaged in supervision of operational and clerical staff.
- Operating Supplies: Includes everyday items required to keep the plant in proper running condition, as well as clothing, tools, and protective devices for operators.
- Administration: The cost of non-process-related administrative and secretarial support.

The above costs were specified as factors of the basic rate. The detailed labour rate of a labour type was estimated as:

Detailed Labour Rate

$$= (\text{Basic Rate}) \times (1 + \text{Benefits} + \text{Supervision} + \text{Supplies} + \text{Administration})$$

The basic labour rate was calculated based on the minimum wage order for skilled worker at Peninsular Malaysia, RM1500/month (effective since 1 May 2022), assuming the amount was according to the market rates and industry standards, and all the operators having the similar skill and experience. The plant operates 24 hours per day. The operators work in 3 shifts of 8 hours. The wages per hour was calculated by dividing the monthly basic rate by the total number of work hours in a month, assuming there are 26 working days per month with 8 hr working duration per day, the average basic labour rate was as below:

Basic rate per hour

$$= \text{Monthly basic rate} / (\text{Total number of work days} \times \text{Total hours in a day})$$

$$= \text{RM } 1500 / (26 \text{ days} \times 8 \text{ hr})$$

$$= \text{RM } 7.21 / \text{hr}$$

The cost factors used were 0.4, 0.2, 0.1 and 0.3 for benefits, supervision, supplies and administration respectively referring to the average multiplier as reported by Heinzle et al. (2007). Hence, the detailed labour rate was updated as below:

Detailed Labour Rate

$$= (\text{Basic Rate}) \times (1 + \text{Benefits} + \text{Supervision} + \text{Supplies} + \text{Administration})$$

$$= \text{RM } 7.21/\text{hr} \times (1 + 0.4 + 0.2 + 0.1 + 0.3)$$

$$= \text{RM } 14.42 / \text{hr}$$

The total labour cost (TLC) was then evaluated by multiplying the detailed labour rate with the summation of labour demand per section.

Total Labour Cost (TLC)

= Detailed Labour Rate x SUM (Labour Demand per Section)

= RM 14.42/hr x SUM (Labour Demand per Section)

Material cost for production of HPP and ESL heat treated milk were estimated based on the total volume of fresh milk and packaging material required for an annual production. The unit cost for milk is RM 2.23/L from Trading Economics (<https://tradingeconomics.com/commodity/milk>), while the unit cost of one polyethylene terephthalate bottle (PET) is RM 1.25/unit based on average wholesale price from Alibaba.com.

Facility-dependent cost was calculated based on the capital investment parameters by summation of depreciation, maintenance and miscellaneous cost. Economic depreciation is used to incorporate the capital expenses into operating costs. It was estimated as the sum of the facility's capital costs divided by the estimated yearly output volume, multiplied by the facility's anticipated economic life, 10 yrs. Maintenance and miscellaneous cost were included as every piece of equipment and the plant in general need to be maintained and repaired. Maintenance cost applied as reported by Sampedro et al. (2014) using a factor of 8 % to the capital cost of HPP treatment to account for the higher cost of the spare parts and labour as well as the vessel replacement required after a number of cycles. In contrast, only 2 % factor applied to the capital cost of the ESL heat treatment with expectation of less maintenance and services required. Other miscellaneous costs, in total 3% were

applied to the capital cost to account for insurance (1%), local taxes (1%) and factory expenses (1%).

Following capital and operational cost calculation, the Net Present Value (NPV), Internal Rate of Return (IRR) and Break-even analysis for both treatments were calculated. NPV is a direct measure of a project's cost-benefit, while IRR measures profitability as a percentage showing the return on money invested. Before calculating NPV and IRR, the present value (PV) was first calculated. Present value is the value of a future cash stream discounted at the appropriate market interest rate (discount rate), calculated using the following equation:

$$PV = \frac{FV}{(1+r)^n}$$

Where PV is the present value of the amount, FV is the future value of the amount at n period, r is the discount rate or the expected date of return and n is the year that the amount occur.

NPV is calculated using the concept of adding the present values of each individual positive or negative cash flow based on the opportunity cost of capital (Chen, 1996).

The formula is as below.

$$NPV = PV \text{ of total benefit} - PV \text{ of total cost}$$

IRR is the discount rate at which the net present value of an investment is zero (Ross, Westerfield, and Jaffe, 2005).

IRR = Discount rate that NPV is 0

Break even analysis determines the number of units or amount of revenue needed to cover the business total cost. The formula for break-even analysis is as follows:

$$\text{Break-Even Quantity} = \frac{\text{Fixed Costs}}{(\text{Sales Price per Unit} - \text{Variable Cost Per Unit})}$$

In this case study, the capital cost considered as fixed cost while operational costs are considered variable costs because they change with the production level. The sale price per unit of milk is assumed at average market price at RM 7.00 (price 2023).

5.2.3 Sensitivity Analysis of HPP Treatment

Sensitivity analysis is a technique used in various field including engineering, economics, finance and other to determine how changes in certain variables affect the outcome of a model, simulation or decision. It is importance to understand and identify whether the changes of the value of certain parameter could influence in a global economic parameter. The purpose of sensitivity analysis is to assess the robustness and reliability of a model by examining the sensitivity of its outputs to changes in input parameters or assumptions. SuperPro Designer could be employed to accomplish a sensitivity analysis with respect to strategic design parameters after the model has been developed.

According to Pérez Sánchez et al. (2023), the unit cost of cow milk is the economic parameter that currently presents the greatest uncertainty and volatility which is expected to increase the future due to restructuring of the internal wholesale market,

variation in national production and other intrinsic commercial and financial factors. As such, a first sensitivity analysis was carried out to evaluate how an increase in cow milk unit cost influences the NPV parameter, and determine at which value the HPP milk production plant becomes unprofitable. In this analysis, the unit cost of cow milk was varied between MYR 2.00 to MYR 8.00, while keeping the unit cost of other components such as bottles, utilities and labour constant.

According to Yao and Oppewal (2016), the unit pricing of a product could influence consumer decisions. In their research, they found that consumers are price sensitive as the presence of unit prices increases the salience of price in decision making which in turn activates a greater motivation to select the cheaper products. Hence, it is importance to review the unit price of a product and identify the economic impact if reduces the product unit prices. A second sensitivity analysis was conducted to evaluate how a reduction in HPP milk unit cost influences the NPV parameter and break even quantity. In this analysis, the unit cost of HPP milk was varied between MYR 4.00 to MYR 7.00, while maintaining all other costs including capital and operational cost constant.

HPP units often have a maximum capacity and throughput rate or it is usually known as HPP unit filling ratio. The filing ratio of HPP refers to the ratio of product volume to the volume of the pressure vessel. This ratio can indeed affect costs and economic feasibility in several ways. For example, if the filling ratio is low, it indicates less product can be processed in each cycle, potentially underutilising the capacity of the HPP equipment and reducing the processing efficiency. This inefficiency might lead to higher energy usage and labour costs per unit of product which further negatively

impacting the economic feasibility. Thus, a third sensitivity analysis was designed to identify the effect of HPP filling ratio on the parameters of operational cost, NPV and break-even quantity. In this study, the filling ratio was modified between 10 to 80%. Assume all unit price including materials (raw milk and bottles), utilities (electricity, cooling water), labour, facility-dependence cost and also HPP milk selling price (MYR 7/ L bottle) remained unchanged.

5.2.4 Environmental Impact

Environment impacts of HPP and ESL heat treatment were evaluated based on carbon footprint, represented by the equivalent CO₂ emissions in kg CO₂/yr and kg CO₂/L during fresh milk processing. It is divided into 2 different emission sources including electricity (equipment) and utilities (steam, cooling water and chilled water). Greenhouse gas emissions for the unit operations were calculated from the energy data using conversion factors. The electricity emission factor in Malaysia is 0.509 kg CO₂/kWh according to Energy Information Administration (Sadegh Shahmohammadi & Noori Houshyar, 2013). The conversion factors used to determine the amount of greenhouse gases generated during the production and transmission of steam were obtained using public available data from the US Department of Energy (Deru, 2007), assuming emission factors are 66.33 kg CO₂/ mmBtu of steam or heat purchased and natural gas fuel is used to generate steam or heat at 80 % thermal efficiency. Meanwhile, the conversion factor of cooling and chilled water was assume similar to the normal water with its conversion factors 0.376 kg CO₂/m³ obtained from Shimizu et al. (2012). The formula used to calculate carbon dioxide emission are summarised as below:

CO₂ emission on electricity consumption:

$$\text{CO}_2 \text{ e (electricity)} = \text{Electricity used (kWh)} \times 0.509 \text{ (kgCO}_2\text{/ kWh)}$$

CO₂ emission on utilities consumption:

$$\text{CO}_2 \text{ e (steam)} = \text{Steam used (mmBtu)} \times 66.33 \text{ kg CO}_2\text{/ mmBtu}$$

$$\text{CO}_2 \text{ e (cooling/chilled water)} = \text{Water used (m}^3\text{)} \times 0.376 \text{ kg CO}_2\text{/m}^3$$

5.3 Results and Discussion

5.3.1 SuperPro Designer Stimulation

In milk production, either batch or continuous modes can be used and implemented depending on the type of the product. In producing HPP milk, the operation mode was batch process as the products were first packed, followed treatment and produce by batch. In contrast, the operating mode used in producing ultra-pasteurised (ESL) milk was continuous process as it can save energy and shorten processing time which preferred by most of the local milk manufacturer.

In both HPP milk and ESL milk production, the only raw material used was raw fresh cow milk. Raw milk was registered as stock mixture with composition of 87.3 % water, 4.0 % fat, 2.8 % casein, 4.6% lactose, 0.7 % ash and 0.6 whey protein. Besides, the plastic bottle needed to fill the milk, HPP milk and ESL milk were also registered as pure components.

After components registration, unit operation were subsequently added on flowsheet.

Figure 5.3a depicts the schematic diagram of commercial high-pressure processing

(HPP) of fresh cow milk with process temperatures. The unit procedures of high-pressure processing (HPP) of fresh cow milk were selected by referring to the process of HPP milk production of a milk brand known as Made by Cow (<https://www.madebycow.com.au/our-cold-pressed-fresh-milk-process>). The fresh milk is first store in a milk storage silo, filled in food graded plastic, polyethylene terephthalate (PET) bottles that can withstand high pressure, followed by placing of 420 bottles of 1 L fresh milk into the plastic baskets (assuming a maximum 80% of filling ratio for the bottled fresh milk). These baskets are then loaded into the HPP vessel (525 L vessel capacity, 80% filing capacity for 1 L bottle milk, 380 mm inside vessel diameter, 15 mins per operation, and 4 cycles/hr) for the high-pressure processing treatment of milk at 600 MPa for 10 mins at room temperature. The pressure and holding time for high-pressure treatment of milk were set based on the results of experimental works on the effect of HPP on milk microbiological properties with a shelf-life of 2 months under cold storage at ± 6 °C. After high-pressure treatment, the bottled milk is directly stored in a cold storage room at 6 °C.

In comparison, Figure 5.3b shows the design of commercial ultra-pasteurisation (ESL) of fresh milk at 125 °C for a holding duration of 5 s. This design was developed by referring to the HTST fluid milk processing system as reported by (Lee & Okos, 2011). The fresh milk is homogenised at pressure 30 MPa and temperature 65 °C before ultra-pasteurisation (ESL) treatment to disintegrate the fat globules in the milk to reduce creaming (Wilbey, 2016). The homogenised milk is then transferred to the indirect thermal pasteurisation system consisting heat exchanger, pump, holding tube and cooling features. The milk is pumped through the heat exchanger to heat the milk to 125 °C and hold for 5 s in a separated holding tube. Following ultra-pasteurisation, the

milk pump to the chilling section where it is generatively chilled by the incoming, untreated cold milk before being cooled with cooling water. The chilled milk is then transferred to the storage tank and filling devices. The bottles of ESL milk produced are stored at 6 °C in the cold storage room before distribution.

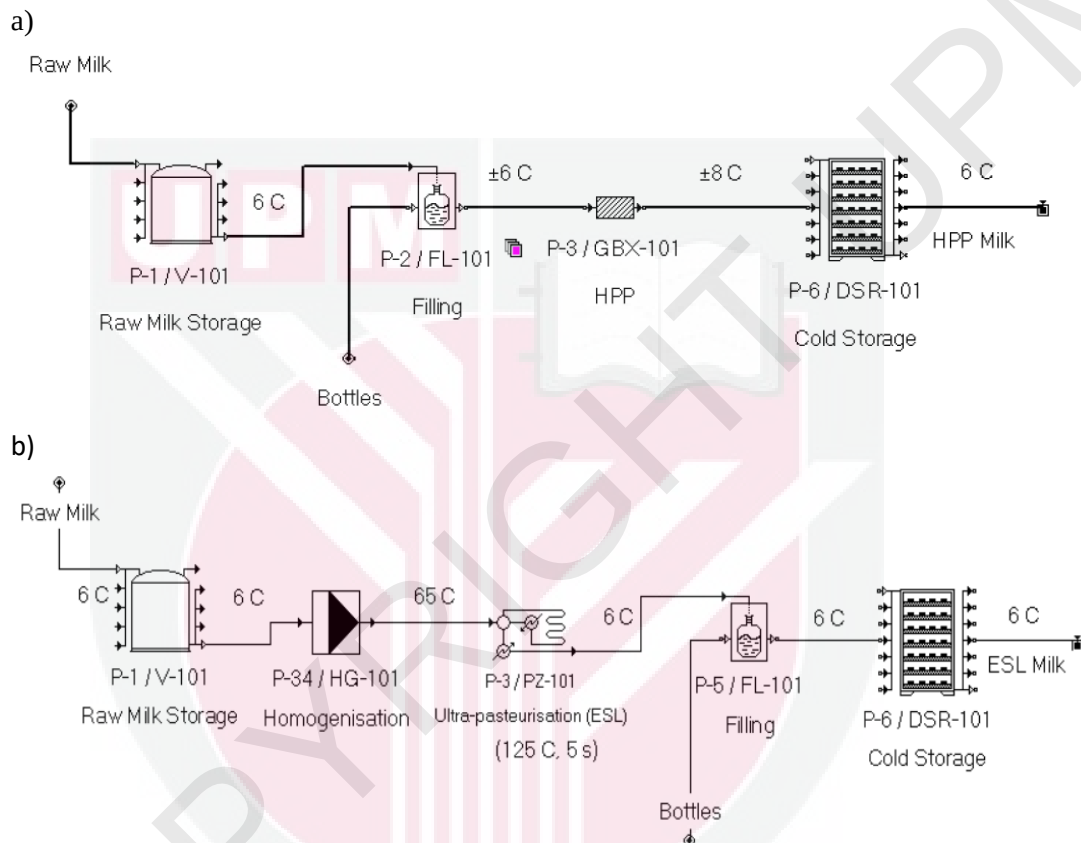


Figure 5.3: Schematic diagram of designed commercial a) HPP treatment of milk and b) ultra-pasteurisation (ESL) treatment of milk.

Modelling and simulation for both HPP and ESL milk production were successfully stimulated using SuperPro Designer. In HPP milk production, one HPP unit produced 420 bottles of 1 L HPP milk per batch, as such, three HPP units in total produce 5000 bottles of 1 L HPP milk per hour. The process flow diagram for HPP milk production was developed using 4 unit procedures including milk storage tank, filling machine, HPP unit and cold storage room that represent the whole operation involved in high

pressure processing. Meanwhile, in ESL milk production, milk entered at flowrate 5000 L/hr and produced 5000 bottles of 1 L ESL milk per hour. The process flow diagram for ESL milk production was developed using 5 unit procedures including milk storage tank, homogeniser, plate heat exchanger, aseptic filling machine and cold storage room that represent the whole operation involved in ESL milk processing.

5.3.2 Cost Analysis

Capital Cost

Capital costs are important cost required to complete a project. For the purposes of preliminary design, the various components of the direct fixed cost were estimated based on the total equipment purchase cost (PC) using a number of multipliers, frequently known as "Lang Factors". The detailed capital costs for HPP and ESL heat treatment of milk were depicted in Table 5.3. The capital cost for an industrial scale HPP milk production line was estimated to be RM 6,226,355 (RM 0.23/L) based on the equipment (milk storage tank, filling machine, HPP unit and cold storage facilities) required to process milk at 600 MPa with 10 mins holding time, while the capital cost for the ultra-pasteurisation (ESL) heat treatment was estimated to be RM 2,179,224 (RM 0.08/L) based on the equipment including storage tank, cold storage room, homogeniser, ultra-pasteurisation unit and aseptic filling machine. This is corresponding to an increase of 3-fold for capital cost of HPP treatment as compared to the ultra-pasteurised heat treatment. The estimated capital cost is in accordance to previous study by Sampedro et al. (2014), showing the total capital cost for HPP treatment (550 MPa for 90 s) was 38-fold higher than the total capital cost for thermal treatment (88 °C with a hold time of 5 s) to process juice at a production rate of 16,500,000 L/yr (3,000 L/hr).

Table 5.3: Capital cost estimation of HPP and ESL heat treatment.

Cost Items	Unit	HPP	ESL
Processed milk	L/yr	27,500,000	27,500,000
<i><u>Total plant direct cost (TPDC)</u></i>			
Equipment Purchase Cost (PC)	MYR	1,040,000	364,000
Installation	MYR	520,000	182,000
Process Piping	MYR	416,000	145,600
Instrumentation	MYR	364,000	127,400
Insulation	MYR	31,200	10,920
Electrical	MYR	156,000	54,600
Buildings	MYR	468,000	163,800
Yard Improvement	MYR	156,000	54,600
Auxiliary Facilities	MYR	520,000	182,000
TPDC	MYR	3,671,200	1,284,920
<i><u>Total plant indirect cost (TPIC)</u></i>			
Engineering (Eng)	MYR	917,800	321,230
Construction (Const.)	MYR	1,284,920	449,722
TPIC	MYR	2,202,720	770,952
Total plant cost (TPC)		5,873,920	2,055,872
Contractor's Fee	MYR	293,696	102,794
Contingency	MYR	58,739	20,559
Total Capital Investment (TCI)	MYR	6,226,355	2,179,224
TCI per litre milk	MYR/L	0.23	0.08

The estimated cost to purchase the major equipment for production of HPP milk and ESL milk were listed in Table 5.4, referring to the prices obtained from online sources Alibaba.com while details and reference link of each equipment can be referred to Appendix F. The total major equipment cost for production of HPP milk was RM 1,040,000, comprising 4 major equipment, including a storage tank to store the fresh milk before treatment; filling machine to fill the milk into bottle; HPP unit to pressurise the milk and a cold storage room to store the bottled HPP milk.

The estimated cost to purchase an HPP unit is approximately RM 291,000, but to produce 5000 L of milk per hour at 600 MPa with 10 min pressure holding time, 3

HPP units with capacity 500 L were required, causing the total HPP unit cost to become RM 874,000. However, higher cost of HPP industrial unit was reported by Picart-Palmade et al. (2019) with the price range from 0.77 to 3.15 million US dollars based on the vessel volume of 55 – 420 L with operational parameter at 600 MPa, room temperature and overall output of 5,000 kg/hr.

Table 5.4: Major equipment cost of HPP treatment and ESL heat treatment in MYR.

Equipment	HPP	ESL
Storage Tank	89,000	89,000
Filling Machine	50,000	-
Cold Storage Room	27,000	27,000
HPP Unit	874,000	-
Homogeniser	-	40,000
Ultra-pasteurisation Unit	-	128,000
Aseptic Filling Machine	-	80,000
Total	1,040,000	364,000

(Source refer Appendix F for equipment cost reference link).

In comparison, the total equipment cost for production of ESL heat treated milk only cost RM 364,000, comprising a storage tank, homogeniser, ultra-pasteurisation unit, aseptic filling machine and cold storage room (Table 5.4). The estimated cost to purchase an indirect steam tubular-type pasteuriser was RM 128,000 which was lower compared to the price of an HPP unit (RM 291,000). A homogeniser is also included in ESL milk treatment to mechanically reduces the size of the milk fat globules by pumping at high pressure through a small valve to produce emulsion of uniform composition and higher stability of milk (Yalçın et al., 2021). In addition, an aseptic filling machine was selected for filling of ESL heat treated milk into bottles to prevent post contamination and providing longer shelf-life to the finished good (Deeth, 2017).

The capital cost also incurred direct cost in installing and commissioning the equipment, providing the necessary process piping, instrumentation, insulation and electrical facility as well as the cost for building, yard improvement and auxiliary facilities. These costs were estimated based on total equipment purchase cost (PC), using a distributed set of PC-Factors as listed in Table 5.1. All the factors used in this capital cost estimation were referred to the cost factor as stated in book entitled Bio separations Science and Engineering by Harrison et al. (2015). The engineering cost was also included in capital cost estimation to develop a design to integrate the system into the other industrial processes, while construction management cost was necessary to organise all of the operations. Contractor's fee and contingency (CFC) cost were estimated using multiplier of 0.05 and 0.01 respectively to the summation of direct cost (DC) and indirect cost (IC). Although the estimated capital cost of HPP treatment was higher compared to ESL heat treatment, however the capital costs for HPP treatment can be declined with time due to a growing demand for high pressure units resulting easier "pre-buying" of raw materials (Sampedro et al., 2014).

Electricity and Utilities Cost

The operating cost estimated for each process was displayed in Table 5.5 based on the electricity consumption, utilities such as steam and cooling water, labour cost and other facility-dependant costs. In comparison, the annual electricity cost for HPP treatment (RM 294,707 or 13.4% of overall operating cost) was higher than the electricity cost for ESL heat treatment (only RM 168,669/yr). High electricity cost of HPP could be due to the high energy consumption for various switching operations during HPP treatment, such as the movement of the vessel and its contents to working position; initial filling with water; closing the plug, and moving the wedge to secure the plugs

(Atuonwu & Tassou, 2018). Similarly, Sampedro et al. (2014) reported the energy cost for HPP at 550 MPa for a holding time of 90 s is 86% more expensive than the production expenses of thermal processing at 88 °C with a hold time of 5 s. However, additional cost on steam (RM 50,950/yr) and higher consumption of chilled water (RM 449,210/yr) caused the total utility cost for ESL heat treatment (RM 861,053/yr) nearly double to the utilities cost of HPP treatment.



Table 5.5: Operating cost estimation of HPP and ESL heat treatment.

Cost Items	Unit of measure	HPP	ESL
<u>Electricity</u>			
Storage Tank	kW h/yr	30,250	30,250
Filling Machine	kW h/yr	19,250	-
Cold Storage Room	kW h/yr	165,000	165,000
HPP Unit	kW h/yr	660,000	-
Homogeniser	kW h/yr	-	247,500
Ultra-pasteurisation Unit	kW h/yr	-	38,500
Aseptic Filling Machine	kW h/yr	-	19,250
Electricity Consumption	kW h/yr	874,500	500,500
Total Electricity Cost	MYR/yr	294,707	168,669
<u>Steam (152°C)</u>			
Steam Consumption	MT/yr	-	5,199
Total Steam Cost	MYR/yr	-	50,950
<u>Cooling Water (25 – 30°C)</u>			
Cooling Water Consumption	L/yr	623,700	82,500,000
Total Cooling Water Cost	MYR/yr	1,247	173,250
<u>Chilled Water (5 - 10°C)</u>			
Chilled Water Consumption	MT/yr	59,293	365,769
Total Chilled Water Cost	MYR/yr	75,895	468,184
Total Utilities Cost	MYR/yr	371,916	861,053
<u>Material costs</u>			
Milk	MYR/L	61,187,500	61,187,500
Bottle	MYR/unit	34,265,000	34,265,000
Total Material Cost	MYR/yr	95,452,500	95,452,500
<u>Labour costs</u>			
Adjustment Rate	MYR/hr	14.42	14.42
Labour Demand	labour.h/yr	41,525	30,250
Total Labour Cost	MYR/yr	598,791	436,205
<u>Facility-Dependent Costs</u>			
Depreciation (10 yrs)	MYR/yr	622,636	217,922
Maintenance	MYR/yr	498,108	43,584
Miscellaneous	MYR/yr	186,791	65,377
Total Facility-Dependent Cost	MYR/yr	1,307,535	326,884
Total Operating Cost (per yr)	MYR/yr	98,026,695	97,469,511
Total Operating Cost (per L)	MYR/L	3.57	3.54

(Refer Appendix F for equipment power consumption)

Labour Cost

The labour cost estimated for production of HPP milk (RM 598,791) was 37% higher than the labour cost to produce ESL milk (RM 436,205) based on the average basic rate at RM14.42/hr which accounted for benefits, supervision, supplies and administration cost. This could be due to the different type of process applied to HPP

and ESL treatment, in which HPP is regarded as a batch process that necessitates more labours for product loading and unloading activities while ESL treatment is a continuous process which does not requires much labour even though more equipment involved in this production line. In this study, 3 labour.hr /hr were allocated at HPP unit section alone for 5,000 L/hr throughput as compared to only 0.5 labour.hr /hr allocated at ultra-pasteurisation (ESL) unit. If the manual operation of loading and unloading process in HPP treatment was replaced by an automated line, it may result a reduction of 50% (1.5 labour.hr /hr) on labour demand for HPP unit section and reduces the total labour cost per year by 20%. However, an automatic line with robot arms and conveyor will increase the total equipment cost by average 20% (Sampedro et al., 2014). Another option to reduce the labour cost is through In-bulk HPP treatment, but details costing and process flow on this treatment require further study and research.

Material Cost

The material cost estimated for production of HPP and ESL milk was same (RM 95,452,500/ yr) as it was calculated based on an annual production of 27,500,000 L milk using 27,500,000 units of PET bottles with assumption of no lost during production.

Facility-dependent cost

The estimated total facility-dependent cost account for HPP (RM 1,307,535) was 75% higher than the ESL heat treatment (RM 326,884). This could be due to the higher capital cost incurred for HPP treatment compared to ESL heat treatment. The high maintenance and miscellaneous cost required by HPP treatment over ESL heat treatment was also a factor contributing to high facility-dependent cost. The nature of HPP in batch process required much operating cycles, which increase the chances of

wear and tears on machine parts, resulting higher maintenance cost. In contrast, ESL heat treatment in continuous process did not involve repeatedly power on and off process, hence the machine will not break down easily. The projected useful life of the processing equipment was assuming to be 10 years, with yearly depreciation expenditures of RM 622,636 and RM 217,922 for HPP and ESL heat treatment respectively.

In this study, the annual operating cost of HPP treatment RM 98,026,695 (RM 3.57/L) was estimated 0.6% more than the ESL heat treatment which costs RM 97,469,511 (RM 3.54/L). The difference between the operational cost of HPP and ESL milk is not high because the cost was balanced in each category with higher electricity, labour and facility-dependent cost charged on HPP, while higher utilities cost including steam, cooling water and chilled water cost charged on ESL treatment (Figure 5.2). In HPP units, cooling water was used as pressure transmitting medium during high-pressure processing. The estimated cooling water consumption for all 3 HPP units is 623,700 L/yr. It was calculated based on filling ratio 80% of HPP units with 525L vessel capacity, assuming the water need to be replaced every 16 cycles to prevent contamination due to product leaking and assuming there is approximately average 2.5 – 3.0% of water loss per cycle of HPP treatment due to factors such as evaporation, leaks or system purging. The details cooling water calculation can refer Appendix G. High-pressure processing starts by filling of water into the HPP vessel. When the vessel is full, more water is pressed into the vessel by powerful pumps until it achieved the targeted pressure 600 MPa. When the HPP treatment is completed, the water is recirculated for the next application, resulting in lower water consumption compared to single-pass systems.

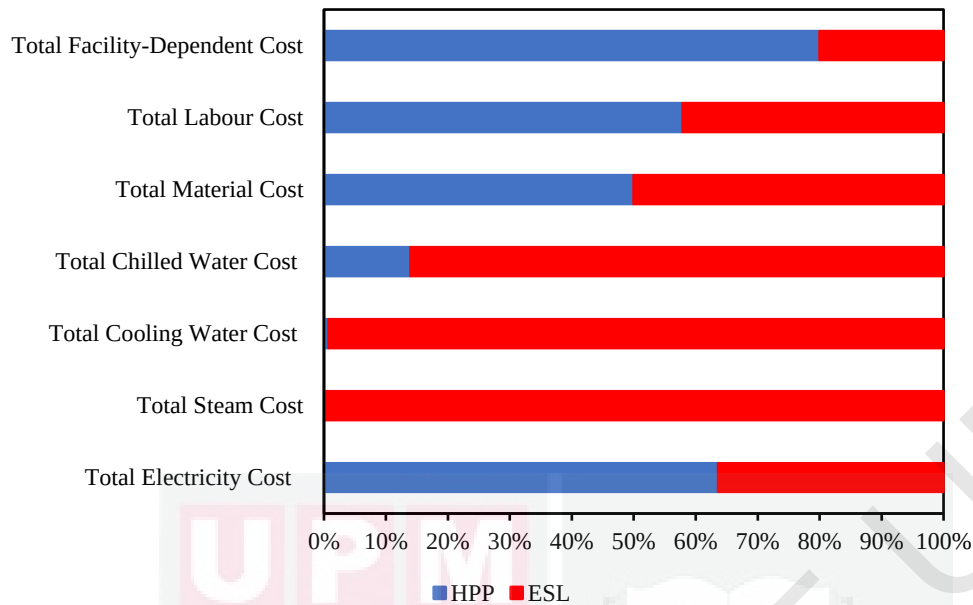


Figure 5.4: Comparison between operational cost of HPP and ESL milk in percentage.

Higher operating cost on HPP products was also reported by Sampedro et al. (2014) with that total cost of production of 1 L of orange juice by thermal pasteurisation (88 °C, 5 s) was estimated to be 1.5 ¢/L, whereas HPP (550 MPa, 90 s) was 10.7 ¢/L which corresponds to 7-fold higher than thermal pasteurisation while Mújica-Paz et al. (2011) estimated the cost for an intensively used HPP unit (600 MPa for 3 min, 5 °C, 300 L vessel) was 7.1 ¢/kg for a 1,440 kg/hr of production. Higher HPP production cost also reported by Ludikhuyze et al. (2002) at 0.1–0.2 Euro/kg of processed goods using pressure 400 MPa. The specific impact on product cost for HPP applications depends on multiple factors such as labour, pressure builds time of HPP unit, holding time, vessel filling rate, vessel capacity and the degree of utilisation (Ludikhuyze et al., 2002; Mújica-Paz et al. 2011).

The capital cost and operational cost obtained were used to study the economic feasibility of the milk processing technologies. The net cash flow per year is presented in Table 5.6, assuming both the milk are sold at the average market price RM 7.00, and having expected rate of return (discount rate) of 10%. The net cash flow of ESL treatment per year is 1% higher than HPP treatment. It suggests that ESL treatment generates more cash inflows or has lower cash outflows compared to HPP treatment.

Table 5.6: Net cash flow of HPP and ESL treatment.

HPP treatment						
Year	0	1	2	3	4	5
Net Economic Benefit	-	192,500,000	192,500,000	192,500,000	192,500,000	192,500,000
Net Cost	6,470,000	98,000,000	98,000,000	98,000,000	98,000,000	98,000,000
Net Cash Flow /year	-6,470,000	94,500,000	94,500,000	94,500,000	94,500,000	94,500,000
ESL treatment						
Year	0	1	2	3	4	5
Net Economic Benefit	-	192,500,000	192,500,000	192,500,000	192,500,000	192,500,000
Net Cost	2,266,000	97,000,000	97,000,000	97,000,000	97,000,000	97,000,000
Net Cash Flow /year	-2,266,000	95,500,000	95,500,000	95,500,000	95,500,000	95,500,000

Table 5.7 showed the comparison between NPV, IRR and break-even quantity of HPP and ESL treatment. Both treatments are acceptable with $NPV > 0$ and $IRR > 10\%$. NPV is a financial metric used to assess the profitability of an investment or project by calculating the net present value of its expected cash flows. A positive NPV indicates that the project is expected to generate more cash inflows than outflows, resulting in a net gain. In this case, both HPP and ESL treatments have an NPV greater than zero, implying that both treatments are expected to be financially profitable.

IRR, on the other hand, is a metric used to evaluate the rate of return on an investment. It represents the discount rate at which the present value of expected cash inflows

equals the present value of cash outflows, resulting in a net present value of zero. An IRR greater than 10% suggests that both HPP and ESL treatments offer a rate of return that exceeds the required rate of return or cost of capital, indicating their attractiveness as investment options.

Table 5.7: Comparison between NPV, IRR and break-even quantity of HPP and ESL treatment.

Analysis	HPP treatment	ESL treatment
NPV	$\text{PV (total benefit) - PV (total costs)}$ $= \sum_{n=1}^5 \frac{94,500,000}{(1+0.10)^n} - 6,226,355$ $= 352,002,994$ $= 352 \text{ million}$	$\text{PV (total benefit) - PV (total costs)}$ $= \sum_{n=1}^5 \frac{95,500,000}{(1+0.10)^n} - 2,179,224$ $= 359,840,912$ $= 359 \text{ million}$
IRR	$\text{IRR = Discount rate at NPV is 0}$ $0 = \sum_{n=1}^5 \frac{94,500,000}{(1+r)^n} - 6,226,355$ $r = 15.18 \%$	$\text{IRR = Discount rate at NPV is 0}$ $0 = \sum_{n=1}^5 \frac{95,500,000}{(1+r)^n} - 2,179,224$ $r = 43.82 \%$
Break-even quantity	$\text{Break-Even Quantity}$ $= \frac{\text{Total Fixed Cost}}{(\text{Sales Price} - \text{Variable Cost})}$ $= \frac{6,226,355}{(7 - 3.57)}$ $= 1,815,264$ $= 1.8 \text{ million}$	$\text{Break-Even Quantity}$ $= \frac{\text{Total Fixed Cost}}{(\text{Sales Price} - \text{Variable Cost})}$ $= \frac{2,179,224}{(7 - 3.54)}$ $= 629,833$ $= 0.6 \text{ million}$

The results of break-even quantity show that it is required to achieve sale target of 1.8 million bottles of HPP milk for the company to make profit on sold units. This amount is three times higher compared to the ESL milk that only required to sell 0.6 million bottles. This is due to the higher equipment purchasing cost of HPP compared to ESL treatment.

In summary, both HPP and ESL treatments are deemed acceptable investments. Their positive NPVs indicate profitability, while their IRRs exceeding 10% suggested

favourable rates of return. The break-even quantity results further assist in assessing the scale or volume of operations needed to achieve the break-even point for each treatment.

5.3.3 Sensitivity Analysis of HPP Treatment

The first sensitivity study was conducted to evaluate how an increase in cow milk unit cost influenced the NPV parameter, and determine at which value the HPP milk production plant became unprofitable. The results of sensitivity study on impact of raw milk unit cost on Net Present Value (NPV) were presented in Figure 5.5. Based on the figure, an increase in the unit cost of raw milk decreased the value of NPV. It can be seen that the unit cost of raw milk above RM 5.50/L led to a negative NPV which indicating the project became unprofitable. The details data for this sensitivity analysis can refer to the first section in Appendix H. This result was in accordance to the finding by Pérez Sánchez et al. (2023), in which the unit cost of raw material cow milk decreased the NPV value of an ice cream production plant, with that a unit cost of cow's milk above 1.50 \$/L caused the project becomes unprofitable.

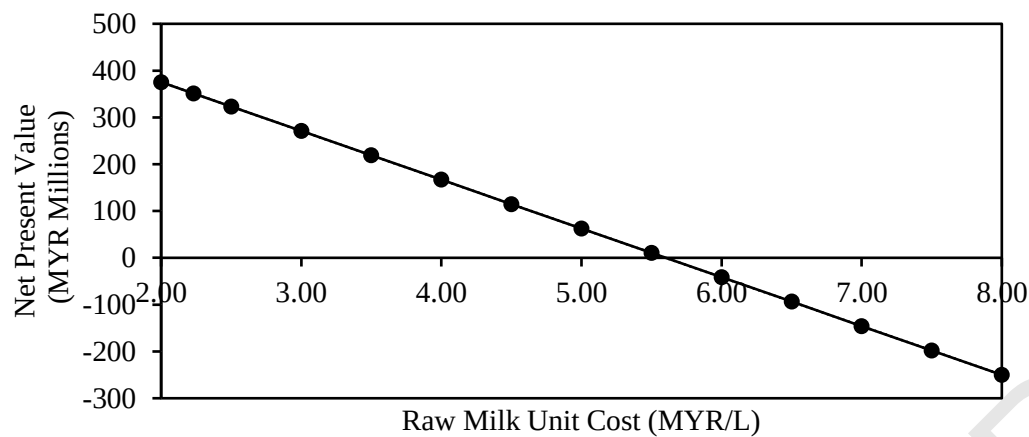


Figure 5.5: Results of sensitivity study on impact of raw milk unit cost on Net Present Value.

Figure 5.6 depicts the results of second sensitivity study which involved the verification of how a reduction of HPP milk selling price influences the NPV indicator and break even quantity (data refer to second section of Appendix H). The figure showed the lower HPP milk selling price resulted in a lower NPV value. It can be seen that the selling price of HPP milk below RM 3.60/L caused the project became not remunerative with a negative NPV. When the HPP was sold for lower money, it lowered the revenue for the business. Lower revenue directly affected the cash flows generated by the sale of HPP milk. These reduced cash flows contributed to a lower NPV when discounted back to the present cost.

A reduction of HPP milk selling price also caused an increase in breakeven quantity. Based on figure 5.6 (b), the breakeven quantity was doubled (from 7 million bottles to 14 million bottles) when the HPP milk selling price reduce by 10%, from RM 4.50/L to RM 4/L. Breakeven quantity was determined by dividing the total fixed costs by the contribution margin per unit. The contribution margin per unit was the difference between the selling price per unit and the variable cost per unit. A decrease in the selling price reduced the contribution margin per unit, as the difference between the

selling price and variable cost per unit decreased. When the contribution margin per unit decreased, it indicated more units of HPP milk need to be sold to cover the fixed costs and reach the breakeven point. In this stimulation, the variable cost of HPP milk was RM 3.57/L. HPP milk selling price below this amount will cause a negative value for breakeven quantity which do not have a conventional interpretation within the context of traditional breakeven analysis.

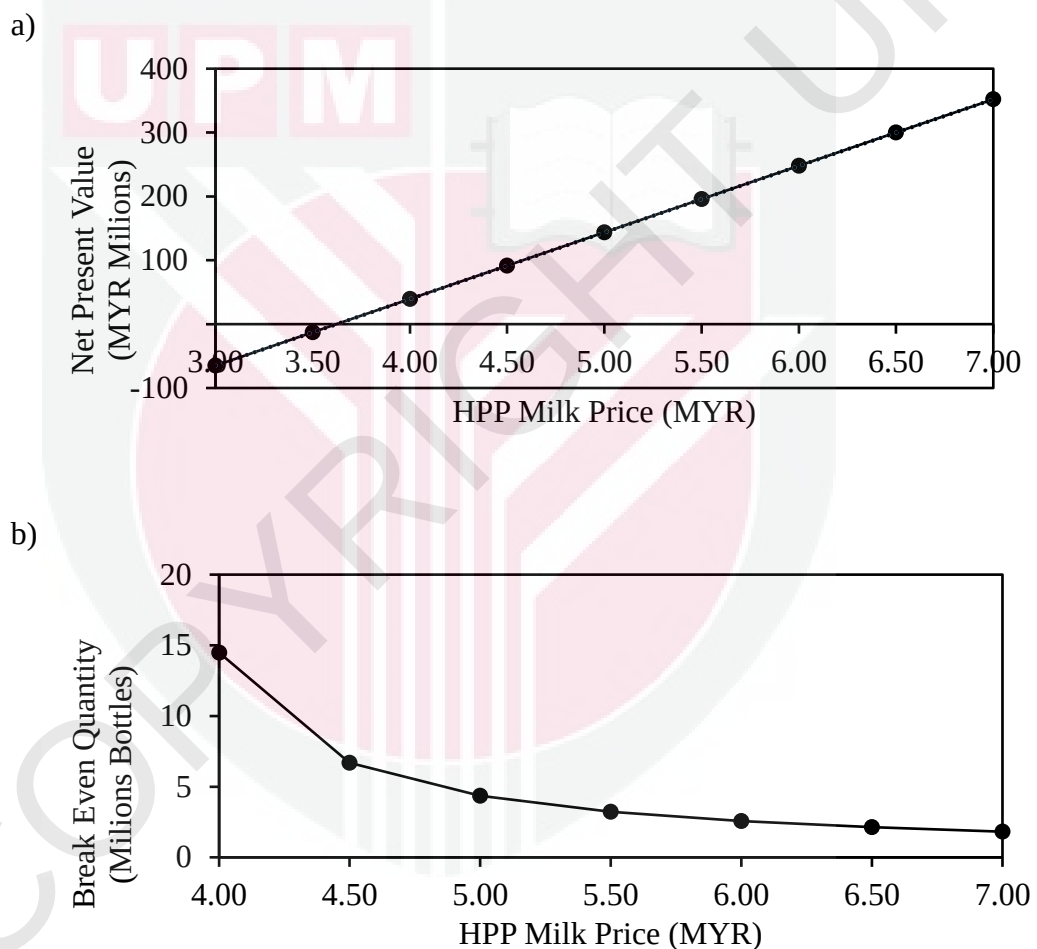


Figure 5.6: Results of sensitivity study on impact of HPP milk price on a) Net Present Value and b) Break Even Quantity.

Sensitivity study on the impact of HPP filling ratio was also conducted to evaluate how changes in this specific parameter can affect the economic feasibility of HPP milk

production. The filling ratio referred to the proportion of the pressure vessel filled with bottles of HPP milk. In this stimulation, it was assumed that the capital cost and all unit cost involved in operation such as utilities cost, materials cost, labour cost and facility-dependent cost remain unchanged. The results of sensitivity study on impact of HPP filing ratio on operational cost, net present value and breakeven quantity were as shown in Figure 5.7 (data details refer to third section of Appendix H).

A decrease in the HPP unit filling ratio means that less quantity of bottles milk were loaded into the HPP unit pressure vessel for treatment and resulted low product output. This generally reduced the total annual operational cost as the amount of materials needed in production such as raw milk and bottles had been reduced (Figure 5.7 a). However, in actual, the operational cost per L of HPP milk was increased when the HPP filing ratio decrease (Figure 5.7 b). When small HPP filling ratio was used, more cooling water was required to fully fill the pressure vessel to achieve the targeted pressure. Additionally, the HPP units have to operate longer periods to produce the same quantity of milk which potentially increasing the energy and labour cost. Typically, larger production runs allowed more efficient use of resources and lower the operational cost.

A decrease in filling ratio also reduced net present value of HPP milk (Figure 5.7 c). The net present value reduced by 94% (from RM 355 Million to RM 21 Million) when the filling ratio reduce by 60% (from 80% to 10%). This implied that a decrease in filling ratio lead to a less attractive investment and making it less economically feasible. When the selling price per unit of HPP milk remain constant, the total revenue generated per cycle of HPP treatment decreased due to the reduced filling ratio. This

reduction in revenue directly affects the cash flows associated with HPP milk production. However, although the filling ratio was reduced to 10%, the NPV was remained positive, indicating this project was still economically viable (Pérez Sánchez et al., 2023).

Apart from this, the breakeven quantity also affected with the change in HPP filling ratio. Based on Figure 5.7 d, the lower the filling ratio, the higher the breakeven quantity of HPP milk. This indicating higher amount of HPP milk needed to be sold to achieve profitability due to the increase in operational cost per L of HPP milk.

In summary, all three parameters including raw milk unit cost, HPP milk selling price and HPP filling ratio examined in sensitivity study could impact the economic feasibility. In this stimulation, raw milk unit cost above RM 5.50/L led to a negative NPV. Meanwhile, the selling price of HPP milk should not below RM 3.60/L which can caused the project became unprofitable. Lastly, a decrease in the filling ratio of HPP milk can lead to higher production costs, reduced revenue, lower cash flows, decreased profitability, and ultimately, a lower NPV for the investment in HPP milk production. This underscores the importance of considering all factors when making decision on any investment.

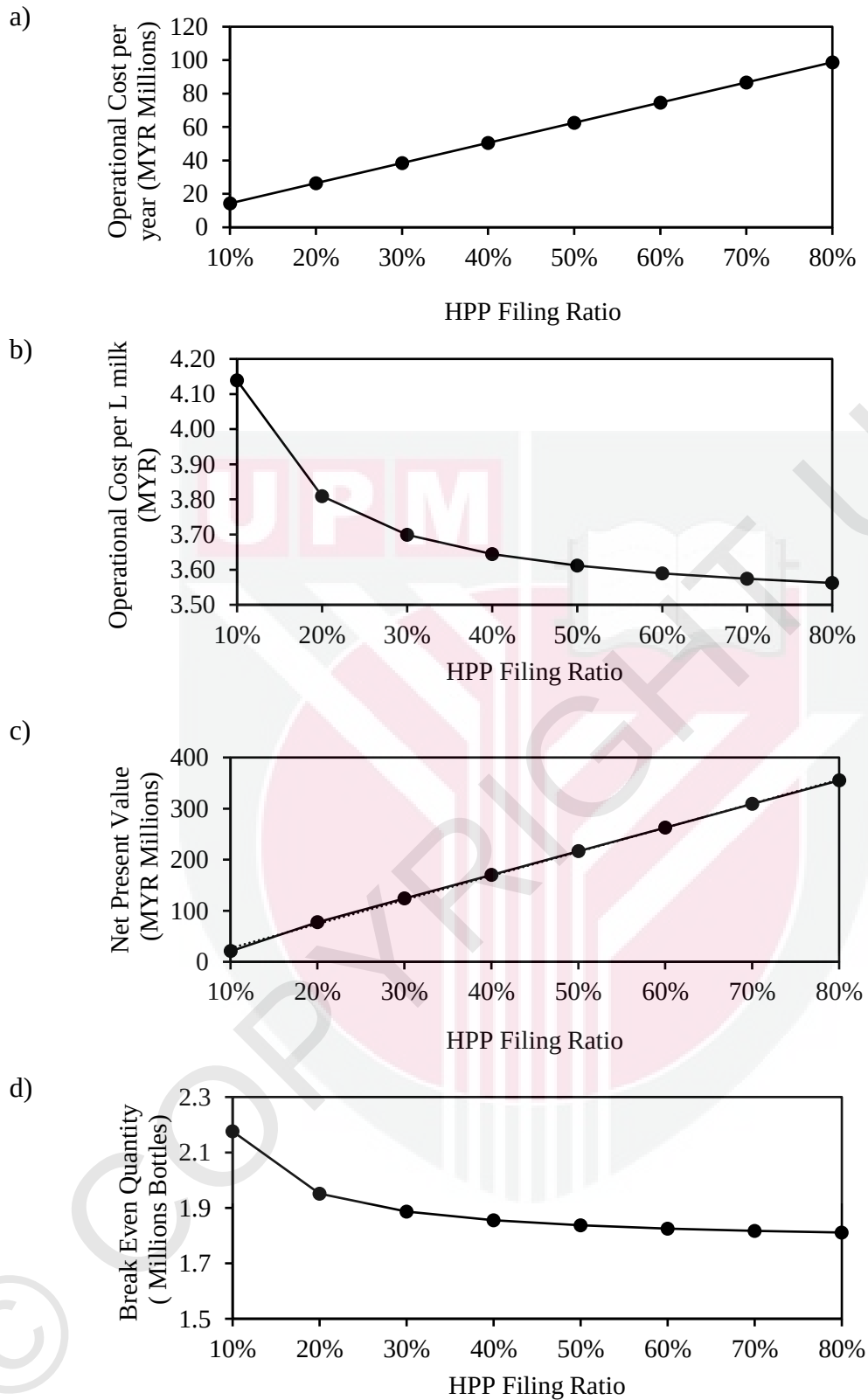


Figure 5.7: Results of sensitivity study on impact of HPP filing ratio on a) Operational Cost per year b) Operational Cost per L milk c) Net Present Value and d) Break Even Quantity.

5.3.4 Environmental Impact

The total carbon footprint of the HPP milk (0.018 kg CO₂/ L of milk) is much lower than the total carbon footprint of the ESL milk (205.4882 kg CO₂/ L) (Table 5.6). This is due to the nature of ESL heat treatment that consume high amount of steam annually for heating of milk. In manufacturing industry, boiler is commonly used to generate steam or heat using fuel such as natural gas as source of energy. During combustion of fuel in boiler, the carbon from the fuel will combine with oxygen from the air to produce carbon dioxide, causing a significant contribution to the carbon footprint. In addition, greenhouse gases such as carbon monoxide (CO) and nitrous oxide (N₂O) might also be produced during combustion of fuel in boiler. However, for a proper tuned boiler, it is common to have nearly all of the fuel carbon (99.9 %) in natural gas is converted to CO₂ during the combustion process (Eastern Research Group, 1998). Even in boilers operating with poor combustion efficiency, the amount of methane (CH₄), CO, and volatile organic compound (VOC) produced is insignificant compared to CO₂ levels (Eastern Research Group, 1998).

For the CO₂ emission from electricity source, HPP (445,121 kg CO₂/yr) is nearby two times compared with ESL treatment (254,755 kg CO₂/yr). Electricity is the main energy source for whole HPP treatment instead of using steam as the main energy source for ESL treatment. High electricity was consumed especially at the stage to build vessel pressure. Rodriguez-Gonzalez et al. (2015) reported most of the energy of HPP is consumed during pressure built up time as during this time the electrical energy is used to achieve desired pressure by the electric motor used in the pump-intensifier systems to operate the vessel at a desired pressure under which suitable reactions are

triggered. Sampedro et al. (2014) also reported higher electricity consumption on HPP (550 MPa for 1.5 min at 60–75% vessel filling ratio) as compared to heat pasteurisation, with an overall electrical energy consumption of 984,500 kWh/yr in processing 16,500,000 L of orange juice per yr.

Table 5.8: CO₂ emission between HPP and ESL

Cold Storage Room	Unit of measure	HPP	ESL
<i><u>Emissions source (Electricity)</u></i>			
Storage Tank	kg CO ₂ /yr	15,397	15,397
Filling Machine	kg CO ₂ /yr	9,798	-
Cold Storage Room	kg CO ₂ /yr	83,985	83,985
HPP Unit	kg CO ₂ /yr	335,940	-
Homogeniser	kg CO ₂ /yr	-	125,978
Ultra-pasteurisation Unit	kg CO ₂ /yr	-	19,597
Aseptic Filling Machine	kg CO ₂ /yr	-	9,798
Total CO ₂ emissions (electricity)	kg CO ₂ /yr	445,121	254,755
Total CO ₂ emissions per liter milk (electricity)	kg CO ₂ / L	0.0162	0.0093
<i><u>Emissions source (Utility)</u></i>			
Steam (152°C)	kg CO ₂ /yr	-	5,650,357,833
Cooling water (25 - 30°C)	kg CO ₂ /yr	22,294	137,529
Chilled water (5 - 10°C)	kg CO ₂ /yr	28,537	176,037
Total CO ₂ emissions (Utility)	kg CO ₂ /yr	50,831	5,650,671,399
Total CO ₂ emissions per liter milk (Utility)	kg CO ₂ / L	0.0018	205.4790
Total CO ₂ emissions per yr (electricity + utilities)	kg CO ₂ /yr	495,951	5,650,926,154
Total CO ₂ emissions per liter (electricity + utilities)	kg CO ₂ / L	0.018	205.488

5.4 Conclusion

The capital cost for an industrial scale HPP milk production was estimated to be RM 6,470,000 (RM 0.24/L), which was 3 folds higher than ESL heat treatment at RM 2,266,000 (RM 0.08/L). The main reason causing high capital cost of HPP is the high equipment purchase cost of HPP equipment at RM1, 040,000, while the equipment purchase cost of ESL heat treatment was only RM 364,000. Overall, the different between operational cost of HPP and ESL milk is not significant because the cost was balanced-up within by the higher electricity, labour and facility-dependent cost for the

HPP with the higher utilities cost including steam, cooling water and chilled water cost for the ESL treatment. Although the yearly net cash flow of HPP was 1% lower than ESL heat treatment, however both treatments are acceptable investment with NPV > 0 and IRR > 10%. The results of break-even quantity showed that it was required to sell 1.9 million bottles of HPP milk which was three times higher compared to the ESL milk that only required to sell 0.6 million bottles to break even or make profit on sold units. Sensitivity study proposed that the HPP milk production begins to be unprofitable from the raw milk unit cost above RM 5.50/L and when the HPP milk selling price lower than RM 3.60/L. Meanwhile, a decrease in HPP unit filling ratio resulted a lower NPV for the investment in HPP milk production. Total electricity consumption was higher for HPP (874,500 kWh/yr) as compared to ESL heat treatment that only consumed 500,500 kWh/yr. The working principle of HPP that uses electricity also contributed to lower carbon emission by approximately 99.99% as compared to ESL treatment that uses steam as source of energy.

CHAPTER 6

SUMMARY, GENERAL CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1 Conclusions

This chapter concludes the study by summarising the key research findings in relation to the research aims and questions with discussion on the value and contribution thereof. It also reviews the limitations of the study and proposes opportunities for future research. The conclusions are drawn from results presented in Chapters 3, 4 and 5.

In response to the first research objective to evaluate the effect of industrial scale HPP treatment on fresh cow milk quality, results showed both HPP and ESL treatments on microbial inactivation of fresh milk are comparable with only minor changes were observed on all physicochemical properties, vitamin and mineral contents following treatments. In storage study, HPP treated milk showed confident results of storage shelf life beyond 60 days with all microbial testing meeting permitted levels, whereas ESL milk was not safe for consumption after 5 days of storage because total plate count, total yeast and moulds, and psychrotrophic counts exceeded industry permitted levels of 4, 1, and 4 log CFU/mL between 5 – 10 days of storage, despite meeting the safety standard on Day 0 of treatment. The ESL milk in this study had an unexpected short shelf life of 10 days instead of anticipated 21 – 45 days as documented in most other researches mostly due to post processing contamination during filling which pose as a processing risk in real industrial environment. Post processing contamination can be prevented in HPP as it is a batch process where products are packed before

treatment. The physicochemical, vitamin and mineral contents between the HPP and ESL milk did not differ much, but less significant degradation was observed in HPP than ESL throughout the 60-day storage period. In general, HPP milk was more stable than ESL milk in term of physicochemical properties, as indicated by steady pH readings and undetectable titratable acidity throughout the storage period. HPP milk also had superior vitamin A and B5 retention at 75% and 65% respectively, as compared to ESL milk with only 25% and 48.9% at the end of 60 days' storage. However, ESL milk showed better vitamin C retention (41.5%) as compared to HPP milk (22.4%) after 60-days storage. In terms of mineral stability, HPP and ESL milk are near to identical.

In relationship to the second research objective to evaluate the influence of HPP treatment on sensory properties, triangle test and acceptance and preference test were conducted to compare with ESL milk. Triangle test results revealed noticeable differences between HPP and ESL milk as there was up to 89.5% of panellists selected the correct sample in the triangle test. In acceptance and preference test, results demonstrated majority panellists have given higher ratings on HPP milk as shown in the hedonic evaluation of overall liking of HPP and ESL milk. The JAR evaluation of acceptance and preference of the treated milk samples' sensory properties showed no significant differences ($p > 0.05$) in all attributes which included milkiness, creaminess, mouthfeel, and aftertaste, with the exception of colour. The colour differences were visible in HPP milk (darker colour) in comparison with ESL milk (lighter colour) because high pressure treatment induced modification of colloidal and emulsion components which affected the light scattering effect that led to colour changes of HPP-treated milk. The overall sensory properties of HPP milk were

evaluated higher for mouthfeel and aftertaste attributes than ESL milk which scored higher for colour, milkiness, and creaminess. The penalty analysis suggested that HPP milk can be improved in terms of creaminess and aftertaste despite the later attribute was still scoring better than ESL milk.

The positive benefits of HPP treated fresh cow milk have brought about the development of the third research objective, i.e. to conduct feasibility study on using the HPP technology for processing milk for the dairy industry. Cost and environmental impacts of using HPP over using heat treatment was assessed. This objective was accomplished using the estimation method. The estimated capital cost of HPP was much higher than ESL heat treatment due to the high equipment purchase cost of the HPP unit. For operation cost, HPP induced higher electricity, labour and facility-dependent cost, while ESL treatment induced higher utilities cost for steam, cooling water and chilled water. Both HPP and ESL treatments are considered economically feasible with positive NPV and $IRR > 10\%$ although HPP has higher break-even quantity (1.9 million bottles) than ESL heat treatment (0.6 million bottles). The working principle of HPP that uses electricity contributes to lower carbon emission and can be declared as a more environmentally friendly treatment compared to ESL heat treatment that uses steam as source of energy.

This study provides a reference or guidance to those exploring new technologies in processing high quality food products, particularly non-thermal treatment in the milk processing industry. The application of HPP for fresh cow milk processing has been thoroughly studied in all aspects including technical operation and safety, consumer market acceptance, economic and costing, and environment and sustainability aspects.

Results suggest that the HPP technology is highly considerably as an alternative of heat treatment if cost is not the main challenge weighing the other benefits gained including consumer and environmental aspects.

6.2 Study Limitations

There are few potential limitations in this study. Firstly, it was a great challenge to assess a commercial scale HPP unit to conduct this research as HPP is not a common treatment for food preservation in Malaysia. This has not only resulted a high equipment rental cost but also raised the issue of industrial sensitivity due to industrial oriented research. There was constrain of more generous sample numbers and runs as desired and preferred in the research environment.

Similar issue was encountered for the ultra-pasteurisation unit as it was privately owned and required to be operated following the conduct and protocols of provided. The runs were not accessible first hands unlike laboratory scale equipment thus it was anticipated that the spoilage of milk much earlier than the standard ESL milk shelf life of 30 days was due to unforeseen contamination during filling. This reflects reality in milk industry as dairy products in the market do experience spoilage before the best before dates when such miss-handling occurs during processing.

In sensory evaluation, the result accuracy could be influenced by panellists, sample handing and environment. Due to the pandemic restriction, the sensory test was conducted in simplified method. For example, the sensory test was conducted in simple kitchen instead of a proper sensory tasting room with special facilities such as sample preparation room, sample deliver platform, dedicated seat for each panellist, adequate

lightning and other. Covid-19 pandemic also causes difficulties in finding panellist. The panellist participate in this sensory evaluation were of close proximity figures who know the objective of this research, hence, it might cause result bias.

For cost and environment assessment, the limitation is that the study has referred to a local scenario and this may give variance for a different country and period of time. Conducting a cost and environmental assessment may be challenging as the costs associated with HPP can change during the study, making it difficult to capture the most recent economic and environmental factors accurately.

6.3 Recommendations

Future researches can focus on studying the effect of HPP on vitamin and mineral contents of milk as they are only limited literature found on this topic. It is also recommended to increase the days of analysis for the storage study of vitamin and mineral contents of HPP milk.

Sensory evaluation should be conducted in standard sensory tasting room that provides a controlled environment. As sensory evaluation is fully depending on the panellist, hence panellist should be selected carefully based on their sensory acuity and ability to articulate their perceptions. Comprehensive training programs can be conducted for sensory panellists to enhance their sensory perception, discrimination abilities, and understanding of sensory attributes referring to the standard handling procedures and requirements to provide reliable result. For example, in triangle tests, 4 - 8 tasters are considered enough to carry single testing, 4 - 6 panellists are suggested for flavour

profiling and 50 - 150 panellists for consumer acceptance and preference test (Mihafu et al., 2020).

For the limitations in the cost and environmental assessment, it can be improved by developing a cost or environment prediction model. This model then allows user to do cost and environment estimation at any time by inputting relevant and consistent data. In cost estimation, prediction model can produce precise cost estimation by considering each element including the material costs, labour costs, energy usage, and maintenance needs whether individually or simultaneously. This aids businesses in cost optimisation, budgeting, and financial planning. In environmental assessment, prediction model will assist in quantifying and comparing environmental consequences whilst investigating factors which include resource consumption, emissions, trash generation, and land usage where then making classification on the environmental impact into different degrees such as friendly and not friendly in terms of Sustainable Development Goals (SDG) con-current to the world's or country's direction. This enables decision-makers to identify environmentally friendly alternatives, set reduction targets, and prioritise sustainable initiatives.

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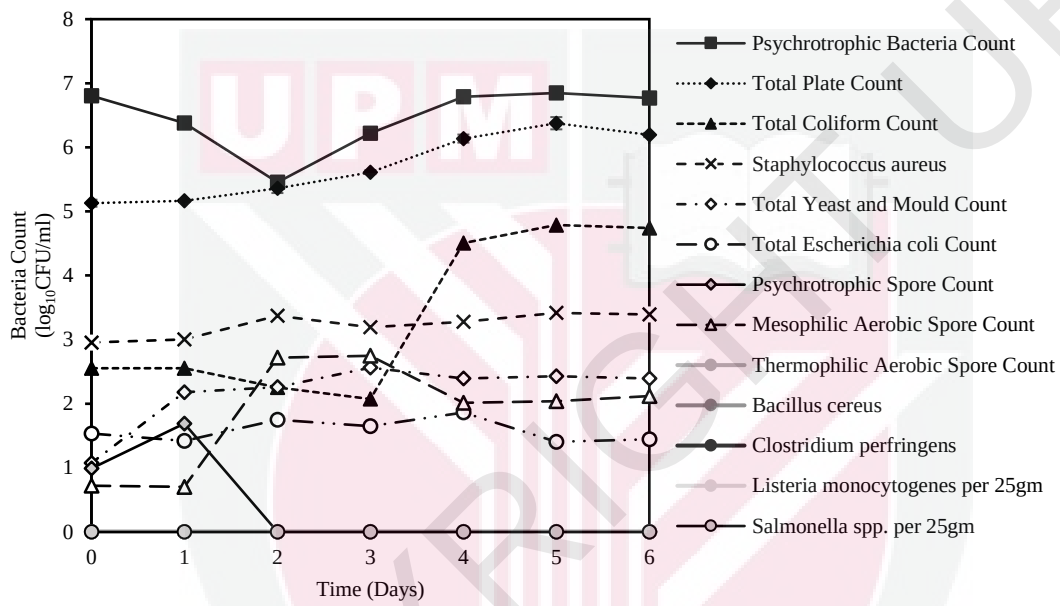
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APPENDICES

Appendix A: Microbiological Analysis Data

Appendix A1



Changes in microbiological properties of raw milk during 6 days' storage at 6±1 °C

Appendix A2

Data of changes in microbiological properties of fresh milk during 6 days' storage.

Bacteria count (log ₁₀ CFU/mL)	Storage (Days)						
	0	1	2	3	4	5	6
Total Plate Count	5.13 ± 0.06 ^a	5.17 ± 0.02 ^a	5.36 ± 0.08 ^{ab}	5.61 ± 0.29 ^{abc}	6.14 ± 0.06 ^{bc}	6.38 ± 0.10 ^c	6.19 ± 0.03 ^{bc}
Total Coliform Count	2.56 ± 0.02 ^a	2.56 ± 0.03 ^a	2.25 ± 0.03 ^a	2.70 ± 0.07 ^a	4.41 ± 0.08 ^b	4.79 ± 0.05 ^b	4.74 ± 0.02 ^b
Total <i>Escherichia coli</i> Count	1.53 ± 0.12 ^a	1.42 ± 0.06 ^a	1.75 ± 0.05 ^a	1.65 ± 0.33 ^a	1.86 ± 0.33 ^a	1.41 ± 0.09 ^a	1.44 ± 0.05 ^a
Total Yeast and Molds Count	1.61 ± 0.11 ^a	2.18 ± 0.14 ^a	2.26 ± 0.04 ^a	2.56 ± 0.03 ^a	2.40 ± 0.10 ^a	2.43 ± 0.06 ^a	2.40 ± 0.08 ^a
Psychrotrophic Bacteria Count	6.81 ± 0.05 ^a	6.38 ± 0.07 ^a	6.16 ± 0.07 ^a	6.22 ± 0.08 ^a	6.79 ± 0.02 ^a	6.85 ± 0.03 ^a	6.77 ± 0.02 ^a
Psychrotrophic Spore Count	0.99 ± 0.15	1.69 ± 0.01	< 1	< 1	< 1	< 1	< 1
Mesophilic Aerobic Spore Count	0.72 ± 0.06 ^a	0.70 ± 0.10 ^a	2.72 ± 0.06 ^c	2.75 ± 0.08 ^c	2.01 ± 0.01 ^b	2.04 ± 0.02 ^b	2.12 ± 0.06 ^b
Thermophilic Aerobic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Staphylococcus aureus</i>	2.96 ± 0.03 ^a	3.01 ± 0.07 ^{ab}	3.37 ± 0.03 ^c	3.19 ± 0.08 ^{abc}	3.28 ± 0.04 ^{bc}	3.42 ± 0.04 ^c	3.39 ± 0.09 ^c
<i>Bacillus cereus</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Clostridium perfringens</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Listeria monocytogenes</i> per 25gm	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<i>Salmonella spp.</i> per 25gm	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Mean ± standard deviation in same row with different superscripts letters (a,b,c) are significantly different at p≤0.05.

Appendix A3

Changes in microbiological properties of HPP milk during 60 days' storage at 6±1 °C.

Bacteria count (log ₁₀ CFU/mL)	Storage (Days)												
	0	5	10	15	20	25	30	35	40	45	50	55	60
HPP Milk													
Total Plate Count	1.26 ± 0.04 ^{ab}	1.75 ± 0.13 ^{cd}	1.84 ± 0.03 ^d	1.49 ± 0.09 ^{bc}	1.17 ± 0.11 ^{ab}	1.15 ± 0.07 ^a	1.15 ± 0.08 ^a	1.07 ± 0.01 ^a	1.04 ± 0.02 ^a	1.76 ± 0.03 ^{cd}	1.09 ± 0.05 ^a	1.08 ± 0.02 ^a	1.03 ± 0.01 ^a
Total Coliform Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Total <i>Escherichia coli</i> Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Total Yeast and Molds Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Psychrotrophic Bacteria Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Psychrotrophic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Mesophilic Aerobic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Thermophilic Aerobic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Staphylococcus aureus</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Bacillus cereus</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Clostridium perfringens</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Listeria monocytogenes</i> per 25gm	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<i>Salmonella spp.</i> per 25gm	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

^{a-d} Mean ± standard deviation in same row with different superscripts letters are significantly different at p≤0.05.

Changes in microbiological properties of ESL milk during 60 days' storage at 6±1 °C.

Bacteria count (log ₁₀ CFU/mL)	Storage (Days)												
	0	5	10	15	20	25	30	35	40	45	50	55	60
ESL Milk													
Total Plate Count	1.23 ± 0.09 ^a	2.59 ± 0.05 ^b	4.18 ± 0.06 ^c	6.64 ± 0.01 ^{de}	6.85 ± 0.03 ^{ef}	7.16 ± 0.04 ^h	7.72 ± 0.07 ^{ij}	7.88 ± 0.03 ^j	7.51 ± 0.04 ⁱ	7.10 ± 0.01 ^{gh}	6.92 ± 0.01 ^{fg}	6.79 ± 0.01 ^{ef}	6.47 ± 0.04 ^d
Total Coliform Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Total <i>Escherichia coli</i> Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Total Yeast and Molds Count	< 1	1.90 ± 0.01 ^a	2.72 ± 0.06 ^b	3.89 ± 0.03 ^f	4.48 ± 0.07 ^g	4.93 ± 0.04 ^h	4.39 ± 0.03 ^g	3.08 ± 0.02 ^c	3.41 ± 0.02 ^d	3.52 ± 0.11 ^{de}	3.13 ± 0.08 ^c	3.71 ± 0.01 ^{ef}	3.86 ± 0.04 ^f
Psychrotrophic Bacteria Count	< 1	1.82 ± 0.10 ^a	5.54 ± 0.19 ^b	6.71 ± 0.02 ^{de}	7.69 ± 0.01 ^g	7.03 ± 0.01 ^{ef}	8.07 ± 0.05 ^h	7.68 ± 0.14 ^g	7.26 ± 0.03 ^f	7.03 ± 0.01 ^{ef}	6.76 ± 0.03 ^{de}	6.31 ± 0.04 ^c	6.42 ± 0.04 ^{cd}
Psychrotrophic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Mesophilic Aerobic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Thermophilic Aerobic Spore Count	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Staphylococcus aureus</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Bacillus cereus</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Clostridium perfringens</i>	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
<i>Listeria monocytogenes</i> per 25gm	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
<i>Salmonella spp.</i> per 25gm	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

^{a-h} Mean ± standard deviation in same row with different superscripts letters are significantly different at p≤0.05.

Appendix B: Physicochemical Analysis Data

Appendix B1

Changes in physicochemical properties of fresh milk during 6 days 'storage at 6±1 °C.

Properties	Storage (Days)		
	0	3	6
pH Value (pH)	6.57 ± 0.03 ^{ab}	6.60 ± 0.00 ^a	6.50 ± 0.00 ^b
Acidity as Lactic Acid (%)	0.20 ± 0.00 ^a	0.20 ± 0.00 ^a	0.37 ± 0.03 ^b
Fat (g/100g)	3.57 ± 0.12 ^a	3.50 ± 0.06 ^a	3.57 ± 0.12 ^a
Protein (g/100g)	3.10 ± 0.00 ^a	3.27 ± 0.09 ^a	3.10 ± 0.00 ^a
Total Solids (g/100mL)	12.07 ± 0.12 ^a	12.57 ± 0.09 ^b	12.37 ± 0.12 ^{ab}
Non-Fat Milk Solids (g/100g)	8.40 ± 0.15 ^a	8.77 ± 0.03 ^a	8.60 ± 0.00 ^a
Specific Gravity (g/mL)	1.02 ± 0.00	1.02 ± 0.00	1.02 ± 0.00

^{ab} Mean ± standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$.

Appendix B2

Effects of treatments on physicochemical (main) properties of fresh milk.

Properties	Fresh Milk	Treatments	
		HPP	ESL
pH Value (pH)	6.57 ± 0.03 ^a	6.50 ± 0.00 ^a	6.50 ± 0.00 ^a
Acidity as Lactic Acid (%)	0.20 ± 0.00	< 0.1	< 0.1
Fat (g/100g)	3.57 ± 0.12 ^a	3.70 ± 0.15 ^a	3.60 ± 0.06 ^a
Protein (g/100g)	3.10 ± 0.00 ^a	3.27 ± 0.03 ^a	3.27 ± 0.09 ^a
Total Solids (g/100mL)	12.07 ± 0.12 ^a	12.27 ± 0.22 ^a	12.13 ± 0.09 ^a
Non-Fat Milk Solids (g/100g)	8.40 ± 0.15 ^a	8.50 ± 0.15 ^a	8.33 ± 0.09 ^a
Specific Gravity (g/mL)	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a

^a Mean ± standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$.

Appendix B3

Changes in physicochemical properties of HPP and ESL milk during 60 days' storage at 6±1 °C.

Properties	Unit	Storage (Days)										
		0	6	12	18	24	30	36	42	48	54	60
HPP Milk												
pH Value	pH	6.50 ± 0.00 ^a	6.60 ± 0.00 ^a	6.57 ± 0.00 ^a	6.57 ± 0.00 ^a	6.57 ± 0.00 ^a	6.60 ± 0.00 ^a	6.57 ± 0.00 ^a	6.57 ± 0.00 ^a	6.57 ± 0.00 ^a	6.57 ± 0.00 ^a	6.60 ± 0.00 ^a
Acidity	%	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	0.10 ± 0.00
Fat	g/100 g	3.70 ± 0.15 ^a	3.90 ± 0.00 ^a	3.80 ± 0.15 ^a	3.53 ± 0.13 ^a	3.80 ± 0.10 ^a	3.90 ± 0.06 ^a	3.50 ± 0.06 ^a	3.53 ± 0.19 ^a	3.63 ± 0.03 ^a	3.43 ± 0.03 ^a	3.80 ± 0.06 ^a
Protein	g/100 g	3.27 ± 0.03 ^{ab}	3.03 ± 0.07 ^a	3.17 ± 0.12 ^{ab}	3.27 ± 0.07 ^{ab}	3.33 ± 0.07 ^b	3.13 ± 0.03 ^{ab}	3.13 ± 0.03 ^{ab}	3.13 ± 0.07 ^{ab}	3.17 ± 0.03 ^{ab}	3.27 ± 0.03 ^{ab}	3.40 ± 0.00 ^b
Total Solids	g/100 mL	12.27 ± 0.22 ^{bc}	12.90 ± 0.06 ^{bc}	10.20 ± 0.35 ^a	12.63 ± 0.15 ^{bc}	11.73 ± 0.07 ^b	12.90 ± 0.25 ^{bc}	10.43 ± 0.18 ^a	12.57 ± 0.35 ^{bc}	12.40 ± 0.20 ^{bc}	13.07 ± 0.37 ^c	12.37 ± 0.20 ^{bc}
NF Solids	g/100 g	8.50 ± 0.15 ^{ab}	8.70 ± 0.06 ^{ab}	8.63 ± 0.30 ^{ab}	8.87 ± 0.07 ^b	7.70 ± 0.10 ^a	8.70 ± 0.26 ^{ab}	9.13 ± 0.18 ^b	8.67 ± 0.47 ^{ab}	8.70 ± 0.26 ^{ab}	9.57 ± 0.09 ^b	8.50 ± 0.17 ^{ab}
SG	g/mL	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.03 ± 0.00 ^a	1.03 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a
ESL Milk												
pH Value	pH	6.50 ± 0.00 ^a	6.60 ± 0.00 ^a	6.53 ± 0.00 ^a	6.50 ± 0.00 ^a	6.57 ± 0.00 ^a	6.57 ± 0.00 ^a	6.53 ± 0.00 ^a	6.53 ± 0.00 ^a	6.50 ± 0.00 ^a	6.57 ± 0.00 ^a	6.53 ± 0.00 ^a
Acidity	%	< 0.10	0.20 ± 0.00	< 0.10	0.10 ± 0.00	0.10 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	< 0.10	< 0.10	0.10 ± 0.00	0.20 ± 0.00
Fat	g/100 g	3.60 ± 0.06 ^a	3.63 ± 0.12 ^a	3.87 ± 0.03 ^a	3.53 ± 0.07 ^a	3.63 ± 0.20 ^a	3.80 ± 0.15 ^a	3.77 ± 0.09 ^a	3.67 ± 0.17 ^a	3.53 ± 0.09 ^a	3.80 ± 0.12 ^a	3.70 ± 0.17 ^a
Protein	g/100 g	3.27 ± 0.09 ^a	3.23 ± 0.09 ^a	3.23 ± 0.09 ^a	3.20 ± 0.10 ^a	3.27 ± 0.09 ^a	3.33 ± 0.07 ^a	3.17 ± 0.03 ^a	3.13 ± 0.09 ^a	3.13 ± 0.03 ^a	3.23 ± 0.03 ^a	3.40 ± 0.00 ^a
Total Solids	g/100 mL	12.13 ± 0.09 ^b	12.73 ± 0.07 ^{bc}	10.60 ± 0.35 ^a	12.60 ± 0.12 ^b	14.23 ± 0.43 ^d	11.97 ± 0.07 ^b	10.30 ± 0.15 ^a	12.70 ± 0.10 ^b	12.97 ± 0.28 ^{bc}	12.13 ± 0.15 ^b	13.77 ± 0.03 ^{cd}
NF Solids	g/100 g	8.33 ± 0.09 ^a	8.83 ± 0.09 ^{abc}	8.80 ± 0.31 ^{abc}	8.90 ± 0.10 ^{abc}	10.23 ± 0.30 ^d	7.87 ± 0.12 ^a	8.57 ± 0.24 ^{ab}	8.67 ± 0.24 ^{abc}	9.53 ± 0.26 ^{bcd}	8.47 ± 0.26 ^{ab}	9.70 ± 0.15 ^{cd}
SG	g/mL	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.03 ± 0.00 ^a	1.02 ± 0.00 ^a	1.03 ± 0.00 ^a	1.02 ± 0.00 ^a	1.02 ± 0.00 ^a	1.03 ± 0.00 ^a

^{a-d} Mean ± standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$. NF is non-fat. SG is specific gravity.

Appendix C: Vitamin Content Analysis Data

Appendix C1

Changes in vitamin content of fresh milk during 6 days' storage at 6±1 °C.

Vitamin Content	LOR Unit	Storage (Days)		
		0	3	6
Vitamin A (as Retinol) *	0.1 mg/100 mL	0.04 ± 0.00 ^b	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a
Beta-carotene *	1 mg/100 mL	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a
Vitamin B1 *	0.25 mg/100 mL	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a
Vitamin B2 *	0.25 mg/100 mL	0.17 ± 0.00 ^a	0.18 ± 0.01 ^a	0.17 ± 0.01 ^a
Vitamin B3 (as Niacin) *	0.2 mg/100 mL	0.11 ± 0.00	< 0.2	< 0.2
Vitamin B5	0.25 mg/100 mL	0.40 ± 0.00 ^a	0.58 ± 0.01 ^b	0.58 ± 0.00 ^b
Vitamin B6 *	0.25 mg/100 mL	0.05 ± 0.01 ^b	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a
Vitamin B7 (Biotin)	1 µg/100 mL	4.00 ± 0.00 ^a	4.33 ± 0.58 ^a	4.33 ± 0.58 ^a
Vitamin B9 (Folic Acid)	1 µg/100 mL	6.00 ± 0.00 ^a	7.50 ± 0.50 ^b	7.50 ± 0.50 ^b
Vitamin B12 (Cyanocobalamin)	1 µg/100 mL	1.67 ± 0.58	< 1	< 1
Vitamin C *	2 mg/100 mL	1.00 ± 0.00 ^c	0.47 ± 0.06 ^b	0.20 ± 0.10 ^a
Vitamin D	0.1 µg/100 mL	2.50 ± 0.00 ^a	2.52 ± 0.01 ^a	2.51 ± 0.01 ^a
Vitamin E (Alpha-Tocopherol) *	0.1 mg/100 mL	0.07 ± 0.00 ^a	0.08 ± 0.00 ^b	0.08 ± 0.00 ^b
Vitamin K	0.1 µg/100 g	0.30	0.45	0.46

^{a-c} Mean ± standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$.

* Results showing vitamin content lower than LOR. LOR is limit of reporting.

Appendix C2

Changes in vitamin content of HPP and ESL milk during 60 days' storage at 6±1 °C.

Vitamin Content	Unit	Storage (Days)				
		0	15	30	45	60
HPP Milk						
Vitamin A (as Retinol)	mg/100mL	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.03 ± 0.00 ^a	0.02 ± 0.33 ^b	0.03 ± 0.00 ^a
Beta-carotene	mg/100mL	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
Vitamin B1	mg/100mL	0.03 ± 0.00 ^a	0.04 ± 0.00 ^a	0.03 ± 0.01 ^a	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a
Vitamin B2	mg/100mL	0.18 ± 0.00 ^a	0.17 ± 0.00 ^a	0.17 ± 0.00 ^a	0.17 ± 0.00 ^a	0.18 ± 0.00 ^a
Vitamin B3 (as Niacin)	mg/100mL	0.11 ± 0.00 ^b	0.05 ± 0.03 ^a	0.05 ± 0.00 ^a	0.04 ± 0.00 ^a	0.01 ± 0.00 ^c
Vitamin B5	mg/100mL	0.40 ± 0.00 ^b	0.40 ± 0.01 ^b	0.48 ± 0.00 ^c	0.53 ± 0.03 ^c	0.26 ± 0.00 ^a
Vitamin B6	mg/100mL	0.06 ± 0.00 ^d	0.03 ± 0.00 ^c	0.02 ± 0.00 ^b	0.20 ± 0.00 ^b	0.01 ± 0.00 ^a
Vitamin B7 (Biotin)	µg/100mL	4.00 ± 0.00 ^a	3.00 ± 0.00 ^a	4.00 ± 0.00 ^a	4.33 ± 0.88 ^a	5.00 ± 0.58 ^a
Vitamin B9 (Folic Acid)	µg/100mL	6.33 ± 0.33 ^a	14.33 ± 0.88 ^b	18.0 ± 1.00 ^c	11.33 ± 0.33 ^b	12.00 ± 0.58 ^b
Vitamin B12(Cyanocobalamin	µg/100mL	2.00 ± 0.00 ^b	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	2.00 ± 0.00 ^b	2.00 ± 0.00 ^b
Vitamin C	mg/100 mL	1.67 ± 0.33 ^c	1.00 ± 0.00 ^{bc}	0.87 ± 0.03 ^b	0.51 ± 0.00 ^{ab}	0.15 ± 0.08 ^a
Vitamin D	µg/100mL	2.50 ± 0.00 ^b	0.42 ± 0.07 ^a	2.52 ± 0.01 ^b	0.50 ± 0.00 ^a	2.50 ± 0.00 ^b
Vitamin E (Alpha-Tocopherol)	mg/100 mL	0.07 ± 0.00 ^a	0.07 ± 0.00 ^b	0.07 ± 0.00 ^{ab}	0.07 ± 0.00 ^{ab}	0.06 ± 0.00 ^a
Vitamin K	µg/100g	0.65	0.46	1.05	0.90	0.30
ESL Milk						
Vitamin A (as Retinol)	mg/100mL	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.03 ± 0.00 ^a	0.01 ± 0.03 ^b	0.01 ± 0.00 ^a
Beta-carotene	mg/100mL	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
Vitamin B1	mg/100mL	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.05 ± 0.00 ^a	0.04 ± 0.00 ^a
Vitamin B2	mg/100mL	0.18 ± 0.00 ^a	0.18 ± 0.00 ^a	0.17 ± 0.00 ^a	0.17 ± 0.00 ^a	0.18 ± 0.00 ^a
Vitamin B3 (as Niacin)	mg/100mL	0.11 ± 0.00 ^e	0.07 ± 0.04 ^d	0.05 ± 0.00 ^c	0.04 ± 0.00 ^b	0.01 ± 0.01 ^a
Vitamin B5	mg/100mL	0.47 ± 0.03 ^b	0.42 ± 0.02 ^b	0.48 ± 0.00 ^b	0.51 ± 0.04 ^b	0.23 ± 0.01 ^a
Vitamin B6	mg/100mL	0.06 ± 0.00 ^d	0.03 ± 0.00 ^c	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	0.01 ± 0.00 ^a
Vitamin B7 (Biotin)	µg/100mL	4.00 ± 0.00 ^{ab}	3.00 ± 0.00 ^a	4.00 ± 0.00 ^{ab}	7.00 ± 0.58 ^c	5.00 ± 0.00 ^b
Vitamin B9 (Folic Acid)	µg/100mL	6.33 ± 0.33 ^a	14.67 ± 0.88 ^c	17.33 ± 0.33 ^d	12.00 ± 0.00 ^b	12.67 ± 0.33 ^{bc}
VitaminB12(Cyanocobalamin)	µg/100mL	2.00 ± 0.00 ^b	1.00 ± 0.00 ^a	1.00 ± 0.00 ^a	2.00 ± 0.00 ^b	1.67 ± 0.33 ^{ab}
Vitamin C	mg/100 mL	2.00 ± 0.00 ^c	1.00 ± 0.00 ^{ab}	1.13 ± 0.03 ^b	1.13 ± 0.07 ^b	0.83 ± 0.12 ^a
Vitamin D	µg/100mL	2.50 ± 0.00 ^b	0.35 ± 0.08 ^a	2.52 ± 0.00 ^b	0.50 ± 0.00 ^a	2.50 ± 0.00 ^b
Vitamin E (Alpha-Tocopherol)	mg/100 mL	0.06 ± 0.00 ^b	0.07 ± 0.00 ^d	0.07 ± 0.00 ^{cd}	0.05 ± 0.00 ^a	0.06 ± 0.00 ^{bc}
Vitamin K	µg/100g	0.24	0.46	0.9	0.8	0.7

^{a-d} Mean ± standard deviation in same row with different superscripts letters are significantly different at p<0.05.

* Results showing vitamin content lower than LOR. LOR is limit of reporting.

Appendix D: Mineral Content Analysis Data

Appendix D1

Changes in mineral content of fresh milk during 6 days' storage at 6 ± 1 °C.

Mineral Content	Storage (Days)		
	0	3	6
Potassium (mg/100 mL)	131.67 ± 0.88^a	145.33 ± 2.03^b	126.00 ± 0.58^a
Calcium (mg/100 mL)	119.67 ± 1.86^b	147.67 ± 2.33^c	111.00 ± 1.53^a
Phosphorus (mg/100 mL)	73.63 ± 0.37^b	86.10 ± 1.78^c	62.73 ± 1.27^a
Magnesium (mg/100 mL)	10.73 ± 0.17^a	12.97 ± 0.18^b	10.90 ± 0.10^a
Zinc (mg/kg)	3.13 ± 0.07^b	2.37 ± 0.03^a	3.27 ± 0.24^b
Selenium (mg/kg)	< 0.2	< 0.2	< 0.2

^{ab} Mean $\pm$ standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$.

Appendix D2

Effects of treatments on mineral content of fresh milk.

Mineral Content	Unit	Fresh Milk	Treatment	
			HPP	ESL
Potassium	mg/100 mL	131.67 ± 0.88^a	133.00 ± 0.58^a	131.67 ± 1.20^a
Calcium	mg/100 mL	119.67 ± 1.86^a	118.67 ± 0.67^a	114.67 ± 2.33^a
Phosphorus	mg/100 mL	73.63 ± 0.37^a	73.23 ± 0.98^a	71.00 ± 0.31^a
Magnesium	mg/100 mL	10.73 ± 0.17^a	10.60 ± 0.06^a	10.37 ± 0.13^a
Zinc	mg/kg	3.13 ± 0.07^a	3.13 ± 0.07^a	3.40 ± 0.00^b
Selenium	mg/kg	< 0.2	< 0.2	< 0.2

^{ab} Mean $\pm$ standard deviation in same row with different superscripts letters are significantly different at $p \leq 0.05$.

Appendix D3

Changes in mineral content of HPP and ESL milk during 60 days' storage at 6±1 °C.

Mineral Content	Unit	Storage (Days)				
		0	15	30	45	60
HPP Milk						
Potassium	mg/100 mL	133.00 ± 0.58 ^{bc}	130.00 ± 1.53 ^b	135.33 ± 0.33 ^c	122.00 ± 0.58 ^a	124.67 ± 0.88 ^a
Calcium	mg/100 mL	118.67 ± 0.67 ^{ab}	129.33 ± 1.20 ^c	123.00 ± 1.53 ^b	115.67 ± 0.33 ^a	121.67 ± 0.88 ^b
Phosphorus	mg/100 mL	73.23 ± 0.98 ^{bc}	70.57 ± 0.84 ^b	71.03 ± 0.52 ^{bc}	59.33 ± 0.50 ^a	74.43 ± 0.91 ^c
Magnesium	mg/100 mL	10.60 ± 0.06 ^{ab}	11.03 ± 0.07 ^{bc}	11.23 ± 0.12 ^c	10.57 ± 0.03 ^a	12.10 ± 0.15 ^d
Zinc	mg/kg	3.13 ± 0.07 ^b	3.03 ± 0.03 ^b	3.10 ± 0.15 ^b	2.93 ± 0.03 ^b	2.53 ± 0.03 ^a
Selenium	mg/kg	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
ESL Milk						
Potassium	mg/100 mL	131.67 ± 1.20 ^{ab}	128.33 ± 3.18 ^{ab}	134.00 ± 0.58 ^b	126.00 ± 0.58 ^a	125.33 ± 0.88 ^a
Calcium	mg/100 mL	114.67 ± 2.33 ^a	124.67 ± 7.17 ^a	124.67 ± 1.86 ^a	119.67 ± 1.45 ^a	120.67 ± 0.67 ^a
Phosphorus	mg/100 mL	71.00 ± 0.31 ^{bc}	68.10 ± 2.25 ^{ab}	71.87 ± 0.60 ^{bc}	65.17 ± 0.24 ^a	75.73 ± 0.58 ^c
Magnesium	mg/100 mL	10.37 ± 0.13 ^a	10.70 ± 0.56 ^{ab}	11.40 ± 0.12 ^{ab}	11.00 ± 0.06 ^{ab}	11.90 ± 0.06 ^b
Zinc	mg/kg	3.40 ± 0.00 ^c	3.10 ± 0.06 ^{bc}	3.13 ± 0.13 ^{bc}	2.97 ± 0.03 ^{ab}	2.70 ± 0.06 ^a
Selenium	mg/kg	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2

^{a-c} Mean ± standard deviation in same row with different superscripts letters are significantly different at p≤0.05

Appendix E: Sensory Analysis

Formula of mean drops:

The percentage of consumers in each of the 3 categories is calculated and corresponding mean liking (L_{TL} , L_{JAR} , L_{TM}) scores for the “Too Little” (TL), “JAR”, and “Too Much” (TM) categories estimated. Mean drops is calculated as follows:

$$\text{Mean drops TL} = L_{JAR} - L_{TL}$$

$$\text{Mean drops TM} = L_{JAR} - L_{TM}$$

Formula of penalty:

The penalty is a weighted difference between the means (mean of liking for JAR—mean of liking for the two other levels taken together).

Example:

$$\text{Penalty value for colour attribute of HPP milk} = \frac{(16)(0.889) + (73)(0.326)}{16 + 73} = 0.439$$

Appendix F: Equipment Cost Reference Links

Equipment	Capacity	Power (kW)	Reference Links
<u>HPP treatment</u>			
Storage Tank	10000 L	5.5	https://www.alibaba.com/product-detail/Milk-Storage-Tank-10000L-Milk-Storage_62020220129.html?spm=a2700.galleryofferlist.normal_offer.d_title.1866ed00LDECdV&s=p
Filling machine	5000 BPH	3.5	https://m.made-in-china.com/product/5000bph-Automatic-Rotary-Complete-Production-Bottle-Filling-Machine-for-Juice-Milk-859762145.html
HPP unit	525 L	40	https://www.alibaba.com/product-detail/GYC-food-HPP-UHP-Ultra-high_1600261246418.html?spm=a2700.details.0.0.28833ad1zpWRda https://www.hiperbaric.com/en/hpp-technology/equipment/hpp-in-pack/hiperbaric-525/
Cold room	50 ton	30	https://www.alibaba.com/product-detail/Cold-Room-Avocado-Air-Control-Cold_1600237920916.html?spm=a2700.7724857.0.0.4f323084e6DzYc
<u>ESL heat treatment</u>			
Storage Tank	10000 L	5.5	https://www.alibaba.com/product-detail/Milk-Storage-Tank-10000L-Milk-Storage_62020220129.html?spm=a2700.galleryofferlist.normal_offer.d_title.1866ed00LDECdV&s=p
Homogeniser	5000 L	45	https://www.alibaba.com/product-detail/Milk-Homogenizer-Dairy-High-PressureHomogenizer_1600446013482.html?spm=a2700.galleryofferlist.normal_offer.d_title.63995d22PKlwNH
Indirect steam tubular-type pasteurizer	5000 kg	7	https://www.alibaba.com/product-detail/Stainless-steel-tubular-milk-pasteurizer-and_754122445.html?spm=a2700.galleryofferlist.normal_offer.d_image.34c9598aj8fIIg
Aseptic milk bottle filling machine	5000 BPH	3.5	https://www.alibaba.com/product-detail/2019-New-condition-milk-bottle-filling_62483652264.html?spm=a2700.galleryofferlist.normal_offer.d_title.5c9365dbBJ65N8
Cold room	50 ton	30	https://www.alibaba.com/product-detail/Cold-Room-Avocado-Air-Control-Cold_1600237920916.html?spm=a2700.7724857.0.0.4f323084e6DzYc

Appendix G: HPP Cooling Water Cost Calculation

HPP vessel capacity = 525 L

Filling ratio = 80% for 1 L bottle milk = 420 bottles 1 L milk

Volume of cooling water required per cycle = 525 L x 20 %

$$= 105 \text{ L}$$

Assume the cooling water is required to replace every 16 cycles, hence, total 80 cycles in a day will required to change 5 times.

Total cooling water required per day = 105 L x 5

$$= 525 \text{ L}$$

Assume cooling water lost per cycle is 2.5 – 3.0 % of volume of cooling water required per cycle, taking 2.75 % as average, the estimated water loss per cycle is as below:

Estimate total water loss per day = 105 L x 2.75% x 80 cycles

$$= 231 \text{ L}$$

Total volume of cooling water required per day = 756 L

Assuming there is 275 working days per year, the total volume of cooling water required per year for one unit HPP is as below:

Total volume of cooling water required per year (1 HPP unit) = 756 L x 275

$$= 207900 \text{ L}$$

In order to cover the production throughput of 5000 L/hr, 3 HPP units with 525 vessel capacity were used. Hence, the total volume of cooling water required per year for 3 units is as below:

Total volume of cooling water required per year (3 HPP units) = 207900 L x 3

$$= 623700 \text{ L}$$

Unit cost of cooling water is RM 0.002/ L based on Malaysia water cost. Hence, the total cost of cooling water required per year is as below:

Total cost of cooling water per year = 623700 L x RM 0.002/L

$$= \text{RM } 1247.40$$



Appendix H: Sensitivity Analysis of HPP Treatment Data

a) Data of sensitivity study of impact of raw milk price on net present value.

Raw Milk Price (MYR per L)	2.00	2.23	2.50	3.00	3.50	4.00	4.50	5.00	5.50	6.00	6.50	7.00	7.50	8.00
Total Raw Milk Cost (MYR Million)	55	61	69	83	96	110	124	138	151	165	179	193	206	220
Operational Cost (MYR Million)	92	98	106	119	133	147	161	174	188	202	216	229	243	257
Operational Cost (MYR per L milk)	3.34	3.57	3.84	4.34	4.84	5.34	5.84	6.34	6.84	7.34	7.84	8.34	8.84	9.34
NPV (MYR Million)	375	351	323	271	219	167	115	63	10	-42	-94	-146	-198	-250

b) Data of sensitivity study of impact of HPP milk price on net present value and break-even quantity.

HPP Milk Price (MYR per L)	7.00	6.50	6.00	5.50	5.00	4.50	4.00	3.50	3.00
NPV (MYR Millions)	352	300	248	196	144	91	39	-13	-65
Break Even Quantity (Million bottle)	1.82	2.13	2.56	3.23	4.35	6.70	14.48	-88.95	-10.92

- a) Data of sensitivity study of impact of HPP filling ratio on operational cost, net present value and break-even quantity.

HPP Milk Filling Capacity	80%	70%	60%	50%	40%	30%	20%	10%
Milk Vessel Capacity (L)	525	525	525	525	525	525	525	525
Output Per Batch (bottle)	420	368	315	263	210	158	105	53
Output Per Hour (bottle)	1680	1470	1260	1050	840	630	420	210
Output Per Year (Millions bottle)	9.2	8.1	6.9	5.8	4.6	3.5	2.3	1.2
Total Output Per Year (3 HPP units) (Millions bottle)	27.7	24.3	20.8	17.3	13.9	10.4	6.9	3.5
Cooling Water Required Per Batch (L)	105	158	210	263	315	368	420	473
Cooling Water Required Per Day (Replace every 16 cycles) (L)	525	788	1050	1313	1575	1838	2100	2363
Estimate total water loss per day (2.75% per cycle) (L)	231	346.5	462	577.5	693	808.5	924	1039.5
Total volume of cooling water required per day (L)	756	1134	1512	1890	2268	2646	3024	3402
Total volume of cooling water required per year (1 HPP unit) (L)	207,900	311,850	415,800	519,750	623,700	727,650	831,600	935,550
Total volume of cooling water required per year (3 HPP units) (L)	623,700	935,550	1,247,400	1,559,250	1,871,100	2,182,950	2,494,800	2,806,650
Total cost of cooling water (3 HPP) (MYR)	1247.4	1871.1	2494.8	3118.5	3742.2	4365.9	4989.6	5613.3
Raw Milk (Millions L)	27.7	24.3	20.8	17.3	13.9	10.4	6.9	3.5
Raw Milk Total Cost (MYR Millions)	61.8	54.1	46.4	38.6	30.9	23.2	15.5	7.7
Bottle (Millions bottle)	27.7	24.3	20.8	17.3	13.9	10.4	6.9	3.5
Bottle Total Cost (MYR Millions)	34.7	30.3	26.0	21.7	17.3	13.0	8.7	4.3
Operational Cost (MYR Millions)	98.7	86.7	74.6	62.6	50.5	38.5	26.4	14.3
Operational Cost (per L milk) (MYR)	3.56	3.57	3.59	3.61	3.64	3.70	3.81	4.14
NPV (MYR Millions)	355	309	263	216	170	124	78	21
Break Even Quantity (Millions bottles)	1.8	1.8	1.8	1.8	1.9	1.9	2.0	2.2

LIST OF PUBLICATIONS

- Lim, S. H., Chin, N. L., Sulaiman, A., Tay, C. H., & Wong, T. H. (2022). Sensory analysis for cow milk product development using high pressure processing (HPP) in the dairy industry. *Foods*, 11(9), 1233.
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