UNIVERSITI PUTRA MALAYSIA

PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF HIGH PRESSURE TREATED COW AND GOAT MILK

TAN SOK FERN

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183792

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ABSTRACT

High pressure processing (HPP) on cow and goat milk was conducted at 450 MPa for 7 min, 600 MPa for 5 min and 600 MPa for 7 min to investigate the effects on physico-chemical and microbiological properties in comparison to high-temperature-short-time (HTST) pasteurization at 72°C for 15 sec. Both HPP and pasteurization effectively reduced the bacterial and spore populations to beyond the permissible limit, with highest total plate count reduction of 99.99% by HPP treatment at 600 MPa and 5 min. All treatments caused no significant changes to most of the physico-chemical properties such as titratable acidity, specific gravity, total protein, total fat, total solid and solid-non-fat. However, pH of pasteurized cow milk has decreased significantly (from 6.59 to 6.52) while both pasteurized and HPP treated goat milk have significant increased pH (from 6.34 to 6.38–6.42). During storage at 8°C, HPP treated cow and goat milk both achieved microbial shelf life of 22 days with no increase in *Bacillus cereus*, mesophilic aerobic spores, coliform, yeast and mould but slight increase in psychrotrophic bacteria and total plate count. However, pasteurised goat milk was spoilt at the end of the storage as both psychrotrophic bacteria (9.0 x 10^8 CFU/ml) and total plate count (3.5 x 10^8 CFU/ml) have exceeded the permissible limits. HPP treated cow milk exhibited higher physico-chemical stability than goat milk which is most evident in the significant increment (average 0.04%) in goat milk's titratable acidity while no significant changes observed in cow milk.

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CHAPTER 1

INTRODUCTION

1.1 Overview

Milk is well-known as a major source of dietary energy, protein and fat. It contributes 134 kcal of energy/capita per day on averagely (Weaver et al., 2013). Apart from cow milk which has accounts for 83 percent of global production, goat milk has also started to gain interest worldwide in recent years (Muehlhoff et al., 2013). Goat milk contains lower amount of caseins, hence give a greater digestive utilization compared to cow milk (Ulusoy, 2015). However, the nature of milk and its chemical composition render it as one of the ideal culture media for microbial growth and multiplication (Woldemariam & Asres, 2017). Hence, the major challenge in milk industries is to ensure delivery of safe and healthy milk products of consistent quality to an ever-increasing demand of milk.

Thermal treatments such as pasteurization (HTST – 72°C/15 s, LTLT – 63°C/30 min) and UHT (135°C/3 s) are usually used to treat raw milk in order to be rendered safe for consumer and increase its shelf life (Barraquio, 2014). Studies have shown that microbial population counted is significant beyond 20 days of storage for pasteurized milk (Holsinger et al., 1997; Nagla et al., 2009; Ziamo et al., 2005).

UHT treated milk typically has up to 4–6 months of commercial shelf life at ambient temperature (Incecco et al., 2018). However, these conventional heat treatments could have detrimental effects on the nutrient content of milk. Studies have proven that thermal treatments caused 5–15% of whey protein denaturation, 10–25% of water soluble vitamins destruction, disturbed structure of casein micelles and also enhancement of allergenic properties of milk proteins (Barraquio, 2014; Bogahawaththa et al., 2018; Tamime, 2014).

High pressure processing (HPP) is a promising alternative to conventional thermal treatments as it is capable to inactivate foodborne pathogens while minimising the loss of nutrients, such as water-soluble vitamins and maintaining the fresh-like characteristics of food products (Alexandros et al., 2019; Considine et al., 2008; Wang et al., 2015). During HPP, pressure is allowed to remain in contact with the product for a specific holding time to activate the destruction of spore and microbes, and formation of non-covalent bonds of food components to gelatinize the enzyme (Chawla et al., 2011; Elamin et al., 2015; Wang et al., 2015). Pressure regime of 200–600 MPa with holding time of 3–10 min is usually applied in HPP of milk. Generally, higher pressure resulted in higher rate of microbial destruction as well as longer shelf life. Pereda et al. (2007) reported a shelf life of 14–18 days for milk treated at 200 MPa at storage temperature of 4°C. Mussa and Ramaswamy (1997) reported that pressure of 350 MPa renders a milk shelf life of 18 days at similar storage temperature. When milk was treated at 600 MPa, shelf life of 28 days was achieved at similar storage temperature (Alexandros et al., 2019). Storage temperature of milk also plays a role in milk's shelf life. Rademacher and Kessler (1997) reported that milk subjected to pressure treatment of 400 MPa for 15 min or

600 MPa for 3 min could only achieve 10 days of shelf life when stored at 10°C, which is 18 days shorter than that of storing at 4°C.

While studies about the HPP effects on nutrient content like fat, protein and moisture are very limited, several studies have proven that HPP is able to preserve vitamins in milk better than pasteurization and UHT (Amador-espejo et al., 2015; Sharabi et al., 2018). From the microbiological perspective, a more pronounced microbial inactivation was obtained by increasing HPP pressure levels and holding time. Alexandros et al. (2019) reported that HPP above 550 MPa and 3 min reduced the number of *E. coli*, *Salmonella* spp. and *L. monocytogenes* to below limit of detection while pressure regime of 400 MPa for 1 min did not result in statistically significant differences in reduction levels. Yang et al. (2012) reported that holding time of 30 min at 300 MPa is sufficient to inactivate *Salmonella* spp., *E. coli*, *Shigella* and *S. aureus*. Hence, it is obvious that the pressure and holding time is critical for the safety for consumption in milk.

1.2 Problem Statement

Most HPP studies conducted on milk are based in countries with seasonal climates. For a hot tropical country like Malaysia, HPP for milk is a new technology. Milk collected from different geographical locations may have different properties shown, particularly the microbiological properties due to the milking practice, climates and the hygiene of the environment. In a hot and humid country like Malaysia, the growth of the microbial populations may be very rapid and causes milk spoilage in a time frame lesser than usual if the milk was stored at improper conditions. Hence, it is necessary to carry out a comprehensive study on the physico-chemical and microbiological properties of milk after treated by high pressure in a hot tropical country like Malaysia to ensure the safety of HPP milk consumption.

1.3 Objectives

The general objective of this study is to investigate the properties of high pressure treated cow and goat milk in Malaysia in order to improve the milk quality. To achieve the general objectives, the following specific objectives were established:

1) To investigate the effects of HPP on local cow and goat milks in terms of its physico-chemical and microbiological properties.
2) To study the changes in milk physico-chemical and microbiological properties during 22 days of storage.
3) To compare the physico-chemical and microbiological properties of HPP treated milk with pasteurized milk.

1.4 Scope of Work

In this study, the focus is given to the HPP of cow and goat milk which was then compared with the pasteurized milk. The fresh cow and goat milk collected from local farm were analysed for its physico-chemical and microbiological properties as control samples. Then, both cow and goat milk was subjected to pressure of 450 MPa for 7 minutes, 600 MPa for 5 minutes and 600 MPa for 7 minutes to be compared with the pasteurized samples treated at 72°C for 15 seconds. Physico-chemical and microbiological analysis were carried out at day 0, day 11 and day 22. All the samples were stored at 8°C until further analysis.

1.5 Thesis Structure

The thesis begins with a general introduction that recognizes the current problem and establishes the objectives of the study. Chapter 2 is the literature review which contains the background information about the research. Chapter 3 is the methodology for this research which consists of the details about the analysis and the treatments applied throughout this research. Chapter 4 reports the results obtained and discussions about the findings. The final chapter wraps up the research by providing the general conclusions on the research.

CHAPTER 2

LITERATURE REVIEW

2.1 Cow Milk

Cow milk is a white liquid food produced by cow's mammary gland initially for their offspring. Today, cow milk is widely consumed by human for dietary needs, from toddlers to adults. It is well-known for its various nutrients which are necessary for human nutrition and contributes significantly to the required nutrient intakes for calcium, magnesium, selenium, riboflavin, vitamin B_{12} and pantothenic acid (Weaver et al., 2013). An adequate intake of high-quality protein, such as that contained in cow milk, is also essential to counteract the progressive loss of muscle mass and strength which typically happen to the elderly. Besides, milk fat contributes half of the energy in whole milk which is why milk can play an important role in the diets of infants and children in populations with very low fat intake, where the availability of other animal-source food is limited (Marangoni et al., 2018).

Malaysia's dairy market has developed very positively with constant growth rates in the past few decades. While demand for dairy products has surged, domestic supply of milk was still very limited and unable to keep up with the increasing demand. More than 90% of milk is imported, mainly from Australia and New Zealand (Sim & Suntharalingam, 2015). In particular, Malaysia's market structure for drinking milk product is now dominated by a few international brands such as Nestle, Dutch Lady, F&N and Fonterra, who entered Malaysia's growing dairy market at an early stage and has good reputation among Malaysians (Boniface & Umberger, 2012). A range of Malaysian government research and development programs and initiatives has been implemented over the years in an attempt to encourage the growth of the dairy industries. This includes the establishment of Milk Collection Centres (MCC), the introduction of more productive dairy breeds and the improvement of veterinary services (Boniface & Umberger, 2012).

Table 1 represents the annual growth of drinking milk products sales in percentage. From year 2009 to 2014, the total turnover of drinking milk products has clearly increased with an average annual growth rate of 4.4%. It is also obvious that the liquid milk drinks, which included both cow and goat milk, has experienced stable growth rate with an average annual growth rate of 6.3%. Hence, the Malaysia's market for drinking milk products is expected to continue its strong growth as experienced in the past.

Table 1: Annual growth of drinking milk products sales from year 2009–2014 in percentage (Malaysian-German Chamber of Commerce, 2015)

Type of Milk	**2009/10**	**2010/11**	**2011/12**	**2012/13**	**2013/14**
Flavoured Milk Drinks	7.4	5.5	6.0	5.8	6.0
Flavoured Powder Milk Drinks	2.0	3.0	2.4	2.6	2.8
Milk (incl. cow & goat milk)	7.0	5.6	6.3	6.3	6.4
Powder Milk	5.0	5.5	4.0	3.8	3.6
Total	4.7	4.6	4.2	4.2	4.4

2.2 Goat Milk

Besides cow milk, a niche market in the Malaysian market for drinking milk products is goat milk. Consumed mainly for health purposes, goat milk is found mainly in Malaysian Indian communities, who represent about 7.3% of Malaysian's population (Malaysian-German Chamber of Commerce, 2015). A special feature of goat milk in Malaysia is that goat milk is usually sold in small plastic bags without company brands by farmers, which is largely differed from cow milk that is usually well marketed and sold in milk cartons.

Goats are considered the first ruminant to be domesticated and produce about 2% of the world's total annual milk supply (Arora et al., 2013). India is the main producer of goat milk (30%), followed by Bangladesh (17%) and Sudan (11%). However, industrialization of the goat milk is not well succeeded because of its poor and insufficient volume. More than any other mammalian farm animal, the goat is a main supplier of dairy and meat products for rural people, which is why demand for goat milk is mainly for home consumption (Haenlein, 2004).

In fact, goat milk is rich in mineral and vitamin content and has creamy texture. It also has better digestibility due to its smaller fat globule size, buffer capacity, alkalinity and therapeutic values than cow milk. In term of appearance, goat milk is always whiter than cow milk due to its higher vitamin A content (Lad et al., 2017).

2.3 Physicochemical Properties

The quality of milk which is in conformity with consumer's requirement is determined largely by nutritional, physicochemical, sensory properties and microbiological quality (Hamiti et al., 2014). It is generally acceptable that the higher the quality of raw milk and the better the physicochemical properties, the greater the benefit for both milk industry and consumer. However, assuring high quality and desirable physicochemical properties of raw milk is very challenging as they depend on various factors such as breed, interval of milking, stage of lactation, feeding, season and many other factors. It is also vital to ensure the physicochemical properties are complying with law and regulation before marketing a milk product. According to Malaysians Food Regulations 1985, the milk fat content of cow milk should be more than 3.25% while the non-fat milk solids should be more than 8.5%.

2.3.1 Milk Composition

According to Food and Agriculture Organization of the United Nations (FAO), the proximate composition of cow milk generally consisted of an average of 87.8% of water, 3.3% of total protein, 3.3% of total fat, 4.7% of lactose and 0.7% of ash. However, these values may differ slightly from cow milk produced at different geographical areas. According to Gasmalla et al. (2013), the cow milk in Sudan has an average of 86.8% of water, 3.3% of protein, 3.7% of fat, 4.5% of lactose and 0.5% of ash. Arora et al. (2013) reported that cow milk in India has an average moisture content of 87.8%, 3.9% of fat, 3.2% of protein, 4.8% of lactose and 0.8% of ash. Hence, it is common to have different composition for milk at different geographical areas.

On the other hand, the proximate composition of goat milk is very similar to that of cow milk. As shown in Table 2, goat milk generally consists of 87.7% of water, 3.4% of total protein, 3.9% of total fat, 4.4% of lactose and 0.8% of ash (Wijesinha-Bettoni & Burlingame, 2013).

Table 2: Proximate composition of cow and goat milk (Wijesinha-Bettoni & Burlingame, 2013)

Proximate	**Cow**		**Goat**	
	Average	**Range**	**Average**	**Range**
Energy (kJ)	262	247–274	270	243–289
Energy (kcal)	62	59–66	66	58–74
Water (%)	87.8	87.3–88.1	87.7	86.4–89.0
Protein (%)	3.3	3.2–3.4	3.4	2.9–3.8
Fat (%)	3.3	3.1–3.3	3.9	3.3–4.5
Lactose (%)	4.7	4.5–5.1	4.4	4.3–4.5
Ash (%)	0.7	0.7–0.7	0.8	0.8–0.8

Milk fat contributes about half of the energy in the whole milk. For this reason, milk fat is an important indicator of the milk quality. This is also why animal milk can play an important role in the diet of infants and children especially within the populations where the availability of other animal-source food is limited. In the 3 percent of cow milk fat, it consists primarily of triacylglycerol (97–98% of total lipids by weight), which are composed of fatty acids of various lengths and saturation levels.

Moreover, the estimation of milk solid-non-fat (SNF) is very desirable in the manufacturing of cheese, ice-cream, condensed and powdered milk. Most commercial ice cream products contain between 10–12% of SNF. The protein and lactose in the SNF contributes to the finer texture and better body of the products. While its content such as vitamins is the main concern of consumers, SNF is usually the prior concern of milk products manufacturers.

Besides, the major proteins found in milk are casein and whey proteins, with casein accounting for approximately 80% of the protein in cow milk and whey proteins accounting for about 20% of the total protein (Tamime, 2014). For the sake of human nutrition, milk is considered as an excellent source of essential amino acids which cannot be produced by human body unlike non-essential amino acids.

2.3.2 pH

Milk pH gives an indication of milk hygiene as well as milk spoilage. The pH of normal milk from a healthy cow is in the range of 6.6–6.7 (Mahmood & Usman, 2010); lower pH values would suggest growth of microorganisms that ferment lactose to lactic acid and extensive lipolysis that hydrolyses fat and esters, especially phosphoric esters (Tamime, 2014). However, higher pH values in milk may be encountered in times of physiological stress for the producing animal, when the mineral balance of milk is altered by changes in the permeability of the blood-milk barrier, for example, during late stages of the lactation cycle or during mastitis infection. Milk pH is also affected by temperature, generally decreasing with increasing temperature, due to changes in dissociation of ionisable groups. In contrast, the pH of goat milk is generally lower than cow milk, which is usually in the range of 6.2–6.8 (Imran et al., 2008; Lai et al., 2016).

2.3.3 Titratable Acidity

The titratable acidity of milk is determined in the dairy industry mainly for two reasons. Firstly, ensure and determine the freshness of milk and milk products. Secondly, to control the manufacture of cultured dairy products. The acidity of milk is usually expressed as percentage lactic acid. A high initial acidity in the absence of lactic acid development suggests that the milk is rich in proteins and other indigenous buffering constituents. The general titratable acidity of fresh cow milk usually falls in the range of between 0.14–0.17% (Fox, 1997; Mahmood & Usman, 2010).

For goat milk, the titratable acidity is found to be varying between different studies. From a study conducted in Pakistan, the acidity of goat milk falls between the range of 0.97–1.73% (Imran et al., 2008). However, a study conducted in Malaysia found that the titratable acidity is only around 0.07% for raw goat milk (Lai et al., 2016). A more consistent titratable acidity value between 0.14–0.18 was reported by Mahmood and Usman (2010) for goat milk.

2.3.4 Specific Gravity

Specific gravity is the ratio between the density of the substance and that of water. The specific gravity of milk is influenced by the proportion of its constituents, each of which has a different specific gravity. Cow milk specific gravity at 20°C ranges from 1.027–1.033 (Hui, 2007; Mahmood & Usman, 2010). On the other hand, Imran et al. (2008) reported that the specific gravity of goat milk is generally around 1.030, while Mahmood and Usman (2010) reported the value ranged from 1.028–1.032.

2.4 Microbiological Properties

Milk is a nutritious liquid food serves as the dietary basis for human being, but at the same time it also serves as an excellent medium for microorganism growth, especially bacterial pathogens. Fresh milk drawn from a healthy cow normally contains a low microbial load, which is usually less than 1000 ml^{-1}, but the loads may shoot up to 100 fold or more once it is kept for a period of time at normal temperatures (Richter et al., 1992). The presence of food-borne pathogens in milk also may be due to direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal (Elrahman & Medani, 2009). In particular, literatures that related on the microbiological quality of goat milk today are insufficient for the public. Contrarily, cow milk is subjected to strict hygiene and quality regulations controlled while microbiological quality standards for production and distribution of goat milk are seems to be quite unclear (Lai et al., 2016).

2.4.1 Total Plate Count

The total plate count (TPC) is the enumeration of aerobic and mesophilic organisms that grow in aerobic conditions under moderate temperatures of 20 – 45°C. This includes all pathogens, non-pathogens, aerobic bacteria, yeasts, moulds and fungi that grow in the specific agar. A total plate count limit set by the Department of Veterinary Services Malaysia for the Price Incentive Programme is lesser than 10^6 CFU/ml for raw cow milk.

A study conducted in Peninsular Malaysia which has collected 930 raw cow milk samples from 40 Milk Collection Centres reported that the mean count for TPC was 12×10^6 CFU/ml (Chye et al., 2004). This shows that the milks have been heavily contaminated probably because of the unhygienic practices in most of the farm. Dahal et al. (2010) reported that the average total plate count for 520 raw milk samples was 9×10^5 CFU/ml due to conducive ambient temperature and high relative humidity for the growth of bacteria in Nepal. Hence, it is evident that in most cases, the presence of TPC in raw milk is unexceptionally high, which is why treatment must be given to milk before it is marketed.

2.4.2 Psychrotrophic Bacteria

Milk is commonly kept in cold storage to lengthen its shelf life. However, with cooling and extended cold storage of raw milk, spore-forming psychrotrophic bacteria becomes present in 27 – 58 % of cases (Griffiths & Phillips, 1988; D. Samarzija et al., 2012). In cooled raw milk, initially dominant Gram-positive mesophilic aerobic bacteria are replaced by Gram-negative and Gram-positive psychrotrophic bacteria. The domination of psychrotrophic bacteria in the total microbial population is even more pronounced when milk is produced in poor hygiene conditions or contains increased numbers of somatic cells. For these reasons, psychrotrophic bacteria usually account for more than 90% of the total microbial population in cooled raw milk (Samarzija et al., 2012). According to Malaysians Food Act 1983 and Food Regulations 1985, the psychrotrophic bacteria count in milk should not exceed 10^5 CFU/ml.

2.4.3 Coliform and *Escherichia coli*

Coliforms are a group of Gram-negative and non-spore forming bacteria commonly found in the environment, including soil, surface water, vegetation and the intestinal tracts of mammals. They belong to the family of *Enterobacteriaceae*, including the genera of *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter* (Moura & Nelson, 1972; Wanjala et al., 2018). Faecal coliforms are a subset of coliform bacteria that ferment lactose, resulting in production of acid and gas within

48 hours. On the other hand, the presence of faecal coliform in milk is highly associated with the risk of contamination with other enteric pathogens (Belbachir et al., 2015). According to Malaysians Food Regulations 1985, the coliform count should not exceed 50 CFU/ml after incubation at 37°C for 48 hours for raw milk.

A study conducted in Peninsular Malaysia reported that in 930 raw cow milk samples, nearly 90% of the samples are contaminated by coliform bacteria, with a mean count of 1.7×10^5 CFU/ml (Chye et al., 2004). Another study of raw goat milk in Malaysia also reported that an average of 1.87 log CFU/ml of coliform bacteria is presented in the samples (Lai et al., 2016).

Besides, *Escherichia coli* are the major faecal coliforms bacteria that were found in most of the coliform-infected milk. The outbreaks of *E. coli* O157:H7 have been reported in causing several diseases ranging from mild diarrhoea to potentially fatal haemolytic uremic syndrome, haemorrhagic colitis and thrombotic purpura (Keene et al., 1997; Wanjala et al., 2018). Salman and Hamad (2011) reported that out of 644 milk samples, 32% of the Coliform isolates were *E. coli*, which makes up the highest percentage among other bacteria. The presence of *E.coli* is relatively prevalent in milk, bringing high risk to raw milk consumers.

2.4.4 Yeast and Mould

Yeast and mould are a large and diverse group which include several hundred species. Yeasts tend to grow within food matrices in planktonic form and ferment sugars while growing well under anaerobic conditions. Moulds may also be hazardous to human as they are able to produce toxic metabolites known as mycotoxins. A research carried out in Pakistan reported that a mean yeast and mould count of 1.94×10^4 CFU/ml in milk sold at retailer shops (Shah et al., 2016).

2.4.5 Mesophilic and Thermophilic Aerobic Spores

Spore-formers are Gram-positive bacteria that form spores when subjected to environmental stresses such as nutrient limitation, osmotic pressure or extreme temperature deviation. These spores, which facilitate survival, are resistant to chemicals, pH changes, heat, osmotic shock and ultraviolet light penetration (Doyle et al., 2015). When conditions again become suitable for growth, spores can germinate to vegetative cells.

Mesophilic spores are the most prevalent spore-former found in bulk tank raw milk. In spore form, these organisms are capable of surviving environmental stresses including low pH, high temperature, exposure to sanitizers, high pressure and others. Aerobic endospore-forming bacteria of the *Bacillaceae* family have been recognized as major contributors to dairy product quality issues over the past twenty years (Kent et al., 2016). Contrarily, although thermophilic organisms are less harmful to health, but they can cause changes in the organoleptic qualities of milk (Shimeles, 2016). These thermophilic spores can endure heat and survive conventional heat treatments such as pasteurization.

2.4.6 *Bacillus cereus*

Aerobic spore-forming bacteria of the genus *Bacillus* are commonly present in raw milk. Their spores survive pasteurization and subsequently germinate, outgrow, and multiply. Certainly, the presence of *Bacillus* spp. in milk is also undesirable because a number of species have been implicated in food-borne disease. *B. cereus* produces a number of extracellular toxins and other harmful metabolites. Of these, the most significant are the diarrhoeagenic enterotoxins and emetic toxin (Juffs & Deeth, 2007). *B. cereus* was incriminated in a large food poisoning outbreak attributed to pasteurized milk and WHO figures indicate that 5–10% of reported food-borne disease is caused by this organism (McGuiggan et al., 2002).

2.4.7 *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella*

Clostridium perfringens is a spore-forming bacterium and its spores makes it a frequent problem for the food industry. *C. perfringens* causes two very different foodborne diseases: the relatively mild classic type A diarrhoea and Type C human necrotic enteritis. *C. perfringens* lacks the ability to produce 13 of the 20 essential amino acids and is therefore associated with protein-rich food such as milk (Brynestad & Granum, 2002).

Besides, the most common bacterial pathogens causing serious illness associated with non-pasteurized dairy products are *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes* (*L. monocytogenes*) and *Salmonella* spp. (Kunadu et al., 2018; Sonnier et al., 2018). Antimicrobial resistance of Salmonella against 18 antibiotics was proven (Kunadu et al., 2018), and the resistance to the first line antibiotics used in treatment could be very dangerous to the patients infected by the pathogens.

Belbachir et al. (2015) conducted several analyses on the microbiological quality of raw cow milk at three rural communes of the eastern region of Morocco on the *S. aureus*, *L. monocytogenes* as well as *Salmonella* spp. The authors discovered that the pathogenic *S. aureus* have been detected in 23% of samples with an average count of 1.7×10^3 CFU/ml. Besides, *L. monocytogenes* was detected in 3 % of samples while *Salmonella* spp. was not detected in all the samples. Besides, Artursson et al. (2018) reported that the prevalence of samples with *S. aureus* was 71%

and 64%, and *Listeria* spp. was 21% and 29% from dairy cow and goat farms, respectively.

2.5 Pasteurization

Various types of thermal treatments namely pasteurization, ultra-high-temperature (UHT) and sterilization are commonly given to raw milk to inhibit the microbial growth, as well as lengthen its shelf life. Among these treatments, pasteurization is most prevalent among the dairy industries as it preserves the nutrient contents especially vitamins the most (Barraquio, 2014).

Malaysian Standard for pasteurized full-cream and low-fat milk (MS 410:1995) states that pasteurized milk is the milk that has been efficiently heat-treated by the holding method or by the High Temperature Short Time (HTST) method. By holding method, the temperature of the milk is raised to not less than 63°C and not more than 65°C for at least 30 minutes and then immediately and rapidly reduced to 4°C or less. By the HTST method, the temperature of the milk is raised to not less than 73°C and retained at that temperature for at least 15 seconds and then immediately and reduced to 4°C or less. The Grade "A" Pasteurized Milk Ordinance from the U.S. Department of Health and Human Services also states that batch pasteurization shall apply the temperature of 63°C for 30 minutes while continuous pasteurization (HTST) shall apply the temperature of 72°C for 15 seconds.

2.5.1 Effect of Pasteurization on Physico-chemical Properties

In recent years, it is well known that high temperature applied in thermal treatments for milk may bring several negative effects to the physico-chemical properties such as vitamins, calcium and salts. However, the research about the effects of pasteurization on other properties such as protein, fat and pH is still very limited. Tamime (2014) explained that the thermal degradation of fat in milk is generally not observed, because the temperature required for non-oxidative decomposition of fatty acids (>200°C) is well outside the range in which milk products are heated.

Besides, protein which consists of insoluble caseins (80%) and the soluble whey proteins (20%) are susceptible to different degrees of thermal denaturation. Tamime (2014) reported that heating at relatively low temperature (<70°C) causes some reversible changes in the association of micellar caseins, and may result in the interaction of denatured whey protein with the casein micelles. Whey proteins are susceptible to heat-induced denaturation due to its significant degree of tertiary structure; unfolding of the tertiary structure may occur while the native primary structure of the protein is not affected. Heat-induced denaturation of whey proteins in milk is affected by a wide range of environmental conditions. The extent of heat-induced denaturation increases with increasing milk pH, and also increasing level of ionic calcium in the medium.

Moreover, heating milk causes a decrease in its pH, and at temperatures less than 80°C, milk pH decreases in linear fashion with increasing temperature (Ma & Barbano, 2003). Heat-induced reductions in pH at this temperature range are primarily due to shifts in the mineral balance of milk, and are largely reversible on subsequent cooling.

2.5.2 Effect of Pasteurization on Microbiological Properties

Pasteurization of raw milk that attained the minimum temperature and holding time required should eliminates a majority of the bacteria levels to less than 1,000 CFU/ml (Lu & Wang, 2017). Huque et al. (2018) reported that pasteurization reduced the total bacterial count (TBC) from 2.38×10^5 CFU/ml to 3.78×10^2 CFU/ml while the total coliform count (TCC) from 2.5×10^4 CFU/ml to undetectable level.

L. monocytogenes achieved a 5.2D reduction (D-value is defined as the time in minutes needed to kill 90% of the bacteria present) which is more than adequate after the HTST pasteurization at 71.7°C for 15 sec (Juffs & Deeth, 2007). *S. aureus*, *E. coli* and *Salmonella* spp., even the most heat resistant serotype *Salmonella seftenberg* 775W, were destroyed by both LTLT and HTST pasteurization with a wide margin of safety (Doyle et al., 2015; Juffs & Deeth, 2007). Dumalisile et al. (2005) also proved that *E. coli* was totally inactivated by both LTLT and HTST pasteurization.

However, certain thermoduric bacteria strains and spores might survive pasteurization. *B. cereus* survived the HTST pasteurization at 72°C for 10 min, with the initial count declined from 12,000 to 6,500 CFU/ml (Dumalisile et al., 2005). This bacterial strain was so resistant to pasteurization that not even 1 log reduction of its initial concentration was reached.

2.5.3 Changes in Pasteurized Milk Properties during Storage

Ziyaina et al. (2018) reported that the pH and titratable acidity of pasteurized milk increased linearly with the storage period. At storage temperature of 7°C and

10°C, the pH reached the projected level for spoilage values at day 25 and 14 respectively. However, pH alone cannot provide a reliable shelf life index for pasteurized milk since the drop in pH is directly related to production of lactic acid during fermentation. Hence, titratable acidity is a better indicator than pH. The authors reported that at storage temperature of 7°C and 10°C, the titratable acidity reached the projected level for spoilage values at day 24 and 12 respectively. Besides, Brodziak et al. (2017) reported that the storage duration of 7 days for pasteurized milk had no statistically significant effect on its basic nutrients such as fat, protein, lactose and dry matter contents.

In particular, the increase in titratable acidity is linked to the duration of storage and also the total bacterial count. The bacteria count was found to multiply as the storage time increased and causing milk spoilage beyond 20 days of storage (Brodziak et al., 2017; Holsinger et al., 1997; Nagla et al., 2009; Ziarno et al., 2005). The most significant cause of spoilage in pasteurized milk during storage is the post-pasteurized contamination. Filling machine, new contacts and airborne psychrotrophs are all potential sources of post-pasteurized contamination. Psychrotrophs could also present in the pasteurized milk resulted from the thermoduric, gram-positive psychrotrophs that survive pasteurization. During pasteurization, high heat activates the spores, initiating germination and growth in the pasteurized milk. Lu and Wang (2017) explained that as pasteurized milk is stored under refrigerated temperature, these spore-formers predominate, especially the *Bacillus* spp. in the early stages of shelf life (<7 days).

2.6 High Pressure Processing

High pressure processing (HPP), also known as high hydrostatic pressure (HHP), is a relatively new, non-thermal food processing method that subjects food to pressures between 100-1200 MPa at various holding time to inactivate microorganisms and lengthen the shelf life of food products (Chawla et al., 2011; Considine et al., 2008; Wang et al., 2015).

As shown in Figure 1, during HPP operation, the pressure vessel is filled with a food product and pressure is applied for a desired time following which it is depressurized. In a batch operation, a packaged food in a flexible packaging is loaded into the pressure vessel, following which the vessel is pumped with a pressure-transmitting liquid, usually water. Upon filling the vessel, pressure relief valve is closed and pressure is allowed to build up within the vessel. Pressure is allowed to remain in contact with the product for a particular holding time to activate the destruction of spore and microbes, and formation of non-covalent bonds of food components to gelatinize the enzyme (Chawla et al., 2011; Elamin et al., 2015; Wang et al., 2015).

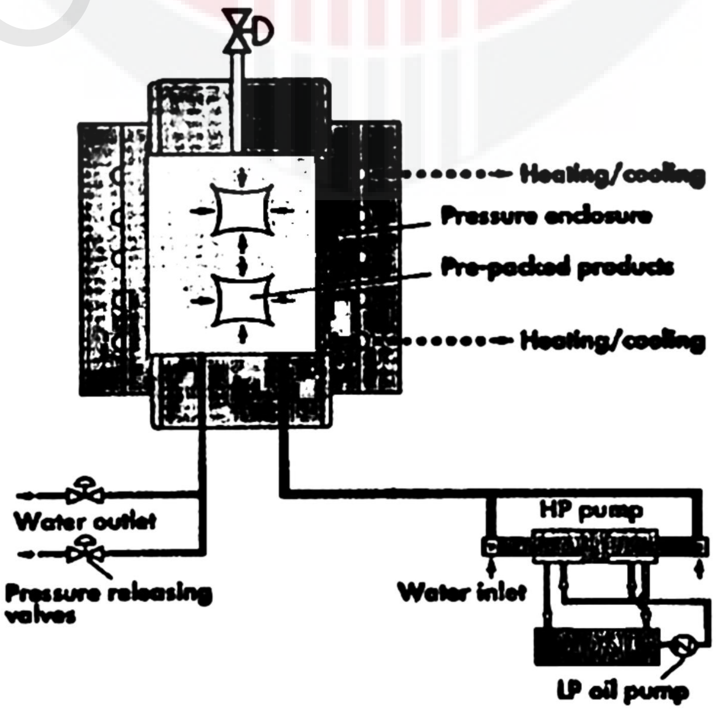

Figure 1: Typical HPP system for treating pre-packed product (Chawla et al., 2011)

The operating principle behind HPP is stated as Le Chatelier's Principle. According to Le Chatelier, any phenomenon in equilibrium chemical reaction, phase transition or change in molecular configuration is accompanied by decrease in volume, which can be enhanced by pressure (Chawla et al., 2011). During HPP, a major inactivation of microorganisms and denaturation of enzymes can occur due to the disruptions of secondary, tertiary and the quaternary structure of proteins; hydrophobic interactions, electrostatic and hydrogen bonds maintaining these structures are irreversibly affected at pressure above 300 MPa (Goyal et al., 2013).

2.6.1 Effect of HPP on Physico-chemical Properties

Zobrist et al. (2005) discovered that HPP induced an increase in milk pH up to 0.08 units at pressure of 600 MPa. The higher the pressure applied, the larger is the effect of HPP to the increment of milk pH. However, the authors also explained that the increase in milk pH were virtually irreversible on subsequent storage of milk at 4°C for up to 48 hours, but were reversed within 4 hours on storage at 20°C. Similar results were obtained by Liepa et al. (2017), who found an increment of 0.04 units at pressure of 600 MPa.

Gaucheron et al. (1997) confirmed that HPP induces an irreversible disintegration of casein micelles into smaller particles and an increase in protein hydrophobicity. Moreover, an increase in casein micelle hydration, slight phosophorus and calcium solubilisation and β-lactoglobulin denaturation were also been confirmed. These changes were related to pressure-induced changes in the hydrogen bonds, hydrophobic and electrostatic interaction which is responsible for maintaining the structural integrity of micelles and the conformation of globular proteins. Similar results were also obtained by Goyal et al. (2013) in recent years.

However, the effects of the disintegration of casein and protein denaturations to the total protein content in milk are still unknown. It is only acknowledged that the primary structure of protein is not influenced by high pressure, so the amino acids chains are still remain intact.

Relatively few studies have examined the effects of HPP on milk fat content. Huppertz et al. (2002) described that HPP does not induced lipolysis of fat in milk, since no damage was incurred to the milk fat globule membrane.

2.6.2 Effect of HPP on Microbiological Properties

HPP is generally used to inactivate the microorganisms present in dairy products. When a microbial cell is subjected to high pressure, the following detrimental changes take place. Firstly, the cell membranes are destroyed via irreversible changes to the structure of the membrane macromolecules, particularly protein. The homogeneity of the intermediate layer between the cell wall and the cytoplasmic membrane is disrupted. Then, the nucleic acids and ribosome involved in the synthesis of proteins are disrupted. In contrast, spores are more resistant to HPP because it contains calcium-rich dipocolinic acid which protects them from excessive ionization (Sakharam et al., 2011).

Considine et al. (2008) exposed that gram-positive bacteria are more resistant to pressure than gram-negative bacteria generally. Pressure of 500 – 600 MPa for 10 min is required to inactivate gram-positive microorganisms, while gram-negative bacteria were inactivated at relatively lower pressures (Evrendilek, 2017). It has been suggested that the complexity of the gram-negative cell membrane could be attributable to its HPP susceptibility. In comparison, yeasts and moulds are relatively HPP sensitive; however, ascospores of heat-resistant moulds such as *Byssochlamys*, *Neosartorya* and *Talaromyces* are generally considered to be extremely HPP resistant (Black et al., 2007; Considine et al., 2008).

Black et al. (2007) reported the response of microbial spores to HPP regarding the *Bacillus*, *Clostridium* and *Alicyclobacillus* species. *Bacillus* spores were found to be resistant to pressure up to 1000 MPa. However, while dormant spores are extremely resistant to pressure and heat, they found out that HPP also triggers spore germination, and this appears to be the major reason that HPP can result in spore killing. Different spores can have different suitable pressure range in spore germination, which then result in spore killing.

Mussa and Ramaswamy (1997) discovered that kinetics of TPC in milk showed a first order rate of destruction up to the pressure of 350 MPa. The corresponding D values were lower at higher levels of operating pressures employed for the treatment. That means the higher the pressure applied in HPP, the lower the time required to obtain the desired reduction in TPC.

Liepa et al. (2018) reported that HPP at 550 MPa for 3 min reduced the TPC significantly for 99.7% less compared with the control sample in skimmed milk. The authors also found out that pressure regime of 250 MPa/15 min and 400 MPa/3 min were insufficient to inactivate *E. coli*. When the holding time is increased to 15 min, pressure of 400 MPa was sufficient to inactivate *E. coli*.

Alexandros et al. (2019) reported that *E. coli*, *Salmonella* spp. and *L. monocytogenes* achieved a more pronounced inactivation with increasing pressure levels (400–600 MPa) and increasing exposure time (1–3 min). HPP application even at lower pressure level (400 MPa) and exposure time (1 min) resulted in significant reduction ($P<0.05$) in the levels for all three bacteria.

2.6.3 Changes in High Pressure Treated Milk Properties during Storage

Liepa et al. (2018) reported that the population of microorganisms grew progressively in all HPP regimes for cow skimmed milk. Rapid growth trends were observed after 5 days of storage. After 7 days of storage, the growth rate of bacteria was in the following order: 250 MPa/3 min < 400 MPa/3 min < 400 MPa/15 min < 550 MPa/3 min. Hence, it is obvious that the higher the pressure and holding time, the stable is the milk during storage. The authors also concluded that the minimum treatment regime for shelf life extension of skimmed milk was not less than 400 MPa with the holding time of at least 15 minutes.

Alexandros et al. (2019) reported that the TPC in HPP milk (600 MPa/3 min) was always lower compared to pasteurization where the TPC in HPP milk only exceeded the detection limit after 28 days compared to pasteurized milk which took 14 days.

CHAPTER 3

METHODOLOGY

3.1 Overview of Research Framework

The research framework is demonstrated as in Figure 2. Firstly, 10 litres each of fresh cow and goat milk were collected from the farms nearby early in the morning. Fresh milk samples were sent to laboratory for physico-chemical and microbiological analysis. Then, bottling was carried out where 27 bottles each for cow and goat milk were prepared for HPP treatment. 9 bottles each of cow and goat milk were loaded into the Hiperbaric 55 for every pressure regime, which were 450 MPa/7 min, 600 MPa/5 min and 600 MPa/7 min respectively. After that, remaining unbottled cow and goat milk were sent for pasteurization at 72°C for 15 seconds. The pasteurized milk was then filled into 18 plastic bottles, 9 bottles each for cow and goat milk. Physico-chemical and microbiological analysis were carried out for treated samples at day 0. Other samples were stored at 8°C until further analysis at day 11 and 22.

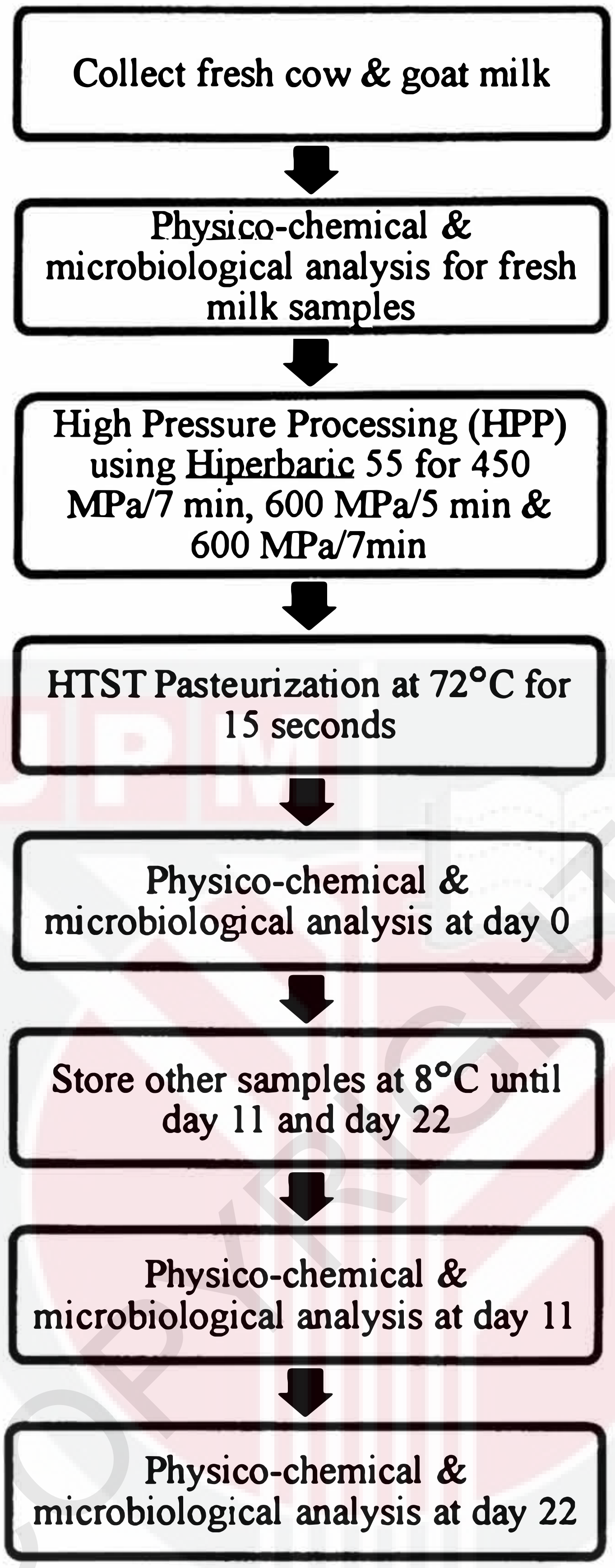

Figure 2: Research framework

3.2 Milk sampling

10 litres of fresh cow milk was collected from the university farm, Ladang 16, Universiti Putra Malaysia while fresh goat milk was collected from a private farm, Janggut Ternak Farm, Dengkil, Selangor, Malaysia. The milk was maintained below 10°C during transportation to the HPP facility (Kara F&B Productions, Sdn. Bhd.)

within 1 hour for bottling into milk samples in 250 ml plastic bottles (HPP grade) after thorough stirring. 3 bottles of milk sample, each for cow and goat was used directly for analysis as control or fresh milk samples.

3.3 High pressure processing

The two HPP pressures applied are 450 MPa and 600 MPa, while the two HPP durations are 5 mins and 7 mins. The three HPP treatments combinations were 450 MPa/7 min, 600 MPa/5 min and 600 MPa/7 min was conducted at 15 ± 2°C. 27 milk samples (3 HPP treatments x 3 storage durations x 3 analysis) each for cow and goat milk were used for analysis on day 0, 11 and 22 for physico-chemical and microbiological properties. Both cow and goat milk samples were loaded into the pressure chamber of HPP unit (Hiperbaric 55, Hiperbaric, Spain) for the same treatment level.

3.4 Pasteurization

Pasteurisation of cow and goat milk was done at 72°C for at least 15 sec (HTST – 72°C/15). Milk was poured into beakers which were pre-heated with boiling water to ensure sterile condition and heated in the water bath (BS-21, Jeio Tech, South Korea) with temperature monitored using clean thermocouples while stirring the milk regularly. After pasteurization, the milk was cooled rapidly to room temperature in an ice cooler box filled with ice cubes. Then, the milk was bottled into 9 plastic bottles (3 storage days x 3 analysis) each for cow and goat milk. All treated milk samples were kept in a chiller (RV710, Hitachi, Japan) at 8 ± 2°C before for storage until analysis.

3.5 Physico-chemical Analysis

3.5.1 pH

The pH values of both cow and goat milk samples were determined using a portable pH meter (Mi805, Milwaukee, Hungary). The pH meter was calibrated to the pH of the buffer solution at 25°C. The electrode was then rinsed with distilled water. After that, the pH values of samples were determined at 25°C.

3.5.2 Titratable Acidity

Titratable acidity was determined according to AOAC Official Method 947.05. 9 ml of sample was measured and poured into a conical flask. 2 ml of phenolphthalein indicator was added and titrated with 0.1M NaOH to first persistent pink.

3.5.3 Specific Gravity

The specific gravity was determined following method of AOAC 925.22 by using pycnometer. It was obtained as the ratio of the weight of a pycnometer (50 ml) filled with milk to the weight of the same pycnometer filled with water.

3.5.4 Total Protein

Total protein was determined using Pyne's Method which is known as formaldehyde titration (Pyne, 1932). This method was proven to be having the same accuracy as compared to Kjeldahl method, as the differences were insignificant between protein contents measured by using both methods (Gomaa et al., 2014). Firstly, 10 ml of sample was added into a conical flask. Then, 0.5 ml of 0.5% phenolphthalein indicator and 0.4 ml of 0.4% potassium oxalate were added into the sample. The mixture was allowed to stand for 2 mins. Then the sample was titrated with 0.1M NaOH until first persistent pink. After that, 2 ml of neutral 40% formalin was added which would discharge the pink colour. The titration was continued with 0.1M NaOH until another persistent pink. The total protein was calculated by multiplying the second titration volume with the factor of 1.74.

3.5.5 Total Solid

The total solid of both cow and goat milk was determined by using oven drying method, in accordance to AOAC Official Method 16.032. Firstly, the clean dry empty dish and lid were heated in the oven (UM500, Memmert, Germany). After that, the dried empty dish and lid were cooled in a desiccator and weighed. 3 – 4 grams of sample was added into the dish, and then the lid was replaced and weighed again. The dish containing sample was placed in a boiling water bath until the moisture was evaporated from the sample. The sample was then placed in the oven at 102 ± 2°C for 2.5 hours. The sample was cooled in the desiccator, weighed and continued drying in the oven repeatedly at hourly intervals until successive weighing did not vary by more than 0.5 mg. The total solid in percentage was calculated using following formula:

$$\text{Total solid (\%)} = \frac{\text{Weight of dish+dried sample} - \text{Weight of empty dish}}{\text{Weight of sample}} \times 100$$

3.5.6 Total Fat

Fat percentage was determined by AOAC Official Method 905.02, which is also known as the Roese-Gottlieb Method. 10g of sample was measured into Mojonnier extraction flask. 1.25 ml of ammonia was added and mixed thoroughly. 10 ml of alcohol was added and mixed well. Then, 25 ml of diethyl ether was added before shaken vigorously for 1 min followed by 25 ml of petroleum ether being added and shaken vigorous with stopper cork. The mixture was then allowed to stand until the upper liquid was practically clear. The ether solution was decanted into a suitable flask. The lip and stopper of extraction flask were washed with mixture of equal parts of the two ethers and added into the flask. The extraction of fat was repeated twice, using 15 ml of each solvent each time. The solvent in the flask was evaporated in a steam bath at temperature that does not cause spattering or bumping. The fat in the flask was dried to constant weight in oven (UM500, Memmert, Germany) at 102 ± 2°C and was recorded. Then, the fat in the flask was removed completely using 15 – 25 ml of warm petroleum ether, dried and weighed as before. The total fat was calculated using the following formula:

$$\text{Total Fat (\%)} = \frac{\text{Weight of flask containing fat} - \text{weight of flask after fat washing}}{\text{Weight of sample}} \times 100$$

3.5.7 Solid-non-fat

Milk solid-non-fat was determined by subtracting the total solid (%) with the total fat content (%).

3.6 Microbiological Analysis

3.6.1 Total Plate Count

The total plate count in milk was determined using the method as described in FDA Bacteriological Analytical Manual (BAM) Chapter 3. A rotated agar plate with liquid sample was inoculated by a mechanical plater in the spiral plate count (SPLC) method. The sample volume dispensed decreases as the dispensing stylus moved from the center to the edge of the rotating plate. The microbial concentration was determined by counting the colonies on a part of the petri dish where they are easily countable and dividing this count by the appropriate volume.

3.6.2 Total Yeast and Mould Count

Total yeast and mould count was determined by using method as described in FDA BAM Chapter 18. 0.1 ml of each dilution was pipetted aseptically on pre-poured, solidified DRBC agar plates and inoculum was spread with a sterile, bent glass rod. Each dilution was plated in triplicate. The plates were incubated in the dark at 25°C. The plates were counted after 5 days of incubation. If there is no growth at 5th day, the plates were re-incubated for another 48 hours. Plates containing 10–150 colonies were counted. If mainly yeasts were present, plates with 150 colonies were countable. However, if substantial amounts of mould were present, depending on the type of mould, the upper countable limit may have to be lowered at the discretion of the analyst.

3.6.3 Total Coliform Count

The total coliform count was determined by using AOAC Official Method 991.14. The dry-film coliform count plate was placed on flat surface. 1 ml of test suspension was inoculated onto center of film base. Top film was carefully placed onto inoculum. Test suspension was distributed over prescribed growth area with downward pressure on the center of plastic spreader device. The plate was left for 1 minute to permit gel to solidify. The plates were incubated for 24 ± 2 hours at 35 ± 1°C. The plates were counted promptly after incubation period. Standard colony counter was used for counting purposes. Coliforms appeared as red colonies that have one or more gas bubbles associated with them.

3.6.4 Mesophilic Aerobic Spore Count

The mesophilic aerobic spore count was determined using method as described in Compendium of Methods for the Microbiological Examination of Foods (CMMEF) Chapter 23. The sample was diluted with a buffer and blended. Multiple extractions were made and placed into duplicate sets of test tubes in a multiple tube format, which have been boiled to remove oxygen. One set was heat shocked for 10–13 minutes at 80°C to detect heat resistant proteolytic strains while the other set was heat shocked at 15–30 minutes at 60°C to detect non-heat resistant non-proteolytic, saccharolytic strains. Inoculated tubes were incubated anaerobically at 35°C for 3 to 5 days and then counted for the spores count.

3.6.5 Thermophilic Aerobic Spore Count

The thermophilic aerobic spore count was determined using the method described in CMMEF Chapter 26. Diluted sample was first heated to boiling point for 5 minutes. After heat shock, 20 ml of the diluted sample was distributed over six tubes containing previously exhausted PE-2 medium. The surface of the medium was covered with a layer of 15 mm Agar Plug (2% agar). When the solidification of the plug was completed, the tubes were incubated at 55 ± 2°C for 72 hours. The presence of spores was observed with the abundant gas production.

3.6.6 Psychrotrophic Spore Count

The psychrotrophic spore count was determined using the method described in CMMEF Chapter 13. Three adequate dilutions were selected and inoculated 1 ml of each dilution in separate Petri dishes. 12–15 ml of previously melted and cooled Plate Count Agar (PCA) was poured to the inoculated plates. The inoculum was mixed with the culture medium and wait until the agar was completely solidified. The plates were inverted and incubated at 17 ± 1°C for 16 hours, followed by 3 more days at 7 ± 1°C. The plates with 25 to 250 colonies were selected and counted the colonies with aid of a magnifying glass on a colony counter.

3.6.7 *Bacillus cereus*

Bacillus cereus was determined using the method described in FDA BAM Chapter 14. Duplicate MYP agar plates were inoculated with each dilution of sample by spreading 0.1 ml evenly onto surface of each plate with sterile glass spreading rod. Plates were incubated for 18–24 hours at 30°C and observed for colonies surrounded by precipitate zone, which indicates that lecithinase is produced. *B. cereus* colonies are usually a pink coloured on MYP and became more intense after additional incubation.

3.6.8 *Escherichia coli*

Escherichia coli were determined using AOAC Official Method 991.14. The dry-film *E.coli* count plate was placed on flat surface. 1 ml of test suspension was inoculated onto center of film base. Top film was carefully placed onto inoculum. Test suspension was distributed over prescribed growth area with downward pressure on center of plastic spreader device. The plate was left for 1 minute to permit gel to solidify. The plates were incubated for 48 ± 4 hours at 35 ± 1°C. The plates were counted promptly after incubation period. The colonies appeared as blue colonies associated with gas bubbles.

3.6.9 *Clostridium perfringens*

Clostridium perfringens was determined using AOAC Official Method 976.30. 5 ml of TSC agar without egg yolk was poured into each of ten 100 x 15 mm petri dishes and was spread evenly by rapidly rotating dish. The plates were labelled after agar has solidified, and 1 ml of each dilution was aseptically pipetted in duplicate onto agar surface in center of dish. 15 ml TSC agar without egg yolk was poured additionally into dish and mixed well with inoculum by gently rotating dish. Plates were placed upright in anaerobic jar when agar has solidified. Jar was incubated for 20 hours at 35°C for TSC without egg yolk and 24 hours for TSC agar with egg yolk. After incubation, plates were removed from jar and observed macroscopically for growth and black colony production.

3.6.10 *Staphylococcus aureus*

Staphylococcus aureus was determined using the method stated in FDA BAM Chapter 12. For each dilution to be plated, 1 ml of sample suspension was aseptically transferred to 3 plates of Baird-Parker agar. Inoculum was spread over surface of agar plate using sterile bent glass streaking rod. Plates were retained in upright position until inoculum is absorbed by agar. Plates were inverted and incubated for 45–48 hours at 35–37°C. Plates containing 20-200 colonies were selected, unless only plates at lower dilutions (>200 colonies) have colonies with typical appearance of *S. aureus*. The colonies are circular, smooth, 2–3 mm in diameter on uncrowded plates, grey to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone.

3.6.11 *Salmonella* spp.

Salmonella spp. was determined using Enzyme-linked Immunosorbent *Assay* (ELISA) Method. First well was left empty, 0.1 ml of negative control and positive control were pipetted respectively into second and third wells. 0.1 ml of each boiled sample was pipetted separately into consecutive wells in the strip. The plates containing the strips were incubated at 37 ± 1°C for 30 minutes. After incubation, the contents of wells were aspirated, removing as much of the liquid as possible. The wells were washed using wash buffer for 5–7 times. 0.1 ml of Conjugate and 0.1 ml of TMB substrate were pipetted into all wells and incubated at room temperature for 30 minutes. After incubation, the reaction was stopped by adding 0.1 ml of Stop solution. The optical densities were read within 10 minutes in a plate reader using 450nm filter.

3.6.12 *Listeria monocytogenes*

Listeria monocytogenes was determined using method as stated in FDA BAM Chapter 10. Analytical sample size for food is generally 25 g, and this can be from individual units or as part of a sample composite. Putative Listeria isolated on selective agars from standard or screen positive enrichments were purified on non-selective agars and confirmed by conventional identification tests or by a battery of such tests in kit form. Isolates may be rapidly confirmed as *L. monocytogenes* by using specific test kits or PCR procedures.

3.7 Experimental Design and Statistical Analysis

All measurements were done in duplicates with samples prepared from the same batch of milk. Minitab software (Version 16, Minitab Statistical Software, United States) was used to perform the analysis of variance (ANOVA). Tukey's test was performed to compare the differences among the mean values at a confidence level of 0.05.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Effects of HPP on Physico-chemical Properties

The physico-chemical properties of cow and goat milk before and after treatment are presented in Table 1. The pH of pasteurized cow milk has decreased significantly ($P<0.05$) from 6.59 ± 0.01 to 6.52 ± 0.00. In contrast, the pH of pasteurized goat milk increased significantly ($P<0.05$) from 6.34 ± 0.00 to 6.38 ± 0.01. Ma and Barbano (2003) reported that pH of cow skim milk decreases linearly with increasing temperature during treatment. However, José et al. (2015) reported that pasteurization increased pH of bovine milk because of the lower whey protein associating with casein micelles. Tamime (2014) explained that the increase of pH is due to reduced casein micelles where heat has denatured its whey protein. For HPP-treated milk, pH for cow milk only changed slightly but statistically insignificant ($P>0.05$). Nonetheless, pH of goat milk has increased significantly ($P<0.05$). This increment is mainly due to the alteration of the distribution of minerals by HPP, which includes calcium, phosphate and other ionized minerals such as calcium (Chopde et al., 2014). Other studies which have observed increase in the milk pH after HPP include Liepa et al. (2017) who found that pressure of 500–600 MPa for

15 min increased the pH of cow milk; while Zobrist et al. (2005) reported that pressure of 100 – 600 MPa for 30 min also caused an average 0.08 units of pH increment in cow milk.

In general, the results show that the titratable acidity, specific gravity, total protein, total fat, total solid and solid-non-fat in both cow and goat milk were not influenced by both HPP and pasteurization. The values changed slightly after HPP treatment but they were statistically insignificant when compared with untreated samples (P>0.05).

Table 3: Physico-chemical properties of cow and goat milk before and after treatment

Analysis	**Before Treatment**	**450MPa / 7min**	**600MPa / 5min**	**600MPa / 7min**	**Pasteurized**
			Cow Milk		
pH	6.59 ± 0.01^{a}	6.56 ± 0.01^{a}	6.58 ± 0.00^{a}	6.61 ± 0.02^{a}	6.52 ± 0.00^{b}
Titratable Acidity	0.16 ± 0.01^{a}	0.15 ± 0.00^{a}	0.16 ± 0.01^{a}	0.17 ± 0.01^{a}	0.16 ± 0.01^{a}
Specific Gravity	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.02 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}
Total Protein	3.13 ± 0.00^{a}	2.87 ± 0.12^{a}	3.04 ± 0.12^{a}	3.04 ± 0.12^{a}	2.96 ± 0.25^{a}
Total Fat	3.33 ± 0.08^{a}	3.29 ± 0.06^{a}	3.24 ± 0.09^{a}	3.30 ± 0.14^{a}	3.32 ± 0.08^{a}
Total Solid	11.65 ± 0.01^{a}	11.61 ± 0.21^{a}	11.55 ± 0.19^{a}	11.64 ± 0.11^{a}	11.88 ± 0.06^{a}
Solid-non-fat	8.32 ± 0.09^{a}	8.32 ± 0.15^{a}	8.31 ± 0.28^{a}	8.34 ± 0.03^{a}	8.56 ± 0.14^{a}
			Goat Milk		
pH	6.34 ± 0.00^{c}	6.38 ± 0.00^{b}	6.42 ± 0.01^{a}	6.38 ± 0.01^{b}	6.38 ± 0.01^{b}
Titratable Acidity	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}	0.21 ± 0.01^{a}	0.23 ± 0.01^{a}
Specific Gravity	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}
Total Protein	3.57 ± 0.12^{a}	3.39 ± 0.12^{a}	3.57 ± 0.12^{a}	3.31 ± 0.00^{a}	3.48 ± 0.00^{a}
Total Fat	4.51 ± 0.05^{a}	4.43 ± 0.49^{a}	4.44 ± 0.41^{a}	4.42 ± 0.45^{a}	4.34 ± 0.61^{a}
Total Solid	13.88 ± 0.06^{a}	13.73 ± 0.14^{a}	13.69 ± 0.00^{a}	13.70 ± 0.13^{a}	13.08 ± 1.42^{a}
Solid-non-fat	9.37 ± 0.10^{a}	9.30 ± 0.35^{a}	9.35 ± 0.41^{a}	9.28 ± 0.58^{a}	8.74 ± 2.03^{a}

Note: Values are mean ± standard deviation. Means that do not share a superscript within a row are significantly different (P<0.05).

4.2 Effects of HPP on Microbiological Properties

Table 4 summarizes the microbiological properties of cow and goat milk before and after treatment. According to the Malaysian Standard for pasteurized milk (MS 410:1995), the total plate count (TPC) should not be more than 10^5 CFU/ml. In this study, the initial TPC of raw cow and goat milk was 1.5×10^5 CFU/ml and 6.9×10^6 CFU/ml respectively. Both HPP and pasteurization produced desirable results of decreased TPC in both milk samples. The pressure regime of 600 MPa/5 min has led to the highest TPC reduction in both milks, i.e. 99.98% for the cow milk and 99.99% for the goat milk. The lowest TPC reduction was found in the pressure regime of 450 MPa/7 min (96%) for the cow milk and for goat milk, it was from the pasteurization process (99.98%). This is in lined with the findings from Alexandros et al. (2019) who found that HPP (600 MPa/3 min) led to a more pronounced decrease of TPC than that of pasteurization.

Psychrotrophic bacteria are undesirable for prolonged storage of milk because they are able to grow below 7°C even though their optimum temperature ranges from 20°C to 30°C. They also produce heat resistant enzymes, including proteolytic and lipolytic enzymes at low temperatures, which are able to hydrolyze milk fat and protein leading to the forming of off-flavours (Samaržija et al., 2016). According to Malaysians Food Act 1983 and Food Regulations 1985, psychrotrophic bacteria count in milk should not exceed 10^5 CFU/ml. In this research, the initial psychrotrophic bacteria count in raw cow and goat milk were 9.2×10^4 CFU/ml and 8.0×10^5 CFU/ml respectively. Both HPP and pasteurization have successfully decreased psychrotrophic bacteria count in all milk samples. The lowest psychrotrophic bacteria count in cow milk was recorded in the pressure regime of 600 MPa/7 min where the bacteria was undetectable (<1 CFU/ml) while the highest count was found in the pressure regime of 450 MPa/7 min (8.3×10^2 CFU/ml). For goat milk HPP treatment at all pressure regimes have reduced the bacteria count to undetectable level (<1 CFU/ml). The highest psychrotrophic bacteria count recorded was in the pasteurized sample which is 3.6×10^1 CFU/ml.

Mesophilic spores are known as the most prevalent spore-former found in bulk milk processing tank. In spore form, these organisms are capable of surviving environmental stresses including low pH, exposure to sanitizers, high pressure and temperature (Kent et al., 2016). Mesophilic spores were undetected in all goat milk samples both before and after treatments. For cow milk, the initial mesophilic aerobic spore count was 70 CFU/ml. All pressure regimes in HPP successfully inactivated the spores. However, pasteurization only managed to reduce it to 20 CFU/ml. McGuiggan et al. (2002) explained that these aerobic spores have the ability to survive in high temperature as well as pasteurization.

Coliforms are gram-negative and non-spore forming bacteria that ferment lactose with the production of acid at 35°C within 48 hours (Wanjala et al., 2018). According to MS 410:1995, the total coliform should be absent in 0.1 ml of sample. In this study, the total coliform count for untreated cow milk was 37 CFU/ml, but after treatment, the coliform became undetected in all HPP-treated and pasteurized samples. While all pressure regimes of HPP were able to reduce the total coliform count to undetectable level in goat milk, pasteurization was capable to reduce the counts from 2.1×10^5 CFU/ml to 26 CFU/ml (99.98% reduction).

Yeast and mould are a large and diverse group which include several hundred species. Although yeast and mould are relatively HPP sensitive (Considine et al., 2008), mascospores of heat-resistant moulds such as *Byssochlamys*, *Neosartorya* and

Talaromyces are generally considered to be extremely HPP resistant. In this research, the total yeast and mould count of untreated cow and goat milk were 60 CFU/ml and 80 CFU/ml respectively. Both HPP and pasteurization were effective in inactivating the yeast and mould in cow and goat milk fully. Similar result was reported by Evrendilek (2017), where most vegetative yeast and mould were inactivated within 5–10 min by 300–400 MPa at 25°C.

Aerobic spore-forming bacteria of the genus *Bacillus* are commonly present in raw milk. Their spores survive pasteurization and subsequently germinate, outgrow, and multiply (McClements et al., 2001). In this study although *B. cereus* was not found in raw cow milk, it was present in HPP-treated and pasteurized milk with the highest count of 20 CFU/ml in milk subjected to 450 MPa for 7 min. The unexpected presence of *B. cereus* in treated cow milk may be due to several factors. McClements et al. (2001) reported that *B. cereus* spores were more resistant to pressure than vegetative cells. The contamination, germination and outgrowth of *Bacillus* spores in milk can occur during simple transfer during milking and bottling when hygiene conditions are not fully observed. Nutrient germinant such as amino acids and unsaturated fatty acids, levels of some indigenous antibacterial factors such as lysozyme, lactoferrin and lactoperoxidase, somatic cells and metal ions have been suggested as factors influencing the germination of *Bacillus* species (Christiansson et al., 1999; McGuiggan et al., 2002). *B. cereus* was not found in both raw and treated goat milk.

C. perfringens, *S. aureus*, *E. coli*, *L. monocytogenes*, *Salmonella* spp. and thermophilic spores were undetected in both cow and goat milk. The absence of these bacteria and spores is due to good milking hygiene, breeds, climates and geographic location. In India, Chaturvedi and Shukla (2015) reported that 51.61% of raw milk samples were found to be highly contaminated with *Clostridium* species due to unhygienic milking practice. Mohamed et al. (2017) observed no germs of *S. aureus* and spore of *Clostridium* spp. in all cow and goat milk samples at Djibouti. However, in the same research, the *E. coli* count of 2.58 log CFU/ml was significantly higher than standard value of 2 log CFU/ml in their milk samples. Chye et al. (2004) reported that only 1.9% and 1.4% of cow milk samples in Malaysia were contaminated by *L. monocytogenes* and *Salmonella* spp. respectively. Higher incidence of *Listeria* was obtained from eastern region while *Salmonella* spp. was found more prevalent in central region of Peninsular Malaysia. Hence, the presence of these bacteria is highly dependent on the location and hygiene.

Table 4: Microbiological properties of cow and goat milk before and after treatment

Bacteria count	**Before Treatment**	**450MPa / 7min**	**600MPa / 5min**	**600MPa / 7min**	**Pasteurized**
			Cow Milk		
Total plate count (CFU/ml)	1.5×10^5	6×10^3	2.6×10^1	2.5×10^2	1.5×10^2
Psychrotrophic bacteria count (CFU/ml)	9.2×10^4	8.3×10^2	1	<1	5.4×10^1
Mesophilic aerobic spore count (CFU/ml)	7.0×10^1	<1	<1	<1	2.0×10^1
Total coliform count (CFU/ml)	3.7×10^1	<1	<1	<1	<1
Total yeast and mould count (CFU/ml)	6.0×10^1	<1	<1	<1	<1
Bacillus cereus (CFU/ml)	<1	2.0×10^1	6	4	8
Clostridium perfringens (CFU/ml)	<1	<1	<1	<1	<1
Staphylococcus aureus (CFU/ml)	<1	<1	<1	<1	<1
Total *E. coli* count (CFU/ml)	<1	<1	<1	<1	<1
Thermophilic aerobic spore count (CFU/ml)	<1	<1	<1	<1	<1
Listeria monocytogenes per 25g	Absent	Absent	Absent	Absent	Absent
Salmonella spp. per 25g	Absent	Absent	Absent	Absent	Absent
			Goat Milk		
Total plate count (CFU/ml)	6.9×10^6	6.4×10^2	6	4.0×10^1	8.0×10^2
Psychrotrophic bacteria count (CFU/ml)	8.0×10^5	<1	<1	<1	3.6×10^1
Mesophilic aerobic spore count (CFU/ml)	<1	<1	<1	<1	<1
Total coliform count (CFU/ml)	2.1×10^5	<1	<1	<1	2.6×10^1
Total yeast and mould count (CFU/ml)	8.0×10^1	<1	<1	<1	<1
Bacillus cereus (CFU/ml)	<1	<1	<1	<1	<1
Clostridium perfringens (CFU/ml)	<1	<1	<1	<1	<1
Staphylococcus aureus (CFU/ml)	<1	<1	<1	<1	<1
Total *E. coli* count (CFU/ml)	<1	<1	<1	<1	<1
Thermophilic aerobic spore count (CFU/ml)	<1	<1	<1	<1	<1
Listeria monocytogenes per 25g	Absent	Absent	Absent	Absent	Absent
Salmonella spp. per 25g	Absent	Absent	Absent	Absent	Absent

4.3 Changes in Physico-chemical Properties in Milk during Storage

The pH for both HPP-treated and pasteurized cow and goat milk decreased significantly (P<0.05) during a 22 days storage period (Figure 3). Similar results were obtained by Siddique et al. (2016), who reported that at the end of 90 days storage of UHT milk, declination of pH occurred. Brodziak et al. (2017) also mentioned that statistically significant reduction in pH of opened drinking cow milk after 7 days of storage. The decreasing trend in pH during milk storage is due to the increasing acidity in milk caused by lactic acid and fatty acid (Zajác et al., 2015). Besides, it is obvious that pH of goat milk was always lower than cow milk which may be due to its higher acidity content, as shown in Figure 4. This is in agreement with the findings from Mahmood and Usman (2010), who also found lower pH in goat milk (6.48) than cow milk (6.59).

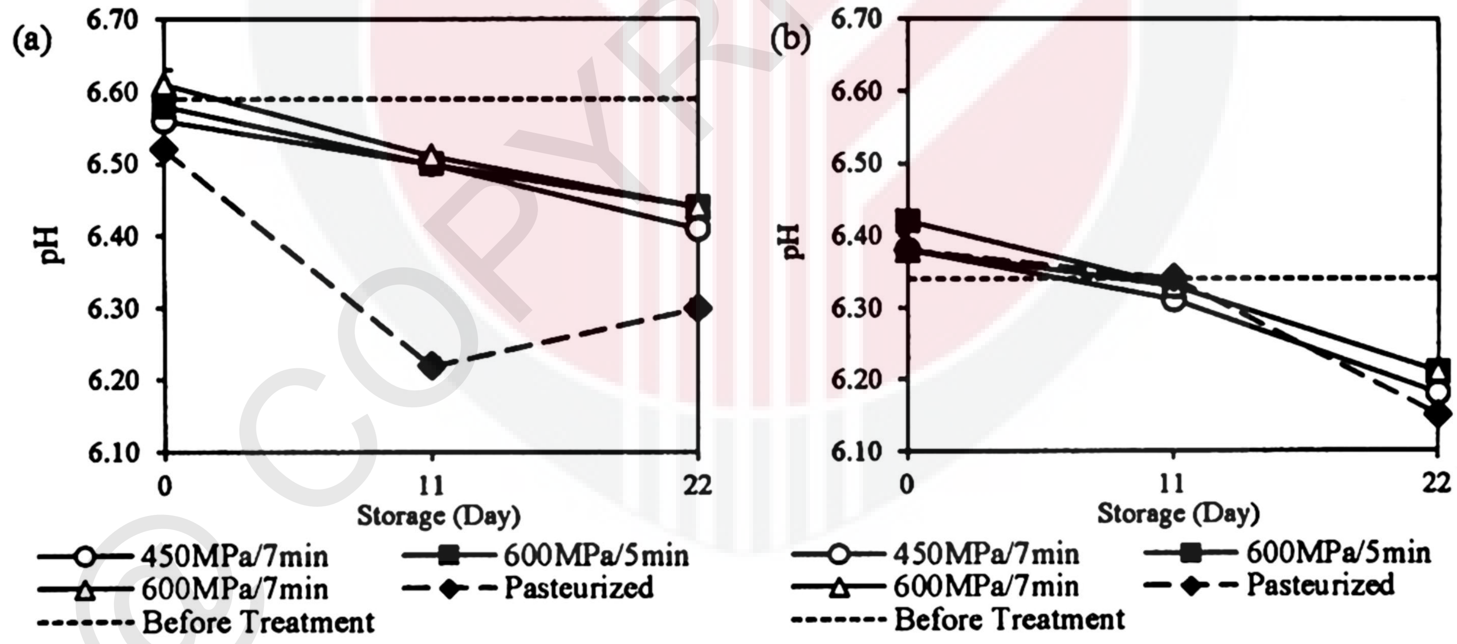

Figure 3: Changes in pH of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min, 600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

Generally, the titratable acidity of both cow and goat milk increased during a 22 days of storage period (Figure 4). The increment found in all HPP-treated cow milk was statistically insignificant (P>0.05) as compared to significant increment

($P<0.05$) for pasteurized cow milk with highest acidity (0.23%) at day 11. For goat milk, all HPP and pasteurized samples titratable acidity was found to have increased significantly, 0.25 to 0.26% and 0.30% respectively at the end of 22's storage from recorded range of 0.21 to 0.23% at day 0. It is thus obvious that both pasteurized cow and goat milk deteriorated faster than HPP-treated milk. Brodziak et al. (2017) also reported increase of titratable acidity in opened drinking cow milk after 7 days' storage in pasteurized milk, followed by micro-filtered milk and UHT milk. The increase in titratable acidity is mainly due to the increasing lactic acid concentration after degradation of lactose caused by the predominant spoilage lactic acid bacteria (LAB) under low oxygen, temperature and acidic condition (Rawat, 2015; Siddique et al., 2016).

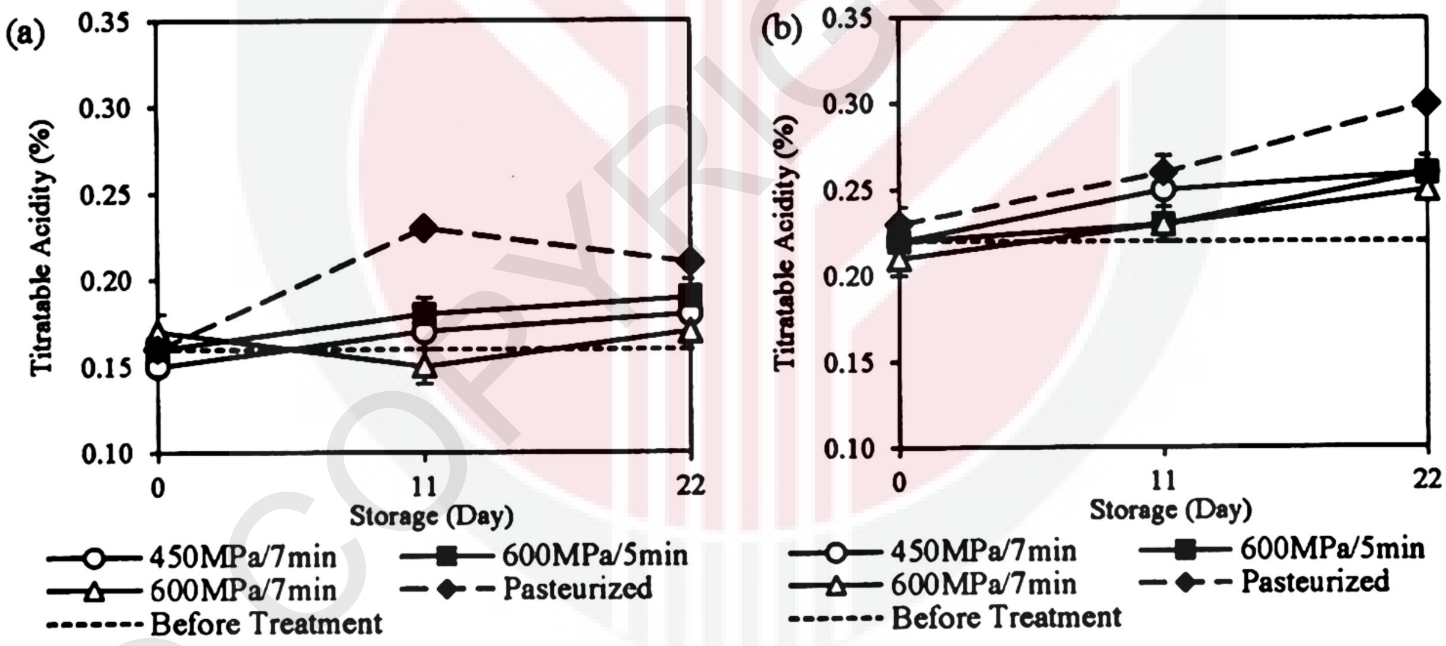

Figure 4: Changes in titratable acidity of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min, 600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

The specific gravity of both cow and goat milk increased gradually when approaching day 22 of storage (Figure 5). All HPP-treated and pasteurized samples have statistically significant increase ($P<0.05$) in the specific gravity except for goat milk in the pressure regime of 450MPa/7min which shows significant decrease ($P<0.05$). The increase in specific gravity are agrees with Aldubhany et al. (2014)'s work who reported increment in specific gravity in cow milk samples after 6 months of storage at various storage temperature. The specific gravity of milk could be influenced by the proportion of its constituents such as fat and lactose which degrade during storage (Rawat, 2015). Ahmad et al. (2005) proved that milk specific gravity decreased due to mastitis. The authors explained that presence of mastitis contributes to the decrease in lactose and increase in chloride contents as specific gravity is positively correlated with lactose and negatively correlated with chloride.

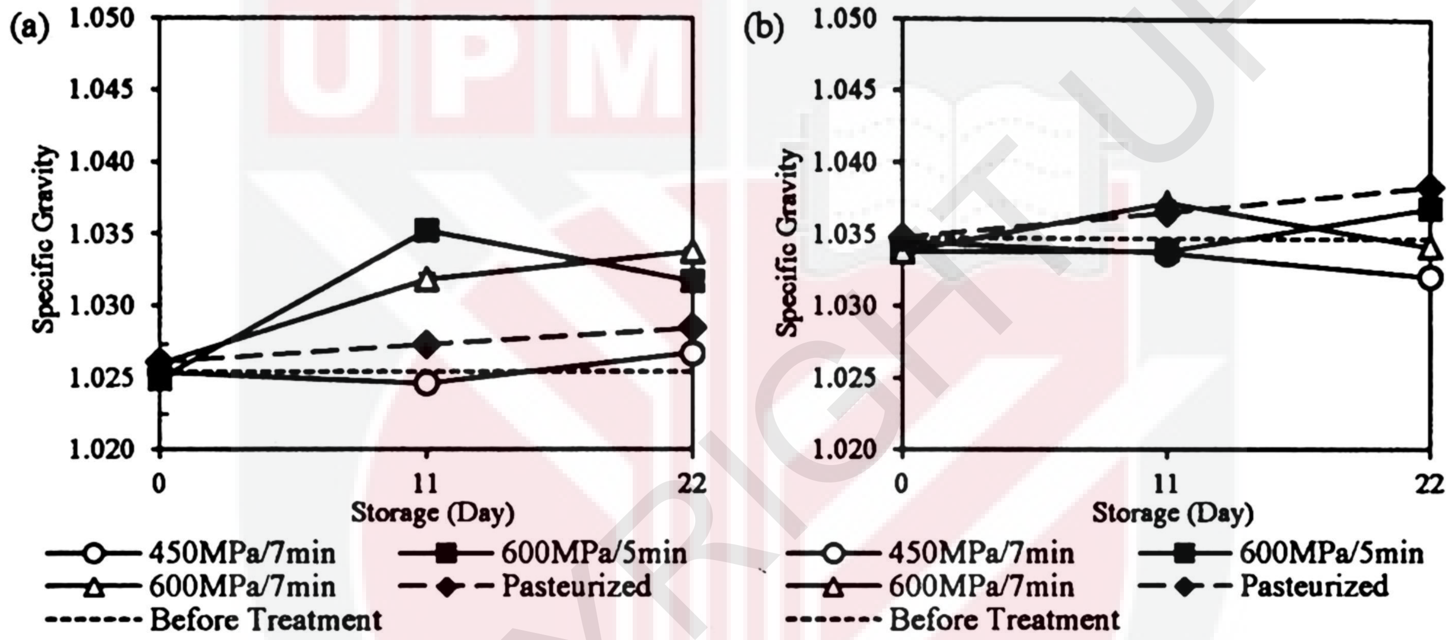

Figure 5: Changes in specific gravity of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min, 600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

Figures 6 shows that the total protein content in both cow and goat milk samples from both HPP and pasteurisation treatment did not have significant change (P>0.05) after 22 days. This is supported by Brodziak et al. (2017) who found that the storage duration of milk had no statistically significant effect on its protein content. Figure 7, however shows that the total fat content of both cow and goat milk samples from pasteurisation treatment have reduced significantly (P<0.05) starting from day 11 for cow milk and day 22 for goat milk despite no significant difference (P>0.05) found for HPP samples. The total fat for both pasteurized cow and goat milk has reduced from 3.32% to 2.94% while for goat milk from 4.34% to 3.11%.

HPP has been proven to have no damage on the milk fat globule membrane where lipolysis incidence could be prevented, thus the milk fat content could be well preserved (Evrendilek, 2017; Huppertz et al., 2002). Zaják et al. (2015) has reported the significant decrease in fat content of raw cow milk after 24 hours of storage at temperature of 4°C to be due to the lipolysis of milk fat initiated by both indigenous milk lipases and also microbial lipases which usually grew in number during storage. Significant increase of free fatty acids as a consequence of lipolysis in UHT cow milk after three weeks of storage also confirmed by Janstová et al. (2004). These analyses have also shown that goat milk has relatively higher total protein and fat contents than cow milk.

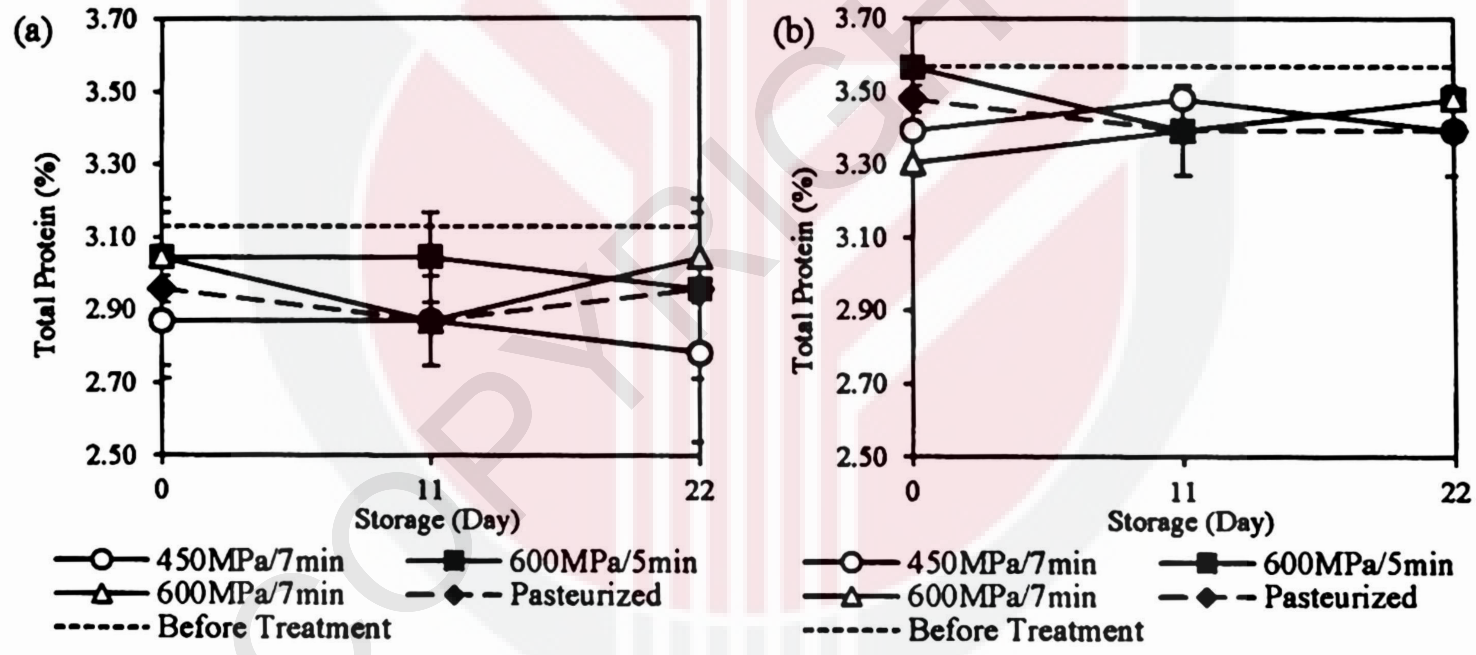

Figure 6: Changes in total protein of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min, 600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

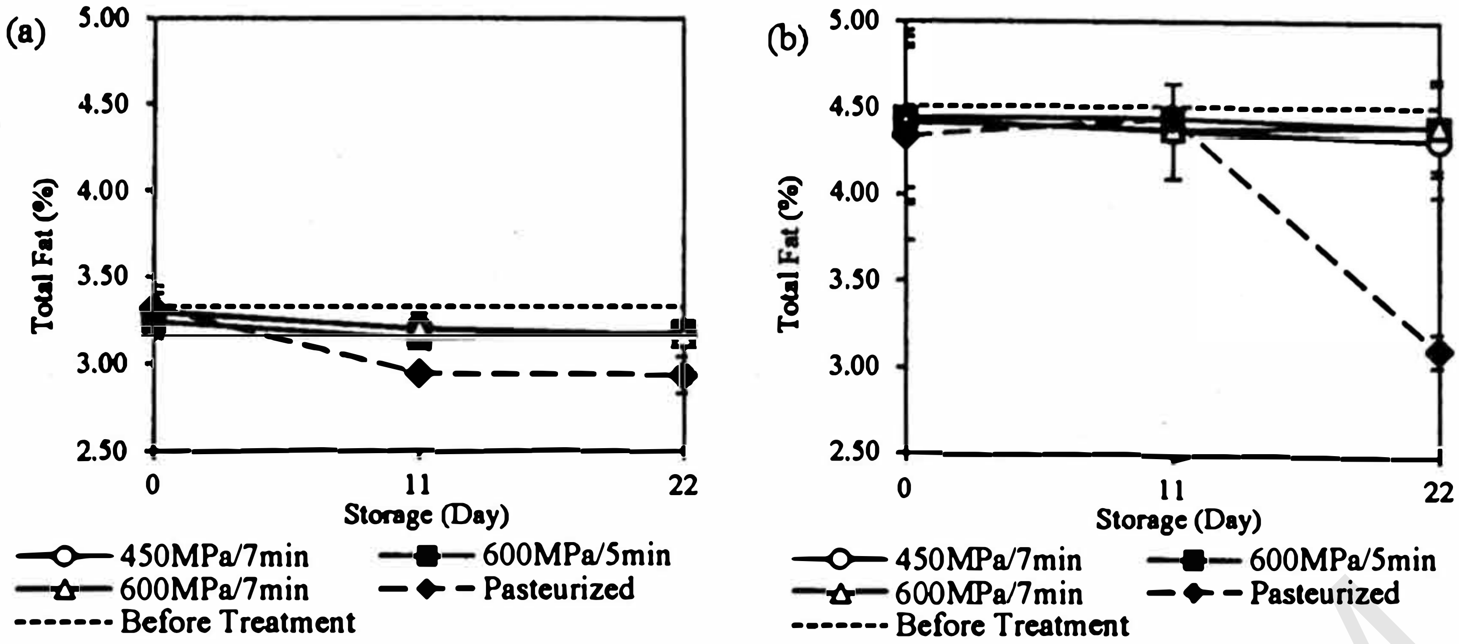

Figure 7: Changes in total fat of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min, 600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

Figure 8 and 9 show a mimicking trend of total solid and solid non-fat contents of cow and goat milk over the fat content trend, *i.e.* no significant difference during 22 storage for HPP treated samples and significant decrease ($P<0.05$) for pasteurized samples. The significant decrease in fat content could affect milk composition in terms of total solid and non-fat contents (Zajác et al., 2015). Omer and Eltinay (2009) have reported similar results where total solid and non-fat content of camel milk reduced significantly after two weeks of storage at 7°C.

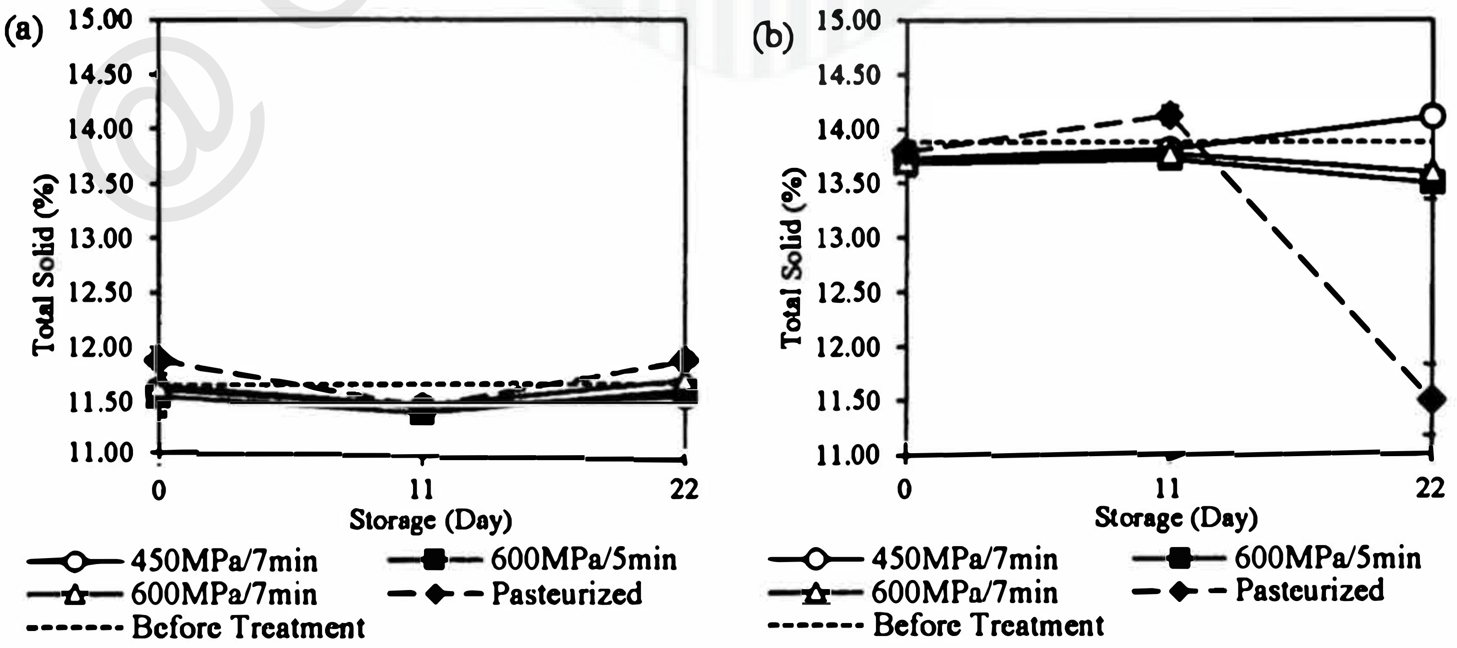

Figure 8: Changes in total solid of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min,

600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

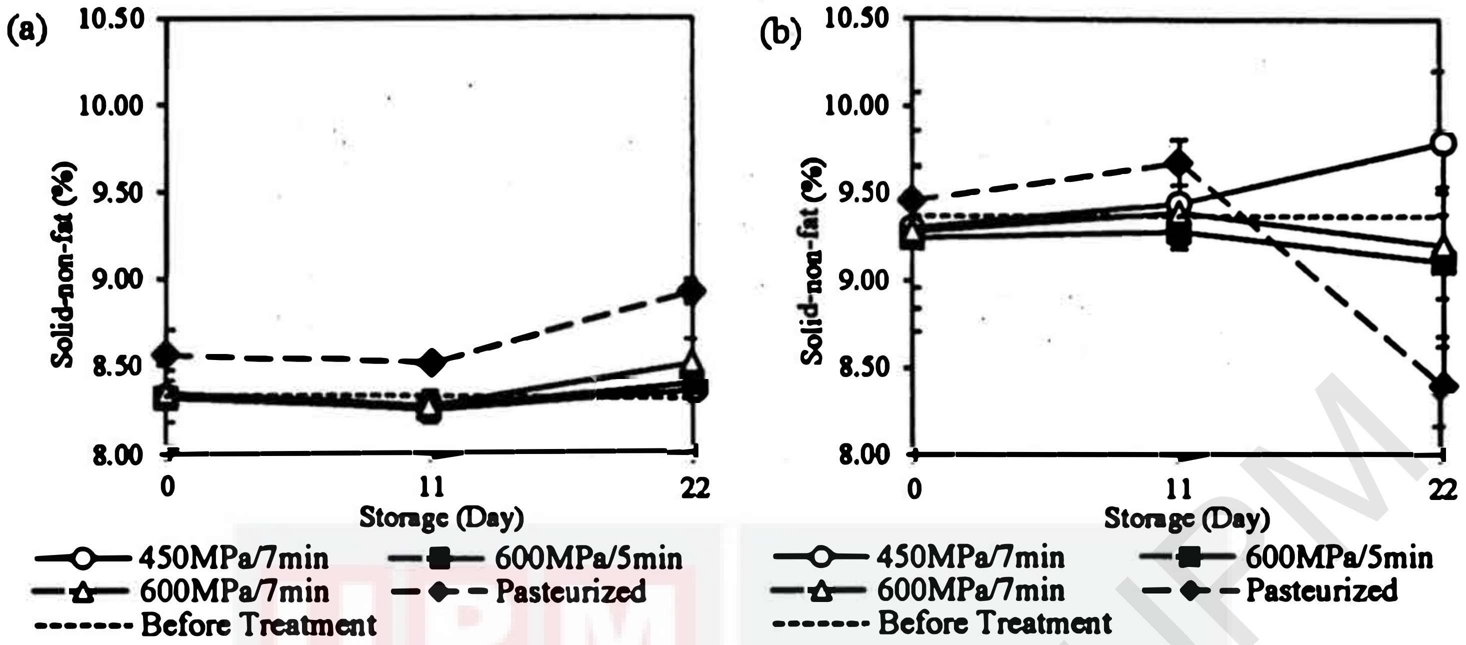

Figure 9: Changes in solid-non-fat of (a) cow and (b) goat milk during storage period of 22 days. Solid lines represent HPP-treated milk (450MPa/7min, 600MPa/5 min, 600MPa/7min), dashed lines represent pasteurized milk and dotted lines represent fresh milk before treatment.

4.4 Changes in Microbiological Properties during Storage

Table 5 presents the count of two microbiological measurements that turned positive during 22 days of storage. The psychrotrophic bacteria population in both cow and goat milk grew progressively throughout the storage period. At the end of 22 days, the best treatment was HPP at 600 MPa/7 min giving the smallest colony count in cow milk, followed by 600 MPa/5 min, pasteurization and 450 MPa/7 min. All these counts however, all still far below the limit of 10^5 CFU/ml set by the Malaysians Food Act 1983 and Food Regulations 1985. In the case for goat milk, the largest colony count of 9.0×10^8 CFU/ml was observed in the pasteurized sample. It has exceeded the permissible limit thus considered spoilt at 22 days of storage. The results are in lined with Doll et al. (2017) who found that 90% of pasteurized cow milk samples stored at 8°C contained 10^6 to 10^7 CFU/ml of psychrotrophs at the end of 24 days storage. Pressure regime of 600 MPa/7 min presented the best result as the psychrotrophic bacteria in goat milk remained undetectable at day 22. Garcia-risco et al. (1998) reported that the bovine milk sample treated at 400 MPa for 3 min has a psychrotrophic bacteria count of 10^6 at day 30 of storage at 7°C.

For TPC, progressive increase was observed during the storage period of 22 days. The lowest TPC observed in the cow milk at day 22 was in the pressure regime of 600 MPa/7 min, followed by 600 MPa/5 min, then pasteurization and lastly 450 MPa/7 min. Despite the increase, the TPC were still below the limit set by MS 410:1995, which states that the TPC in pasteurized milk should not have TPC above 10^5 CFU/ml. For goat milk at day 22, the lowest TPC of 8.8×10^1 CFU/ml was milk treated at 600 MPa/7 min. Pasteurized milk had the highest TPC of 3.5×10^8 CFU/ml, which has exceeded the permissible limit. These results are in agreement with Alexandros et al.'s (2019) work who found that TPC for HPP milk was always lower compared to pasteurized where TPC in HPP milk reached 10^7 CFU/ml after 28 days compared to pasteurized milk which took only 14 days to reach $>10^7$ CFU/ml. From both the psychrotrophic bacteria population and the TPC microbial count, HPP pressure regime of 600 MPa/7 min is the best for shelf life extension of both milk, renders them the shelf life of more than 22 days.

Other monitored microbes which include the *C. perfringens*, *S. aureus*, *E. coli*, *L. monocytogenes*, *Salmonella* spp. and thermophilic aerobic spore remained undetected throughout the 22 days of storage. Yeast and mould that has been inactivated by HPP or pasteurization also remained undetected throughout the storage period. Although the *Bacillus cereus* was found present in low number in cow milk at Day 0 after treatment (Table 4), they were not present in all samples on storage Day 11 and 22. The same situation goes to coliform and mesophilic aerobic spores, which were also present in low number in some pasteurized milk samples at Day 0 after treatment; they were undetected on storage Day 11 and 22. This inconsistent result is most probably due to the randomized sampling and testing of milk from different bottles for each analysis on respective experimental day. The storage results from Table 5 have actually reaffirmed the unexplained presence of coliform and mesophilic aerobic spores after HPP and pasteurization treatments (Table 4) suggesting either it was experimental randomization error or that the microbes were inactivated during storage at low storage temperature which may have prevented microbial growth (Garcia-risco et al., 1998) or reduced the microbial count to undetectable levels.

Table 5: Psychrotrophic bacteria count and total plate count (CFU/ml) in milk for 22 days of storage

Treatment	**Cow Milk**			**Goat Milk**		
	Day 0	**Day 11**	**Day 22**	**Day 0**	**Day 11**	**Day 22**
Psychrotrophic Bacteria Count						
450MPa / 7min	8.3 x 10^{2}	9.0 x 10^{3}	3.0 x 10^{3}	<1	1.0 x 10^{4}	6.8 x 10^{1}
600MPa / 5min	1	1.2 x 10^{4}	4.6 x 10^{2}	<1	1.8 x 10^{2}	2.2 x 10^{1}
600MPa / 7min	<1	6.4 x 10^{4}	1.9 x 10^{2}	<1	3	<1
Pasteurized	5.4 x 10^{1}	1.2 x 10^{4}	2.0 x 10^{3}	3.6 x 10^{1}	9.0 x 10^{5}	9.0 x 10^{8}
Total Plate Count						
450MPa / 7min	6.0 x 10^{3}	2.5 x 10^{3}	8.0 x 10^{3}	6.4 x 10^{2}	2.0 x 10^{3}	7.6 x 10^{2}
600MPa / 5min	2.6 x 10^{1}	6.0 x 10^{3}	3.7 x 10^{2}	6	8.8 x 10^{2}	3.6 x 10^{4}
600MPa / 7min	2.5 x 10^{2}	7.5 x 10^{4}	2.8 x 10^{1}	4.0 x 10^{1}	9.4 x 10^{1}	8.8 x 10^{1}
Pasteurized	1.5 x 10^{2}	1.1 x 10^{4}	3.0 x 10^{3}	8.0 x 10^{2}	6.0 x 10^{5}	3.5 x 10^{8}

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In overall, the results recognized that HPP treatment of 7 minutes at 450 MPa is sufficient to render both cow and goat milk a shelf life of 22 days at 8°C although higher pressure of 600 MPa can give a better safety margin with best treatment results in the order of 600 MPa/7 min, 600 MPa/5 min and lastly 450 MPa/7 min. The HPP treatment has shown better capability of preserving milk than pasteurisation where pasteurized goat milk was considered spoilt at the end of storage since both TPC and psychrotrophic bacteria count exceeded the permissible limit. During storage, the HPP treated milk, particularly the cow milk displayed better physico-chemical properties stability compared to the pasteurized. The HPP treated milk did not show significant changes in total fat, total solid and SNF but pasteurised cow and goat milk demonstrated significant changes in pH, titratable acidity, specific gravity, total fat, total solid and SNF throughout 22 days of storage.

After both HPP and pasteurization, only pH was significantly affected as the pH of both HPP treated and pasteurized goat milk increased significantly, while the reduction was significant for pasteurized cow milk and no significant changes for HPP treated cow milk. In terms of microbiological properties, although both HPP and pasteurization were effective in reducing the numbers of TPC, psychrotrophic bacteria, mesophilic spores, coliform, yeast and mould to far beyond the permissible limit, pasteurization however did not fully inactivate the mesophilic spores and coliform while HPP did. Pressure of 600 MPa for holding time of both 5 and 7 min have resulted in very low TPC and psychrotrophic bacteria count which is favourable to prolong the milk shelf life.

It is now confirmed that the pressure above 450 MPa is favourable for milk preservation although better safety margin is given by pressure of 600 MPa. Further research is recommended to determine the exact shelf life (> 22 days) of cow and goat milk treated at 450 MPa and 600 MPa for holding time above 5 minutes with the focus on the TPC and psychrotrophic bacteria count during the storage study. Besides, it is encouraging to understand more about the effects of HPP on the water soluble vitamins, lactose and minerals as the society has slowly to gain concerns regarding the daily healthy food intake. To promote HPP in the current milk industry, more researches are necessary to support the findings and convince the society.

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APPENDICES

A.1 Physico-chemical Properties

A.1.1 Titratable Acidity of Fresh Cow Milk

	Sample	
	A	B
Initial volume of NaOH (ml)	15.10	16.60
Final volume of NaOH (ml)	16.60	18.30
Volume of titration (ml)	1.50	1.70
Titratable acidity (%)	0.15	0.17

For Sample A

$$\text{Titratable acidity (\%)} = \frac{\text{Volume of 0.1N NaOH} \times 0.009}{\text{Volume of sample}} \times 100$$

$$= \frac{1.50 \times 0.009}{9} \times 100$$

$$= 0.15\ \%$$

For Sample B

$$\text{Titratable acidity (\%)} = \frac{\text{Volume of 0.1N NaOH} \times 0.009}{\text{Volume of sample}} \times 100$$

$$= \frac{1.70 \times 0.009}{9} \times 100$$

$$= 0.17\ \%$$

Therefore, the average titratable acidity (%) = $\frac{0.15+0.17}{2} = 0.16\ \%$

A.1.2 Specific Gravity of Fresh Cow Milk

	Sample	
	A	B
Weight of empty pycnometer (g)	25.3860	25.3860
Weight of pycnometer with water (g)	75.3860	75.3860
Weight of pycnometer with milk (g)	76.6750	76.6360
Specific gravity	1.0258	1.0250

For Sample A

$$\text{Specific gravity} = \frac{\text{Weight of milk}}{\text{Weight of water}}$$

$$= \frac{76.6750-25.3860}{75.3860-25.3860}$$

$$= 1.0258$$

For Sample B

$$\text{Specific gravity} = \frac{\text{Weight of milk}}{\text{Weight of water}}$$

$$= \frac{76.6360-25.3860}{75.3860-25.3860}$$

$$= 1.0250$$

Therefore, the average specific gravity $= \frac{1.0258+1.0250}{2} = 1.0254$

A.1.3 Total Protein of Fresh Cow Milk

	Sample	
	A	**B**
Initial volume of NaOH (ml)	8.70	12.50
Final volume of NaOH (ml)	10.50	14.40
Volume of titration (ml)	1.80	1.80
Total protein (%)	3.13	3.13

For Sample A

Total protein (%) $= 1.80 \times 1.74$

$= 3.13$ %

For Sample B

Total protein (%) $= 1.80 \times 1.74$

$= 3.13$ %

Therefore, the average total protein (%) $= \frac{3.13+3.13}{2} = 3.13$ %

A.1.4 Total Solid of Fresh Cow Milk

	Sample	
	A	**B**
Weight of empty dish + lid (g)	52.897	53.014
Weight of milk + dish + lid (g)	55.874	56.322
Weight of milk sample (g)	2.977	3.308
Weight of dried milk + dish + lid (g)	53.244	53.399
Total solid (%)	11.66	11.64

For Sample A

$$\text{Total solid (\%)} = \frac{\text{Weight of dish+dried sample}-\text{Weight of empty dish}}{\text{Weight of sample}} \times 100$$

$$= \frac{53.244-52.897}{2.977} \times 100$$

$$= 11.66\ \%$$

For Sample B

$$\text{Total solid (\%)} = \frac{\text{Weight of dish+dried sample}-\text{Weight of empty dish}}{\text{Weight of sample}} \times 100$$

$$= \frac{53.399-53.014}{3.308} \times 100$$

$$= 11.64\ \%$$

Therefore, the average total solid (%) = $\frac{11.66+11.64}{2}$ = 11.65 %

A.1.5 Total Fat of Fresh Cow Milk

	Sample	
	A	B
Weight of milk sample (g)	10.146	10.012
Weight of flask containing dried fat (g)	107.881	107.858
Weight of flask after fat washing (g)	107.549	107.520
Total fat (%)	3.27	3.38

For Sample A

$$\text{Total fat (\%)} = \frac{\text{Weight of flask containing fat} - \text{weight of flask after fat washing}}{\text{Weight of sample}} \times 100$$

$$= \frac{107.881-107.549}{10.146} \times 100$$

$$= 3.27\ \%$$

For Sample B

$$\text{Total fat (\%)} = \frac{\text{Weight of flask containing fat} - \text{weight of flask after fat washing}}{\text{Weight of sample}} \times 100$$

$$= \frac{107.858-107.520}{10.012} \times 100$$

$$= 3.38\ \%$$

Therefore, the average total fat (%) = $\frac{3.27+3.38}{2}$ = 3.33 %

A.1.6 Solid-non-fat of Fresh Cow Milk

	Sample	
	A	B
Total solid (%)	11.66	11.64
Total fat (%)	3.27	3.38
Solid-non-fat (%)	8.38	8.26

For Sample A

Solid-non-fat (%) = 11.66 − 3.27

= 8.38 %

For Sample B

Solid-non-fat (%) = 11.64 − 3.38

= 8.26 %

Therefore, the average solid-non-fat (%) = $\frac{8.38+8.26}{2}$ = 8.32 %

A.2 Microbiological Properties

A.2.1 *Listeria monocytogenes* and *Salmonella spp.* during 22 Days of Storage

Treatment	**Cow Milk**			**Goat Milk**		
	Day 0	**Day 11**	**Day 22**	**Day 0**	**Day 11**	**Day 22**
Listeria monocytogenes per 25g						
450MPa / 7min	Abs	Abs	Abs	Abs	Abs	Abs
600MPa / 5min	Abs	Abs	Abs	Abs	Abs	Abs
600MPa / 7min	Abs	Abs	Abs	Abs	Abs	Abs
Pasteurized	Abs	Abs	Abs	Abs	Abs	Abs
Salmonella spp. per 25g						
450MPa / 7min	Abs	Abs	Abs	Abs	Abs	Abs
600MPa / 5min	Abs	Abs	Abs	Abs	Abs	Abs
600MPa / 7min	Abs	Abs	Abs	Abs	Abs	Abs
Pasteurized	Abs	Abs	Abs	Abs	Abs	Abs

A.2.2 *Bacillus cereus*, *Clostridium perfringens* and *Staphylococcus aureus* Counts during 22 Days of Storage

Treatment	**Cow Milk**			**Goat Milk**		
	Day 0	**Day 11**	**Day 22**	**Day 0**	**Day 11**	**Day 22**
Bacillus cereus (CFU/ml)						
450MPa / 7min	20	<1	<1	<1	<1	<1
600MPa / 5min	6	<1	<1	<1	<1	<1
600MPa / 7min	4	<1	<1	<1	<1	<1
Pasteurized	8	<1	<1	<1	<1	<1
Clostridium perfringens (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	<1	<1	<1	<1	<1	<1
Staphylococcus aureus (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	<1	<1	<1	<1	<1	<1

A.2.3 Total *Escherichia coli*, Coliform, Yeast and Mould during 22 Days of Storage

Treatment	**Cow Milk**			**Goat Milk**		
	Day 0	**Day 11**	**Day 22**	**Day 0**	**Day 11**	**Day 22**
Total Escherichia coli Count (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	<1	<1	<1	<1	<1	<1
Total Coliform Count (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	<1	<1	<1	26	<1	<1
Total Yeast and Mould Count (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	<1	<1	<1	<1	<1	<1

A.2.4 Mesophilic and Thermophilic Aerobic Spore Counts during 22 Days of Storage

Treatment	Cow Milk			Goat Milk		
	Day 0	Day 11	Day 22	Day 0	Day 11	Day 22
Mesophilic Aerobic Spore Count (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	<1	<1	<1	<1	<1	<1
Thermophilic Aerobic Spore Count (CFU/ml)						
450MPa / 7min	<1	<1	<1	<1	<1	<1
600MPa / 5min	<1	<1	<1	<1	<1	<1
600MPa / 7min	<1	<1	<1	<1	<1	<1
Pasteurized	20	<1	<1	<1	<1	<1