UNIVERSITI PUTRA MALAYSIA

HIGH PRESSURE PROCESSING AND THERMAL TREATMENT ON GOAT MILK'S QUALITY

SANGITHA NARAYANAN

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SANGITHA NARAYANAN
180338

BACHELOR OF ENGINEERING (PROCESS AND FOOD)
FACULTY OF ENGINEERING
UNIVERSITY PUTRA MALAYSIA
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ABSTRACT

Goat milk spoilage in shorter period of time, an on-going problem of high economy impact to the goat milk industry, is caused by insufficient treatment that exposed to bacterial spoilage. With the increasing demand for high value-added products from goat milk, there is an interest to study non-thermal pasteurization technologies for goat milk; namely High Pressure Processing (HPP).

This research investigated goat milk quality processed by thermal treatment and HPP. Quality assessments before and after processing and during storage were carried out for goat milk -colour properties, rheological properties, total soluble solids, pH and microbiological analyses.

Room temperature HPP processed goat milk at 600MPa for 10 min resulted in 19% bacterial colonies' reduction compared to thermal treatment after storage period of 1 day. When comparing the same process with milk stored at refrigerated condition, HPP processed goat milk showed lower number of bacterial colonies than thermal processed milk which observed bacterial inactivation as more efficient by a HPP process. Moreover, HPP processed goat milk also retained a stable viscosity and total soluble solids than thermal treated milk which declines during extensive heat treatment and storage period. HPP processed (200MPa, 10 min) of goat milk gives lower total colour difference (TCD) than thermal processed milk at (80°C, 10 min) due to brightness factor and (b*) value that changes according to types of treatment.

For processed goat milk, better nutritional preservation and quality was observed from HPP process. HPP processing produced a 30 days' shelf-stable goat milk for usage.

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CHAPTER 1

Introduction

Chapter 1: INTRODUCTION

Consumers worldwide currently more concerning and demanding for quality and safety food products; especially those regarded with healthy lifestyle that maintains the nutritional content of the product. These products should retain its original characteristics and functional properties as flavor, texture, color, nutrient, aroma and offer an extended shelf life (Ozcan et al., 2017; Trujillo et al., 2002). Minimally processed additive –free foods are increasingly attractive to the consumer due to this reason; which is where there is more exploration of physical treatments besides traditional heat treatments (Smelt, 1998). Challenges faced by food processors is to ensure minimally processed foods have the same food attributes and at the same time safe and healthy to be consumed since it is difficult to get rid of microorganisms without addition of high temperature or chemical preservatives.

Thermal processing is one of the most conventional methods used in dairy industries; often referred to sterilization or pasteurization. Inactivation of pathogenic bacteria is one of the main things in dairy milk that relates to the quality of pasteurization process. Heat treatments are still the common method used for inactivating food spoilage and pathogenic bacteria in raw milk (Messens, 2003). Temperatures in between 60 to 85°C are known to inactivate most of the bacterial pathogen present in milk (ACMS, 2007). However, heat applied in thermal processing negatively affects the quality of milk by degrading most of the biochemical components such as vitamin B, lactoferrin and other antioxidant (Da Costa et al., 2003; Silvestre et al., 2008).

Food industry has improvise the conventional heat treatment preservation method by exploring into aseptic packaging systems and HTST (high temperature short time) or UHT (ultra-high temperature) treatments (Ozcan et al., 2017). Various high pressure processed foods have been launched as fruit juices, rice cakes, and raw squid in Japan (Smelt, 1998). However, high pressure processed dairy products as milk, yoghurt and cheese has not been marketed commercially even though various research and studies has been done regarding this (Messens, 2003). Several studies and research have shown that non-thermal alone or in combination with mild thermal processing inactivate microbial and enzyme activity, and prolong shelf life of milk. A detailed review on this topic has been carried out and is presented in Chapter 1. Given the variability of quality evaluation among different types of milk, goat milk has been chosen to be studied in this research, which focuses on quality evaluation by thermal treatment and high pressure processing.

1.1. Thesis objectives

The foundation of this study aims the preservation of goat milk by HPP processing in comparison with the conventional thermal processing. The main focus was the inactivation of the alkaline phosphatase enzyme in goat milk, but the quality of the goat milk was also assessed. The specific objectives of this research were as follows:

i. To evaluate the quality of HPP and thermal treated goat milk in terms of physical, chemical and microbiological properties.
ii. To study the effect of chilled and room temperature storage on HPP and thermally processed goat milk.

1.2. Thesis framework

The thesis contains five chapters. First chapter already explained the introduction of the research. Chapter 2 is literature review focused on effect of different preservation technologies (HPP and thermal treatment) on the quality of preserved goat milk in terms of physical, chemical and microbiological properties. Chapter 3 studies the methodology steps for studying the effect of thermal processing and HPP method on goat milk preservation. The results obtained from the effect of HPP and thermal processing on quality evaluation is described in Chapter 4. Finally, Chapter 5 concludes the research based on detail explanations from Chapter 4.

CHAPTER 2

Literature Review

Chapter 2: LITERATURE REVIEW

2.1. Goat milk

Total goat population of Asia and Africa accounts 91.5% of the world's total goat population. Goat milk production accounts 2.2% of total world milk production (Zervas & Tsiplakou, 2011). 59% of world goat milk production was from Asia based on Food and Agriculture Organization data for 2009 (Joe Stout, 2011). Around 480 million goats worldwide provide more than 5 million tons of milk which account to the world goat milk production (Jandal, 1996).

Table 2.1-1: World numbers of mammalian farm animals (million) since 1980 and annual milk production

	1980 (1, 000 MT)	1999 (1, 000 MT)	Change (%)
Animal numbers			
Goats	458	710	+55
Buffaloes	122	159	+30
Pigs	796	913	+15
Cattle	1216	1338	+10
Sheep	1096	1069	-3
Milk production			
Goats	7720	12161	+58
Buffaloes	44296	60334	+36
Cattle	423034	480659	+14
Sheep	7887	8026	+2

*Data from FAO (2001)

2.1.1. Properties and Characteristics of Goat Milk

2.1.1.1. Composition and characteristics of goat milk

Goat milk have better digestibility, alkalinity, buffering capacity, and certain therapeutic values in medicine and human nutrition differs from cow or human milk (Park, 2007). Goat milk composition vary with diet, breed, individuals, parity, season, feeding, management, environmental conditions, locality, stage of lactation, and health status of the udder (Park, 2007). Table 2.1-2 below shows the average composition of milk from goats, sheep, cows and humans.

Table 2.1-2: Average composition of basic nutrients in goat, sheep, cow and human milk

Composition	**Goat**	**Sheep**	**Cow**	**Human**
Fat (%)	3.8	7.9	3.6	4.0
Solids-non-fat (%)	8.9	12.0	9.0	8.9
Lactose (%)	4.1	4.9	4.7	6.9
Protein (%)	3.4	6.2	3.2	1.2
Casein (%)	2.4	4.2	2.6	0.4
Albumin, globulin (%)	0.6	1.0	0.6	0.7
Non-protein N (%)	0.4	0.8	0.2	0.5
Ash (%)	0.8	0.9	0.7	0.3
Vitamin A (IU g^{-1} fat)	39	25	21	32
Vitamin B_1(mg/100mL)	68	7	45	17
Vitamin B_{12} (mg/100mL)	210	36	159	26
Vitamin C (mg/100mL)	20	43	2	3.60
Vitamin D (IU g^{-1} fat)	0.70	ND	0.70	0.27
Energy (Cal./100mL)	70	105	69	68

*Data from (Saini and Gill, 1991; Posati and Orr, 1976; Larson and Smith, 1974; Jenness, 1980; Haenlein and Cassese, 1984; Jandal, 1996; Park, 2007).

*ND: not detected.

From Table 2.1-2, goat milk contains major nutrient contents than sheep and cow milk. Non-protein nitrogen (NPN) content in goat and human milk is much higher than in cow milk; which is why goat milk has been identified as healthy milk (Slacanac et al., 2010; Joe Stout, 2011).

The fat content in goat milk, percentage wise, is almost similar to cow milk, but differs in physical and chemical structures. The size of fat globules in goat milk is smaller compared with cow milk; which results in softer texture of goat milk products. Apart of this, smaller fat globules size of goat milk are better homogenized; which is better for human digestion by lipases due to high surface area of fat (Joe Stout, 2011).

The digestibility of goat milk is due to the size of casein which is smaller and softer than that produced by cow milk (Yadav et al., 2016). This causes goat milk to be more easily digestible and often suitable for infant (Park, 2007). Other than that, goat milk proteins are more easy to be digested than milk protein of cow milk due to high levels of α_{s2}-casein in goat milk (Haenlein, 2004). Higher proportion of β-casein in goat milk and lower or relative absence of α_{s1}-casein make goat milk closer to human milk; which is why it is considered healthier and nutritious (Joe Stout, 2011). Table 2.1-3 below shows the composition of casein in goat and cow milk.

Table 2.1-3: Comparison of casein fractions (%) in goat and cow milk

	Goat milk	**Cow milk**
α_{s1}-casein	0-28.0	50.0-53.6
α_{s2}-casein	10.0-25.0	12.5-14.3
β-casein	0.6-64.0	37.5-39.3
κ-casein	15.0-29.0	8.3-14.3

*Data from (Yadav et al., 2016).

2.1.1.2. Physicochemical properties of goat milk

Unlike cow milk which is yellowish because of the presence of carotene, goat milk is white in colour (Saini and Gill, 1991). According to Babayan (1981) and Haenlein (1993), goat milk has stronger flavor due to liberation of short – chain fatty acids which give off a goaty smell unlike sheep milk. Goat milk also has alkaline properties due to the different arrangement of phosphates and higher protein content unlike cow milk which is slightly acidic (Saini and Gill, 1991). Table 2.1-4 below shows the difference of goat, sheep and milk physical properties.

Table 2.1-4: Physical properties of goat, sheep and cow milk

Properties	**Goat milk**	**Sheep milk**	**Cow milk**
Density	1.029-1.039	1.0347-1.0384	1.0231-1.0398
Viscosity (cP)	20.12	20.86-30.93	20.0
Surface tension (Dynes/cm)	52.0	44.94-48.70	42.3-52.1
Conductivity ($\Omega^{-1}cm^{-1}$)	0.0043-0.0139	0.0038	0.0040-0.0055
Refractive index	1.450 ± 0.39	1.3492-1.3497	1.451 ± 0.35
Freezing point (-°C)	0.540-0.573	0.570	0.530-0.570
Acidity (lactic acid %)	0.14-0.23	0.22-0.25	0.15-0.18
pH	6.50-6.80	6.51-6.85	6.65-6.71

*Data from (Park, 2007).

From Table 2.1-4, goat milk has almost comparable density to cow milk, but is lower than sheep milk; while both have higher viscosity and acidity. Goat milk has however lower refractive index and pH than cow milk even has greater freezing point compared to cow milk (Parkash and Jenness, 1968; Park, 2007).

2.1.1.3. Health Benefits

Goat milk has few composition and elements in it which contributes to health benefits. It is often prescribed by doctors for children and infants who are sensitive and allergic to cow milk (Saini and Gill, 1991). Goat milk also contains lactose similar to cow and human milk; however goat milk can be consumed by lactose intolerant people (Yadav et al., 2016). This is due to the digestibility of the goat milk; where it can be easily absorbed by the digestive system compared to cow milk (Haenlein, 2004). Some studies mentioned that goat milk may be suitable to be consumed because the patients might not be lactose intolerance, but instead allergic to milk proteins in cow milk; which is usually lower or absent in goat milk (Joe Stout, 2011; Yadav et al., 2016). The good digestibility properties of goat milk, composition of fatty acids and the content of bioactive compounds suitable for medical treatment; which is further shown in Table 2.1-5 regarding the health benefits of goat milk from various studies.

Table 2.1-5: Summary of studies on the health benefits of goat milk.

Composition in goat milk	**Health benefits**	**Results**	**Reference**
Lactoperoxidase	Antimicrobial properties	Effective against microbial disease; eg: cholera, typhoid, pneumonia, dysentery and food poisoning	Yadav et al. (2016)
Oligosaccharides	Anti-inflammatory bowel effect	• Prevent decrease in body weight. • Increase of colon size. • Extension of necrotic lesions. • Decrease diarrhea.	Yadav et al. (2016) Lara Villoslada et al. (2006)
α_{s1}-casein	Alleviation of lactose intolerance, prevention of milk allergy.	Tolerable for people allergic to cow milk.	Haenlein (2004) Yadav Singh, & Yadav (2016) Saini and Gill (1991) Joe Stout (2011)
Medium chain triglycerides (MCT)	Prevent cardiovascular disease	Lower cholesterol level by inhibiting cholesterol deposition in the tissues. Act as anti atherogenic.	Yadav, Singh, & Yadav (2016)

2.2. Thermal processing/pasteurization of milk

Conventional thermal treatment is one of the established methods applied for food processing, because of the ability to kill pathogens and inactivate potentially detrimental enzymes (Giribaldi et al., 2016). During thermal processing, most microorganisms and enzymes are inactivated. Pasteurization is considered effective in controlling pathogenic microorganisms resulted in raw milk as *Staphylococcus aureus, Campylobacter jejuni, Salmonella* spp, *Escherichia coli* and *Yersinia enterocolitica*, and spore formers such as *Bacillus cereus* and *Clostridium* spp (Lewis, 2010). The enzymes are denatured permanently when high temperature is applied which leads to the inactivation (Satin, 1996).

The most common method used to inactivate microorganisms in milk is known as pasteurization as it ensures the milk is safe to be consumed (Arslanoglu et al., 2010; Lewis, 2010). Pasteurization process is targeted to achieve 5- log reduction in viable microorganisms (Goddik and Sandra, 2011). Thus, there is higher chances for inactivation of Campylobacter and Salmonella that have been reported to cause food poisoning outbreaks in milk apart from reducing acid-producing spoilage bacteria as E.coli through pasteurization process (Lewis, 2010). Pasteurization process of milk applies the concept of high temperature, short time (HTST) process; which uses continuous heat processing with combination of separation, standardization, and homogenization process (Goddik and Sandra, 2011).

However, thermal processing particularly under severe conditions may cause physical and chemical change that alter the organoleptic properties as flavor, taste, water-soluble vitamins and texture (Goddik and Sandra, 2011; McAuley, Singh, Haro-Maza, Williams, & Buckow, 2016; Arslanoglu et al., 2013; Tully, Jones and Tully, 2001; Giribaldi et al., 2016)

Much research on thermal processing of milk has been carried out. The conclusion that can be reached from a review of the literature is that the physicochemical properties of milk largely dependent on the thermal component of the treatment (McAuley et al., 2016). Table 2.2-1 summarizes the representative works on thermal processing of milk.

Table 2.2-1: Summary of representative studies on the effect of different treatment on milk.

Treatment	Condition	Treatment time	Results	Reference
Low temperature, long time (LTLT)	72°C	15 s	• Increase in lightness; L*, a* value and b* value. • pH > 6.0 when stored at 8°C even after two weeks.	McAuley, Singh, Haro-Maza, Williams, & Buckow (2016)
Low temperature, long time (LTLT)	63°C	15 s	• Decrease in lightness; L* and b* value. Increase in a* value. • pH < 6.0 when stored at 8°C after two weeks.	
Low temperature, long time (LTLT)	80°C	3 min	• pH is about 4.20 when stored at 4°C after 30 days. • Total soluble solid is about 12.3 °Brix when stored at 4°C after 30 days.	Andrés, Villanueva, & Tenorio (2016)
HPP	600 MPa / 20°C	3 min	• pH is about 4.15 when stored at	

			4°C after 30 days. • Total soluble solid is about 12.2 °Brix when stored at 4°C after 30 days.	
Pasteurized	65°C	30 min	• pH is about 7.19 when stored at 4°C after 15 days.	Giacometti et al. (2016)
Pasteurized plus HPP	Pasteurize at 65°C for 30 min, then HPP at 400 MPa for 100 s.		• pH is about 7.42 when stored at 4°C after 15 days.	

2.3. High pressure processing (HPP) of milk

High pressure processing (HPP) is one of the non-thermal food processing method that has caught consumers interest since the nutritional and sensory qualities of foods as nutrient retention, flavor and colour are not affected by pressure process (Mussa & Ramaswamy, 1997). HPP is also known as ultra - high pressure processing or isostatic processing (Ozcan et al., 2017). It accomplished the use of isostatic non-thermal pressure chamber which is filled with hydraulic fluid, usually water at typical pressures ranging from 200 to 600 MPa (Okpala et al., 2009; Ozcan et al., 2017). HPP acts equally throughout a mass of food regardless of its shape, size and composition by providing uniform and instantaneous effect without heating and can be performed at ambient temperature (Patterson, 2005; Ozcan et al., 2017).

HPP disrupts the non-covalent interaction of proteins by modifying the macro-components; which cause minimal changes to food characteristics and quality (Huppertz et al., 2002). HPP can be used alone to preserve food or in combination with other methods; as thermal to improve the desired effects (Thakur and Nelson, 1998; Rastogi et al., 2007; Evelyn & Silva, 2015). HPP is used to stabilize milk, modification of milk constituents, microorganisms inactivation, water freezing point depression, and recovery of proteins and lactose (Ozcan et al., 2017).

Unlike pasteurization process which cause conformational changes in milk proteins, HPP does not affect the primary structure of protein ; where protein still remain intact during high pressure treatment. HPP also denatures non-casein nitrogen in milk serum with increase in pressure (Ozcan et al., 2017). According to Stapelfeldt e al. (1996), HPP enhances protein digestibility due to the changes in conformation of protein reversed slowly after decompression. This denaturation of protein under pressure associated with the hydrolysis of β-lactoglobulin by pepsin and trypsin (Messens, 2003; Ozcan et al., 2017). Furthermore, Sierra et al. (2000) found no loss in vitamin B1 and B6 due to high pressure processing of milk.

However, application of HPP in milk causes loss of desirable organoleptic properties as texture, colour, and flavor. Needs et al. (2000) stated that milk subjected to HPP treatment turns to yellowish due to reduction in size of casein micelles.

Much research on high pressure processing of milk has been carried out. The conclusion that can be reached from a review of the literature is that high pressure processing of milk may be an alternative for extending the shelf-life with quality advantage (Mussa & Ramaswamy, 1997). Microbiological quantity of raw milk pressurized at 400-600 MPa when compared with pasteurized milk of 72°C at 15 s is more efficient in destruction of spores (Mussa & Ramaswamy, 1997). Table 2.3-1 summarizes the representative works on high pressure processing of milk.

Table 2.3-1: Summary of representative studies on the effect of high pressure processing on milk.

Media	Treatment	Treatment time	Results	References
Goat milk	HPP - 500 MPa / 25°C	4 min	• Decrease amount of whey protein at pH 4.6. • Decrease in amount of β-lactoglobulin due to denaturation. • Reduce growth of microorganisms.	Felipe, Capellas, & Law (1997)
Goat milk	HPP - 350 MPa / 45°C	10 min	• Disintegrate of casein micelles to smaller fragments. • Formation of large casein micelles. • Increase level of serum κ-casein. • Inactivation of microorganisms	Horne and Parker (1982); Zadow et al. (1994); Law et al. (1998); Gervilla et al. (1996)
Milk smoothies	HPP – 450 MPa / 20°C	3 min	• pH of milk is about 4.16 when stored at 4°C after 45 days. • Total soluble solid is about 12.4 °Brix when stored at 4°C after 30 days.	Andrés, Villanueva, & Tenorio (2016)
Milk smoothies	HPP – 600 MPa / 20°C	3 min	• pH of milk is about 4.15 when stored at 4°C after 45 days. • Total soluble solid is about 12.3 °Brix	

			when stored at 4°C after 30 days.	
Cow milk	HPP – 122 MPa / 13°C	83 min	- Destruct microbial vegetative cells in milk	Johnston (1995); (Messens, 2003)
Cow milk	HPP – 400 MPa	20 min	- Disintegration of casein micelles into smaller fragments. - Slight decrease in lightness (L) - values. - 21% increase in viscosity.	Johnston et al. (1992); Mussa & Ramaswamy (1997); Messens (2003)

2.5. Quality of processed milk during storage

More research has been done in extending the shelf-life of pasteurized milk without inducing a cooked flavor. Few factors influence the quality of processed milk during storage; namely raw milk quality, heat treatment conditions, post-processing contamination, and storage temperature (Borde-Lekona et al., 2005).

Short exposure about 15 s to temperature of 72°C shows much more good quality of milk than milk heated for 15s at 80°C due to the heat shocking of spores' activity that reduces milk quality (Gomez Barroso, 1997; Barrett et al. 1999). However, another literature shown that processing time of 2 s at 115°C increase the milk quality more than processing for 15 s at 72°C due to the concept of high temperature, short time processing which did not induce a cooked flavor of milk (Borde-Lekona et al., 2005). In industrial application, it is desirable to pasteurize milk at high temperature for short time in order to preserve the quality and at the same time most efficient condition to inactivate enzyme and microorganisms.

High pressure processing is proven to provide more high quality of processed milk which increases the keeping quality of milk compared to conventional pasteurization process since it does not induce a cooked flavor and at the same time extends the shelf life of milk (Andrés et al., 2016). In general, the quality of processed milk during storage can be preserved when the storage temperature is much lower. Experiments confirmed that pasteurized milk could be stored for up to 20 days at 8°C, between 30 to 40 days at 4°C and for more than 60 days at 2°C (Lewis, 2010). Studies on the processed milk shelf life after thermal and HPP treatment is summarized in Table 2.5-1.

Table 2.5-1: Summary of representative studies on the quality of processed milk during storage.

Treatment	Condition	Treatment time	Results	Reference
Low temperature, long time (LTLT)	72°C	15 s	Milk stored at 10°C became unacceptable after 15 days, but milk stored at 2°C was still acceptable after 22 days.	Borde-Lekona et al. (2005)
High temperature, short time (HTST)	115°C	2 s	Milk stored both at 10°C and 2°C was still acceptable after 22 days.	
High temperature, short time (HTST)	115°C	2 s	Milk has high keeping quality and can be stored for longer period of time.	Lewis (2010); Wirjantoro and Lewis (1996)
Low temperature, long time (LTLT)	72°C	15 s	Milk has low keeping quality and can only be stored for short period of time.	
Low temperature, long time (LTLT)	72°C	15 s	Milk stored at 4°C become unacceptable after 10-14 days, but milk stored at 8°C become unacceptable after 7-10 days.	McAuley, Singh, Haro-Maza, Williams, & Buckow (2016); Lewis (2010)
Low temperature, long time (LTLT)	63°C	15 s	Milk stored at 4°C become unacceptable after 6-7 days, but milk stored at 8°C become unacceptable after 3-4 days.	

Pasteurized	65°C	30 min	Milk stored at 4°C and 12°C become unacceptable after 15 days.	Giacometti et al. (2016)
Pasteurized plus HPP	Pasteurize at 65°C for 30 min, then HPP at 400 MPa for 100 s.		Milk stored at 4°C and 12°C still acceptable after 30 days.	
Low temperature, long time (LTLT)	80°C	3 min	Milk stored at 4°C still acceptable after 45 days.	Andrés, Villanueva, & Tenorio (2016)
HPP	450 MPa / 20°C	3 min	Milk stored at 4°C still acceptable after 45 days but with reduction of microorganisms.	

CHAPTER 3

Methodology

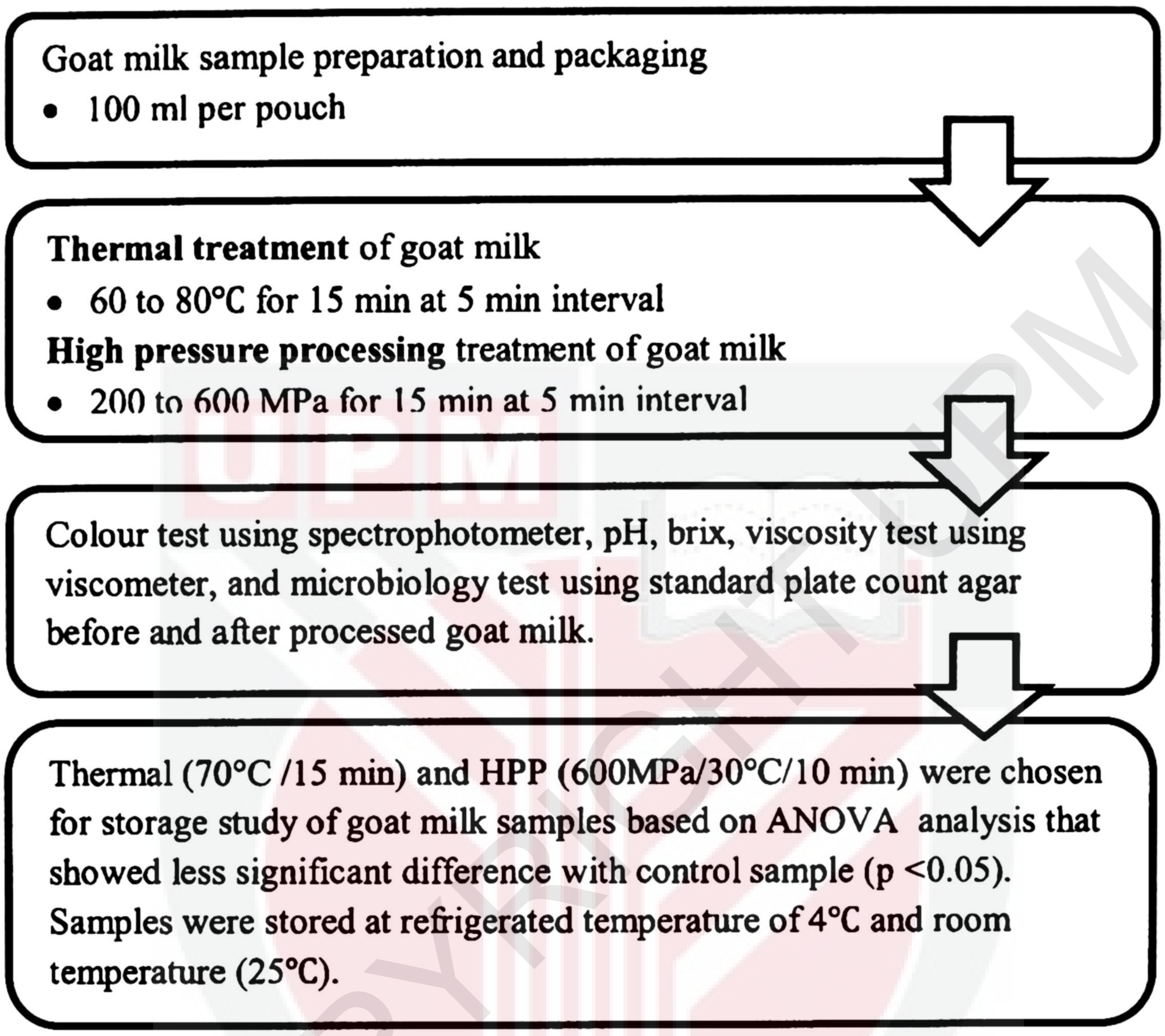
Chapter 3: METHODOLOGY

The effectiveness of different processing technologies (HPP treatment and thermal treatment) on goat milk was determined based on the inactivation of microorganisms which acts as an indicator for successful pasteurization. Impact of High Pressure Processing (HPP) process on goat's milk quality was assessed by functional and physiochemical properties which acts as indicator of the effectiveness of HPP treatment in comparison with conventional thermal treatment. These properties can be expressed by kinetic behaviors that are represented by graphical representation.

3.1. Experimental design

Goat milk samples were brought from a local farm and packed in a quantity of 100ml per pouch. These milk samples were thermal treated 30 min in interval of 5 min at temperature of 60 to 80°C. Goat milk samples also tested for high pressure processing treatment at same time of interval for pressure of 200 to 600MPa which is set at constant room temperature. The optimum pressure based on the high pressure processing earlier was chosen in order to study the effect on both high pressures processing with thermal treatment method. The optimum pressure was varied with different temperatures to identify the optimum temperature where the inactivation of pathogenic bacteria is effective. Enough samples were processed to allow triplication in the quality analyses.

The colour, viscosity, pH, brix, and total sugar content were also analyzed for each different processing method and condition. Three replicates of thermal and high pressure processed samples were analyzed immediately after processing. From those two conditions, samples which were obtained at the optimum condition were stored in chill condition and room temperature for shelf life analysis. Samples were analyzed its quality analyses after 1, 5, 10, 15 and 30 days of storage.



Goat milk sample preparation and packaging

- 100 ml per pouch

Thermal treatment of goat milk

- 60 to 80°C for 15 min at 5 min interval

High pressure processing treatment of goat milk

- 200 to 600 MPa for 15 min at 5 min interval

Colour test using spectrophotometer, pH, brix, viscosity test using viscometer, and microbiology test using standard plate count agar before and after processed goat milk.

Thermal (70°C /15 min) and HPP (600MPa/30°C/10 min) were chosen for storage study of goat milk samples based on ANOVA analysis that showed less significant difference with control sample ($p < 0.05$). Samples were stored at refrigerated temperature of 4°C and room temperature (25°C).

Figure 3.1-1: Experimental design process flow chart

3.2. Preparation of Goat Milk

Fresh raw goat milk was obtained from Mizag Agrofarm situated in Semenyih, Selangor. The fresh goat milk was filled in bottles without being pasteurized and stored at refrigerated condition (4°C) prior to delivery. Milk samples obtained were filled in food grade Quiware vacuum bag (embossed) composed of polyethylene with outer layer of nylon for added strength and rigidity. These vacuum bag can withstand high temperatures and pressure; being suitable for thermal processing and high pressure applications. 100m*l* of goat milk sample was packed in vacuum bag for thermal and

HPP experiments. Packed milk samples were processed for each processing condition (pressure, temperature, time) and the properties of milk was analyzed. Untreated milk samples were stored in refrigerated condition at < -18°C. It will be then applied thermal or pressure treatment to treat the milk each time before use.

3.3. Thermal Processing of Goat Milk

Thermal treatment of milk samples in the absence of high pressure were performed at mild temperature of 60°C, 70°C and 80°C for 5, 10 and 15 min using a thermostatic water bath. This type of pasteurization is also known as batch pasteurization; where milk is heated in a large water bath and held at a specific temperature. Generally, pasteurization of milk is conducted at 63°C for 30 min or heating at 70-80°C for not more than 15 min (Thinley, 2015). However, higher temperature of pasteurization for longer holding time is purposely studied in this research to compare the effectiveness with high pressure pasteurization being performed at longer holding time. For this purpose of study, the pasteurization temperatures selected are different than conventional pasteurization of milk that has been researched previously; thus the quality analysis that might be obtained may differ than other research study.

3.4. High Pressure Processing (HPP) of Goat Milk

Pressures of 200 MPa, 400 MPa and 600 MPa were tested for 5, 10 and 15 min without being applied heat process. The three different extreme pressures were selected to observe the effect of high pressure processing on goat milk quality. The vacuum bag with milk samples were introduced into the HPP Laboratory Food Processing System using distilled water as the pressure medium in the pressure temperature. The temperature was indicated by the thermocouple which was equipped with the HPP chamber during the HPP cycle. It recorded the temperature of pressurization medium; which is water in the chamber at constant pressure phase that describes the relationship of pressure-temperature-time processing conditions.

3.5. Microbiology

Plate count agar (PCA) was used for the enumeration of microorganisms employing a pour plate method. Plate count agar obtained from Sigma Chemicals suspended 17.5g in 1 liter distilled water, brought to boiled, stirred and mixed at speed 1300 rpm and sterilized by autoclaving at 121°C for 15 min. This plate count agar is then poured on petri dish and stored in chiller for the use of spread count later on. Samples of raw goat milk, thermal treated goat milk and high pressure processed goat milk were made into series of milk sample dilutions until 10 degree of dilution factors. 0.1m*l* of the diluted milk samples is poured on plate count agar that has been plated on petri dish and spread evenly. The petri dishes containing the samples are then incubated at 37°C for 36hrs. The colonies are then counted and results are reported. Microbial count is performed by dividing the no. of colonies counted with the dilution factor and multiplying it with the volume of culture plate in order to obtain the colonies in unit CFU/ml. This value is then converted into log CFU/ml to be interpreted in graph.

3.6. Colour

Colour characteristics of goat milk were assessed by the CIE L* a* b* method using Hunter Lab spectrophotometer. The goat milk sample was place in a clear plastic container. The colour coordinates of L*, a* and b* values were measured from the sample. The L-value as used as a measure of lightness and ranges between 0 (black) and

100 (white); measures how light or dark is the colour of the sample. Positive or negative increases of a-value correspond to increase in red or green colour proportions. The b-value represents colour ranging from yellow (+) to blue (-) (Kneifel, Ulberth, & Schaffer, 1992). The total colour difference (TCD) which is the overall colour difference of the processed goat milk sample (L^*, a^*, b^*) when compared to raw goat milk sample (L_0^*, a_0^*, b_0^*) was calculated using Eq. 2.5 (Silva & Silva, 1999):

$$\text{TCD} = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad (2.5)$$

Following classification of TCD was used as additional guidelines for colour grading (Drlange, 1994): 0.0 - 0.2: not perceptible, 0.2 – 0.5: very small, 0.5 – 1.5: small, 1.5 – 3.0: distinct, 3.0 – 6.0: very distinct, 6.0 – 12.0: great and >12: very great difference.

3.7. Rheological

Rheological properties of goat milk samples were evaluated using a rotational viscometer. Approximately 200 mL of the goat milk sample was placed in a small beaker and the rotational viscometer is placed in it and subjected to shear rate. The viscosity values were obtained as the slope from the graph of shear stress against shear rate (Mussa & Ramaswamy, 1997).

3.8. Total soluble solids (°Brix)

The total soluble solids value (°brix) of goat milk samples was determined by using refractometer at 25°C. Distilled water was used to wash the containment before filling the sample in it.

3.9. pH

The pH of goat milk samples was measured using pH meter. The pH meter was calibrated using pH 4 and pH 7 as buffer solutions before testing the pH of samples. Approximately 10 mL of the goat milk sample was placed in a small beaker and stirred manually to ensure the uniformity of the sample, pH was measured at 25°C.

3.9. Storage Study

Shelf-life studies were conducted on thermally treated milk samples and HPP treated milk samples. Thermal treated goat milk samples at temperature 70°C for 15 min and high pressure processed goat milk at pressure 600MPa for 10 min were selected as the optimum condition for each types of treatment for storage study. These specific processing parameters were selected based on the ANOVA analysis results which showed the less significant difference when compared with raw unprocessed milk for all the analyses done with ($p < 0.05$). The treated milk samples were stored in refrigerated condition (4°C) and room temperature (25°C) and evaluated at day 0, 1, 5, 10, 15, and 30 by quality analyses. Milk samples were also heated in boiling water for 10 min to observe any sign of it has been spoilt by observing the signs for coagulation or clotting (Mussa & Ramaswamy, 1997). This indicates whether the milk is safe to be consumed.

3.10. Statistical Analysis

For each parameter analyzed, analysis of variance (ANOVA) (Statistics 12, Statsoft ®, USA) was applied to compare the results for different treatments after processing and storage periods. Statistical significance was set at $p < 0.05$. Results from ANOVA analysis for all the parameters analysed were compared with control sample (raw unprocessed goat milk) and labelled with different alphabets in ascending order according to the one which showed less significant difference of $p < 0.05$. Those alphabets that were indicated in tables and graphs helped to show clear comparison with the control sample.

CHAPTER 4

Results and Discussion

Chapter 4: RESULTS & DISCUSSION

4.1. Effect of different processing methods on goat milk microbial quality

4.1.1. Microbial quality of thermal processed goat milk

The total initial number of aerobic and mesophilic microorganisms in raw unprocessed goat milk sample was 4.07 log CFU/ml. Thermal treatments at 60°C for 5 min reduced the initial total microorganisms significantly ($p< 0.05$) by 2 log CFU/ml. Similarly, thermal treated milk samples at 72°C for 15s showed $\log_{10}$ reduction (21.9%) after processing of raw milk (McAuley et al., 2016). No significant increase in microbial counts were found when further increase in temperature and processing time; which showed complete inactivation of bacteria already occurred before this stage according to Table 4.1-1. Sharma et al. (2017) reported that even pre-heating of raw milk at 55°C for 24s sufficient enough to reduce the numbers of *E.coli* significantly ($p< 0.05$) by 3.4 log CFU/ml.

4.1.2. Microbial quality of HPP processed goat milk

For high pressure processing of goat milk samples, 4.04 log CFU/ml was obtained at 200MPa for 5 min process with log reduction (0.03 log CFU/ml). Microbial inactivation due to pressure has been reported due to mechanisms of HPP that targeted cytoplasmic membrane of microorganisms and starts crystallization of membrane phospholipids causing microbial inactivation; which weakened the microorganisms and unable it to grow (Okpala et al., 2009; Yuste et al., 2001; Lado & Yousef, 2002). However, log reduction of about 1.57 log CFU/ml was observed with total plate count of 2.50 log CFU/ml for pressure treated milk at (200MPa, 15 min) as observed in Appendix A. According to Mussa & Ramaswamy (1997), reduction in microbial counts can also be achieved by treating the milk at lower operating pressure for a longer treatment time. The effect of increasing pressure from 200 to 400MPa showed that inactivation of microorganisms for milk samples of a HPP process (400MPa, 5 min) was more effective compared to one exerted lower pressure treatment. Destruction of microorganisms were more effective at higher operating pressure levels compared to one treated at lower pressure (Andrés et al., 2016; Evelyn & Silva, 2015; Mussa & Ramaswamy, 1997). Evelyn & Silva (2015) reported that 4.8 log reductions were obtained at 600MPa compared to 3.6 log at 200MPa. Besides, Okpala et al. (2009) stated that high pressure greater than 500MPa for 10 min at 20°C demonstrated significant reduction of *L.monocytogenes* present in raw milk.

Table 4.1-1: Microbial counts in goat milk after thermal and HPP processing*.

Treatment	Processing conditions	Microbial count ±SD (log CFU/ml)	
		Raw unprocessed	After processing
Thermal	5 min, 60°C	4.07±0.0	2.00±0.0
	10 min, 60°C		ND
	15 min, 60°C		ND
	5 min, 70°C		ND
	10 min, 70°C		ND
	15 min, 70°C		ND
	5 min, 80°C		ND
	10 min, 80°C		ND
	15 min, 80°C		ND
HPP	5 min, 200MPa		4.04±0.008
	10 min, 200MPa		2.60±0.0
	15 min, 200MPa		2.50±0.12
	5 min, 400MPa		ND
	10 min, 400MPa		ND
	15 min, 400MPa		ND
	5 min, 600MPa		ND
	10 min, 600MPa		ND
	15 min, 600MPa		ND

* Three replicates of the same processing conditions were carried out and bacterial count is expressed as the average reading. ND: Bacterial colonies not detected on plate.

4.2. Effect of different processing methods on goat milk colour properties

4.2.1. Colour properties of thermal processed goat milk

The thermal processed goat milk samples show significant changes in colour. Regarding thermal treatment at 60°C for 5 min, total colour difference (TCD) significantly increased from 0.0 (raw unprocessed goat milk) to 1.20 ($p<0.05$) as in Figure 4.2-1 with increase in lightness (L*). TCD of thermal processed goat milk showed significant change for every increase in temperature due to the effect of lightness (L*) that increases steadily as shown in Figure 4.2-2 and decrease in yellowness as in Figure 4.2-3. Goat milk pasteurized at 80°C was observed having higher L* values than the one treated at 60°C. Similarly, McAuley et al. (2016) reported that milk lightness was lower in less severe thermal treatments where treated milk (63°C, 15 s) was lighter than all other milk samples. Heat denaturation of whey proteins leading to their aggregations, causes increase in lightness of milk and reduction in approaches to yellowness; (b*) value (Wiley, 2017, p. 345). Thermal pasteurization of goat milk samples at 80°C for 5 min showed distinct TCD than raw unprocessed milk. The difference could be due to acidification and heat-induced coagulation process (Kneifel et al., 1992). Acidification of milk was due to production of organic acid from lactose and release of hydrogen ions from calcium phosphate or casein-bound phosphate (Bashir, 1991). Steady increase of TCD was seen at every increase of 5 min. This shows that TCD increases as the processing time increases. Kneifel et al. (1992) also stated increase in TCD of milk after 80°C pasteurized for 5 min compared to reading obtained for (80°C, 1 min). Appendix B shows the value for TCD, lightness (L*) and (b*) for thermal treated goat milk.

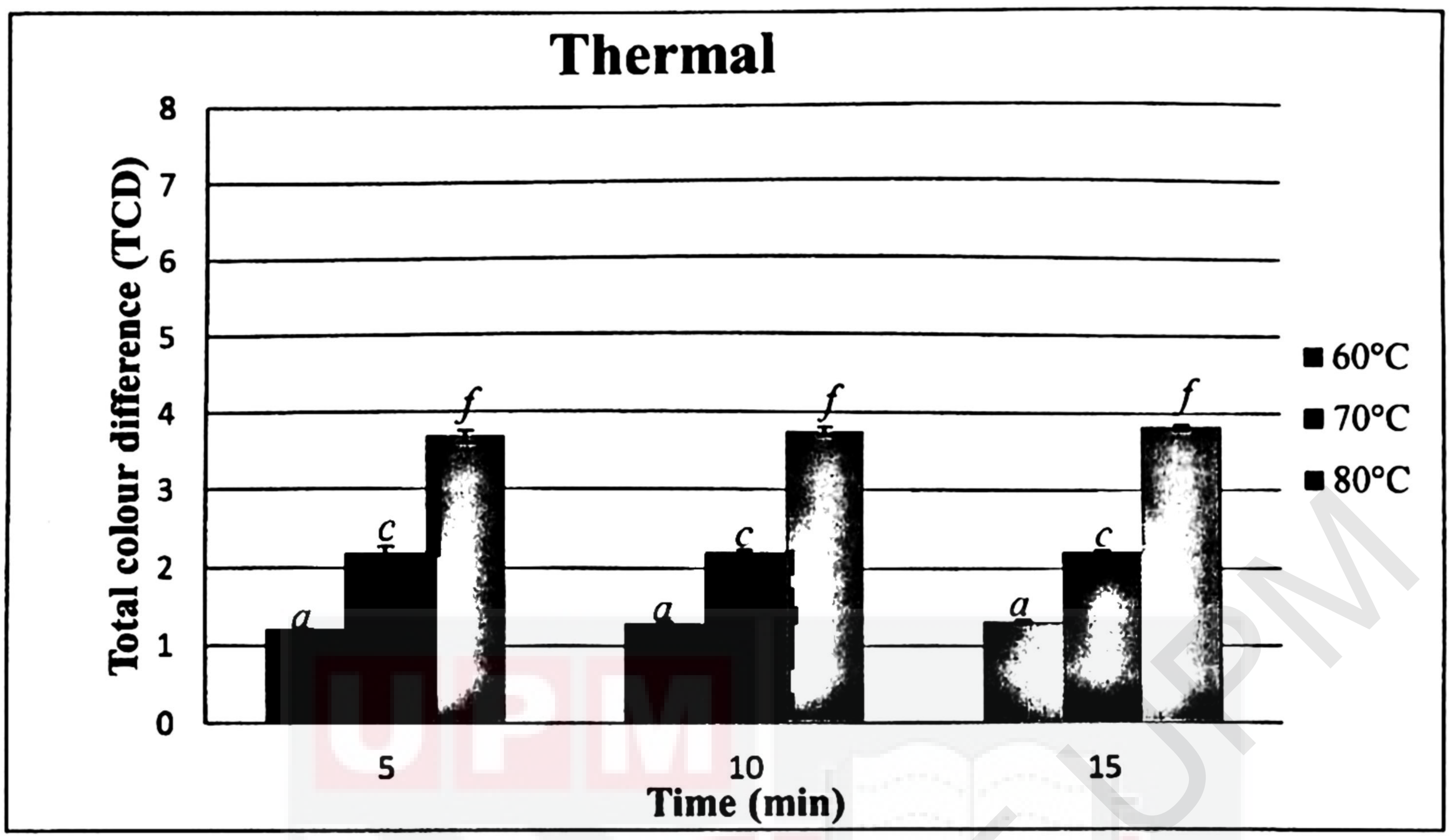


Figure 4.2-1: Total colour difference (TCD) in goat milk after thermal processing (60, 70, 80°C - 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

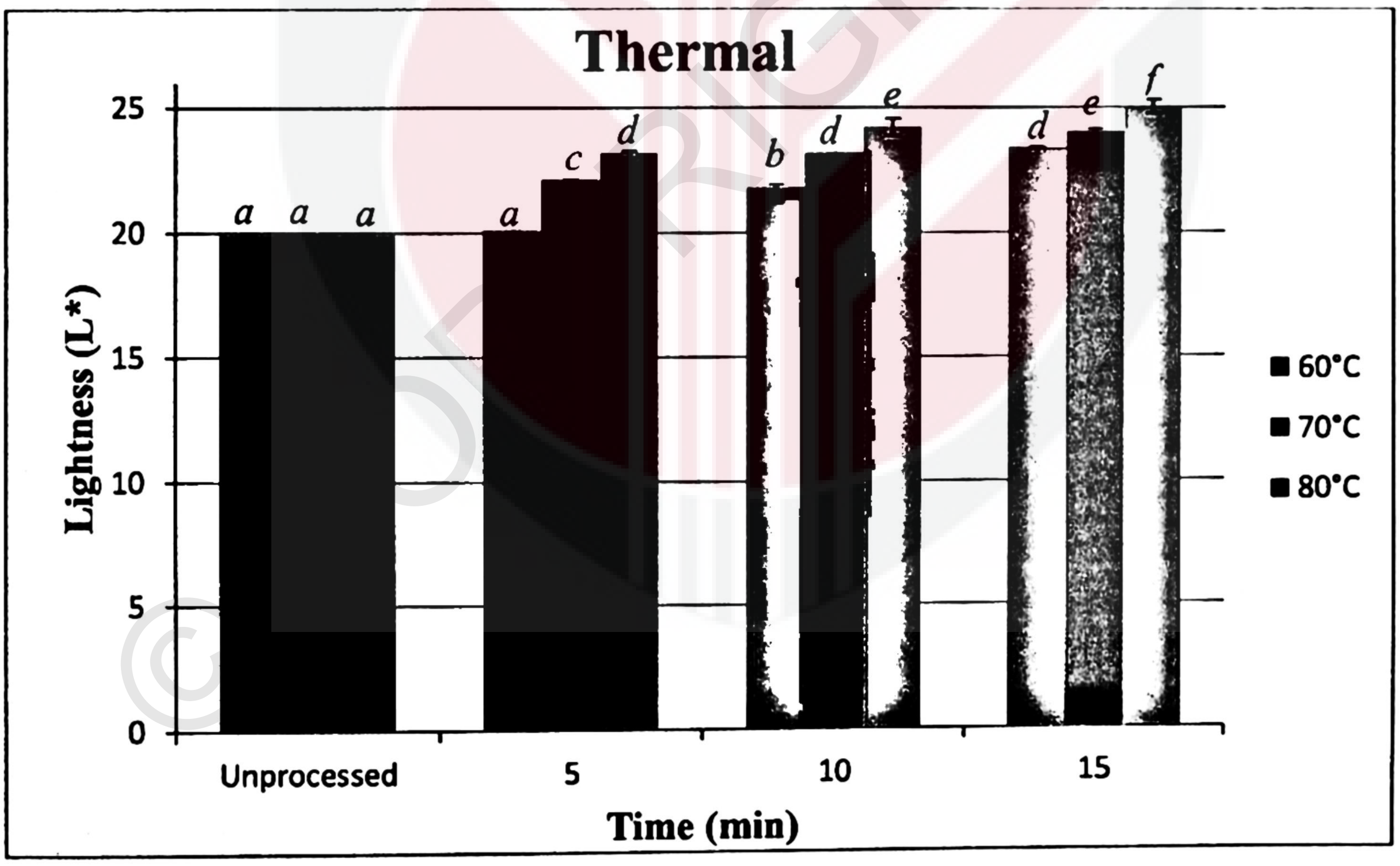


Figure 4.2-2: Lightness (L*) value in goat milk after thermal processing (60, 70, 80°C - 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

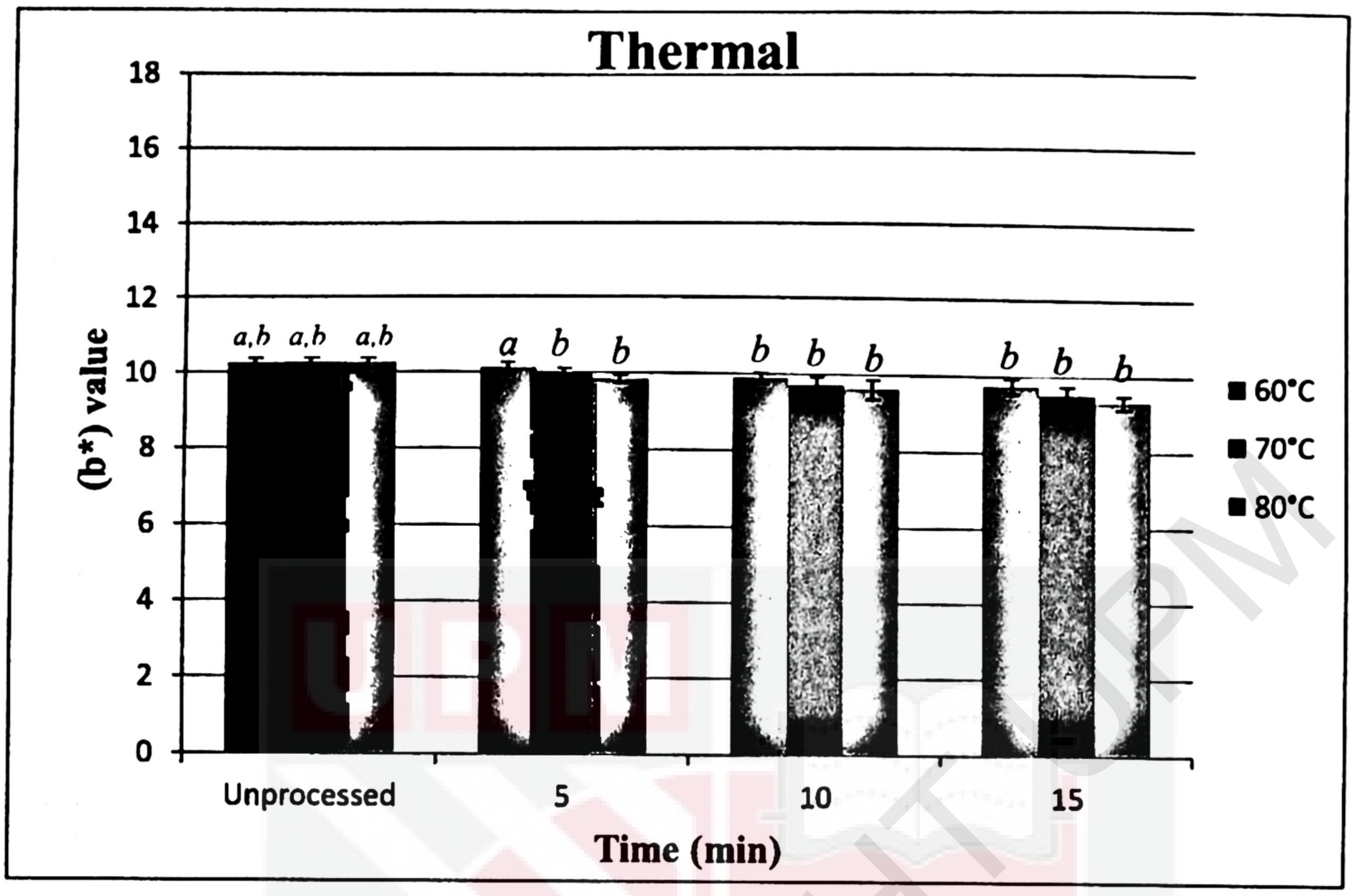


Figure 4.2-3: (b*) value in goat milk after thermal processing (60, 70, 80°C - 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.2.1. Colour properties of HPP processed goat milk

HPP at 200MPa showed a distinct total colour difference (TCD) from raw unprocessed goat milk from 0.0 to 3.59 ($p<0.05$) based on Appendix B. From Figure 4.2-5 and 4.2-6, it were observed that the milk lightness and yellowness increases initially in comparison to untreated raw goat milk. Studies have shown that change in lightness and yellowness due to protein denaturation and change in protein and lipid content because of pressure treatment (Bak et al., 2012; Quinones, 1998).

From Figure 4.2-4, HPP processed goat milk showed steady change in TCD as pressure increased due to effect of lightness (L*) reduction as shown in Figure 4.2-5 that caused the milk to approach yellowness; positive (+) b* value as in Figure 4.2-6. However, goat milk pressurized at 600MPa was observed having lower lightness (L*) value compared to sample pressurized at 200MPa. Similarly, disintegration of casein micelles by HPP treatment on milk into small fragments that increase the translucence of milk causes reduction of milk's lightness (L*) values (Johnston et al., 1992; Shibauchi et al., 1992; Desobry-Banon et al., 1994; Trujillo, Capellas, Saldo, Gervilla, & Guami, 2002). Milk pressurized at 200MPa for 15 min has lower TCD compared to the one pressurized at 600MPa for 15 min. This difference in results showed that TCD value increases as samples pressurized under higher pressure. According to Mussa & Ramaswamy (1997), TCD of milk sample after UHP treatment at 400MPa is higher than the one pressurized at 200MPa mainly due to progressive change in brightness factor as pressure increases. TCD of goat milk samples after a HPP process of (200MPa, 15 min) was 1.97; which was lower than milk samples processed by (200MPa, 5 min) that was about 3.59. This difference in results is due to dairy samples that undergone HPP treatment approached yellowness as treatment time increased due to reduction in size of casein micelles (Okpala et al., 2009; Ozcan et al., 2017). HPP treated milk however showed lower TCD compared to thermal treated milk at 80°C. This might be due to excessive thermal treatment increases the change in colour of milk (McAuley et al., 2016).

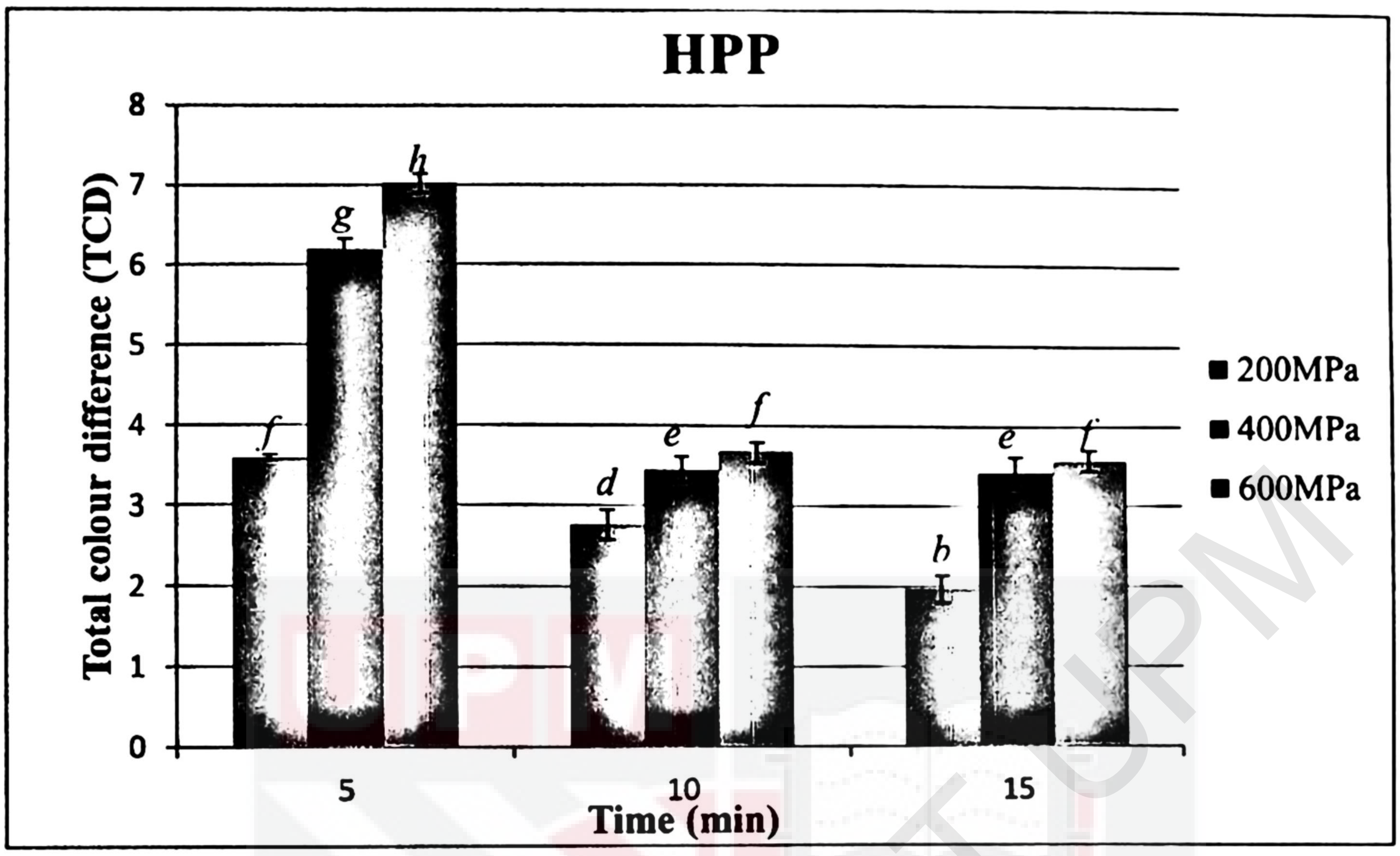


Figure 4.2-4: Total colour difference (TCD) in goat milk after HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters is significantly different ($p< 0.05$).

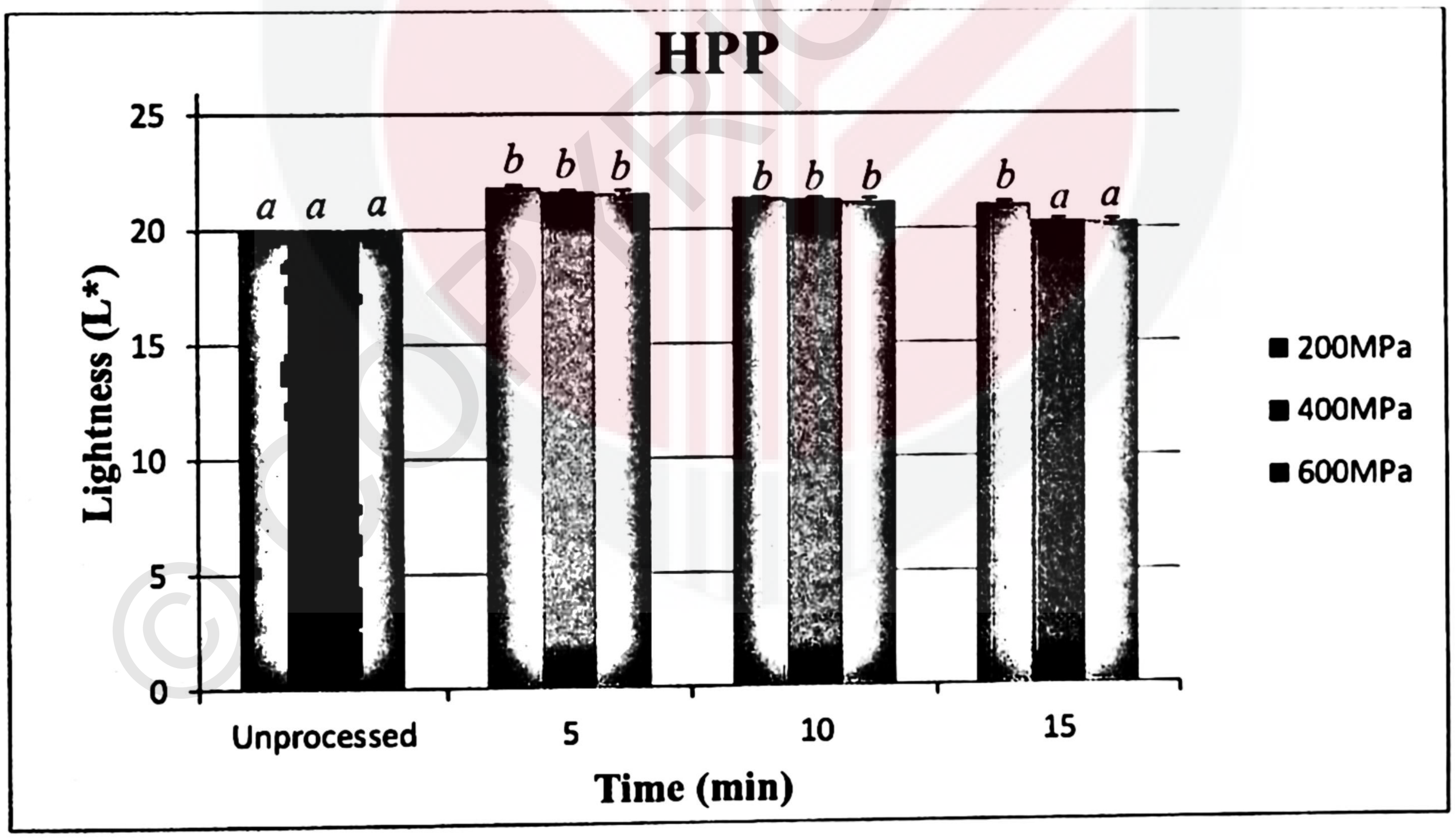


Figure 4.2-5: Lightness (L*) value in goat milk after HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters are significantly different ($p< 0.05$).

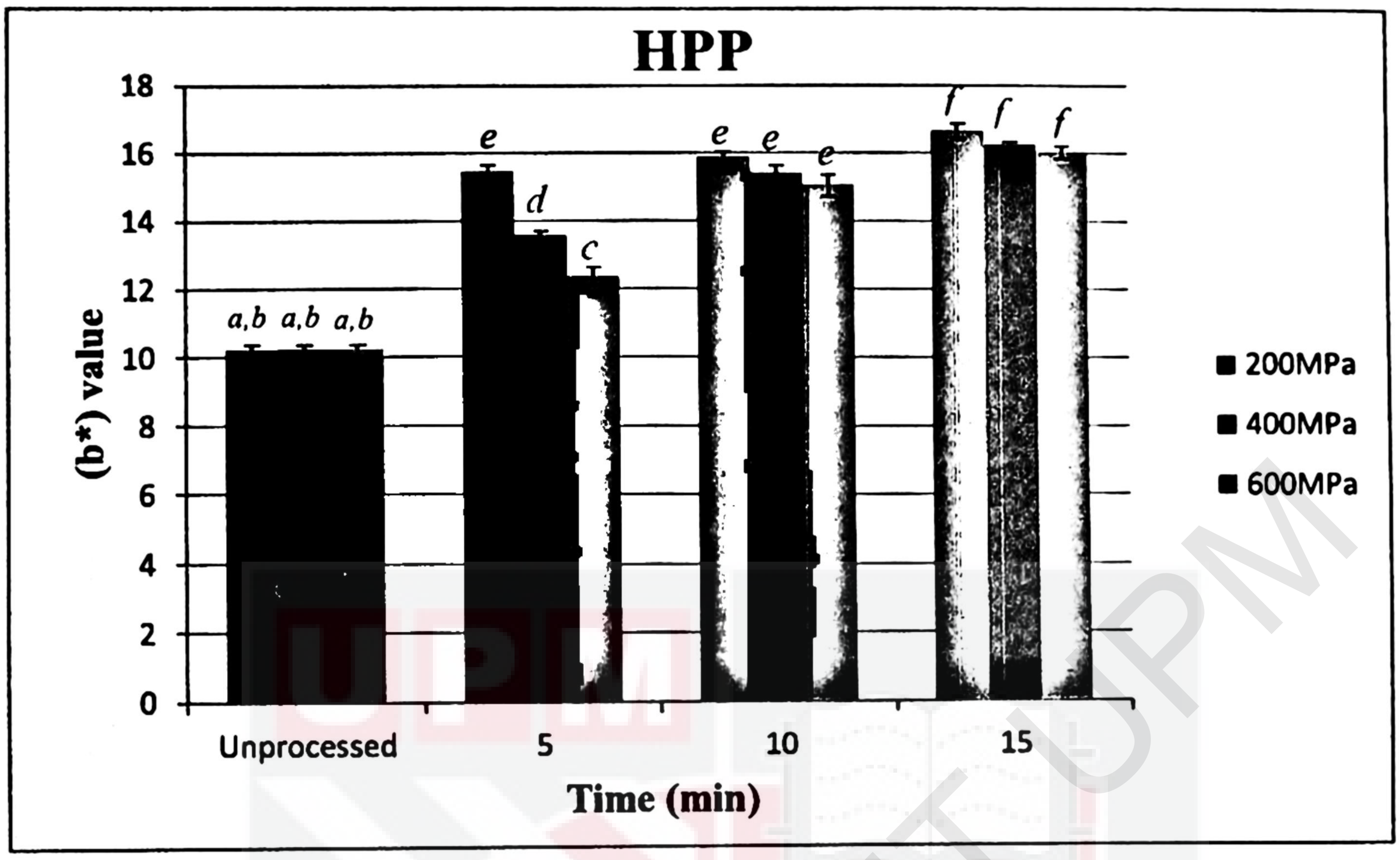


Figure 4.2-6: (b*) value in goat milk after HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters are significantly different ($p< 0.05$).

4.3. Effect of different processing methods on goat milk rheological properties

4.3.1. Rheological properties of thermal processed goat milk

The thermal processed goat milk showed only slight change in viscosity. For thermal treated milk at 60°C for 5 min, the viscosity of milk sample decreased from 21.4 cP (raw unprocessed milk) to 17.6 cP ($p<0.05$). Initial decrease of viscosity was explained by the solubility of casein phosphate that decrease rapidly on heating process and it is known to precipitate on to the casein micelles (Van Dijk, 1990; Jeurnink and Kruif, 1993). However, goat milk samples thermally treated at 60°C for 15 min increased to 18.5 cP as processing time increases which is shown in Figure 4.3-1. Mediwaththe et al. (2018) also reported that milk upon heating at (80°C, 100 s) was slightly lower viscosity than one heated for 1000 s. This difference in results was mentioned due to shear-induced denaturation and aggregation of proteins during thermal processing of milk. Viscosity for thermal pasteurization of goat milk samples at 80°C for 5 min was observed to increase from 17.6 cP (60°C, 5 min) to 19.8 cP (80°C, 5 min). This shows that viscosity increases as temperature increases. Similarly, Sutariya et al. (2017) stated that higher pre-heating temperature increase the viscosity of milk. However, distribution of κ-casein and denatured whey proteins between the serum and micellar phase only slightly influenced the viscosity of milk when undergo thermal processing. No significant change in viscosity was also observed at 80°C for 15 min ($p>0.05$) based on Appendix D. This might be due to the distribution of whey proteins that cause the viscosity of milk at this condition to be similar as the raw milk (Sutariya et al., 2017).

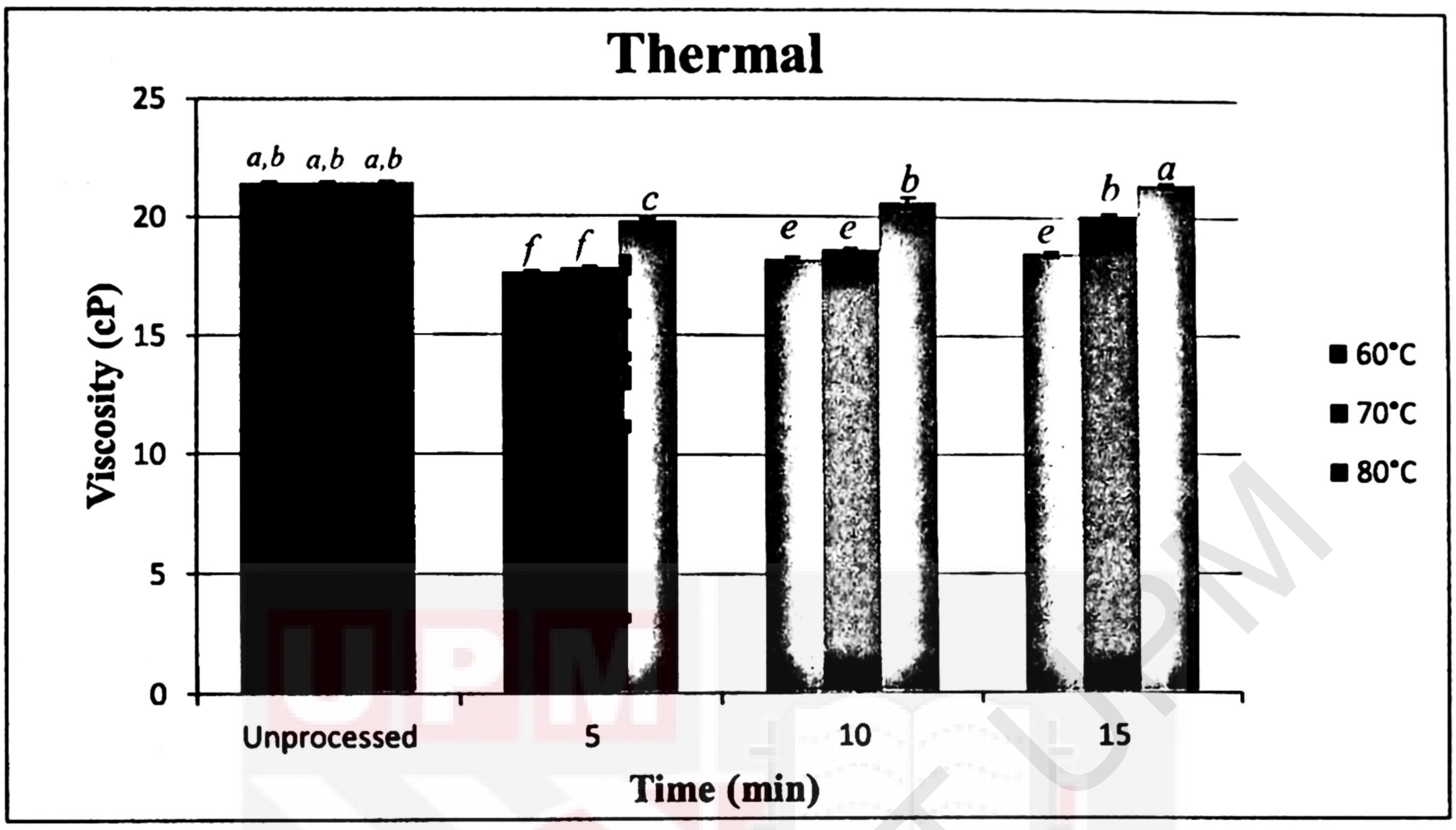


Figure 4.3-1: Viscosity of goat milk after thermal processing (60, 70, 80°C - 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.3.2. Rheological properties of HPP processed goat milk

For goat milk samples that undergone a HPP process at 200MPa for 5 min, the viscosity decreases from 21.4 cP (raw unprocessed milk) to 12.5 cP ($p<0.05$) as shown in Figure 4.3-2. According to Innocente et al. (2009), HPP process that cause the occurrence of complex coacervation between proteins and polysaccharides which led to smaller casein micelles and reduction in polysaccharide molecular weight contributed to initial drop of viscosity.

The viscosity of milk samples however increased as processing time increases; where goat milk samples at a HPP process of (200MPa, 15 min) was 15.8 cP. This significant viscosity change is due to excessive processing conditions of high pressure processing of milk (Mussa & Ramaswamy, 1997). HPP at 600MPa for 5 min showed distinct increase compared to goat milk pressurized at 200MPa for 5 min from 12.5 cP to 20.1 cP according to Appendix D. This difference in results showed that viscosity of milk increases as pressure increases. According to Mussa & Ramaswamy (1997), increase in pressure during UHP treatment increases the viscosity of milk; which was observed when viscosity of pressure-treated milk at 430MPa increased from 19% to 38% (500MPa). Desobry-Banon et al. (1994) and Johnston, D.E. (1995) stated that increase in viscosity of milk processed by high pressure is due to disintegration of casein particles into smaller ones that results in increased fraction of casein micelles in the total volume.

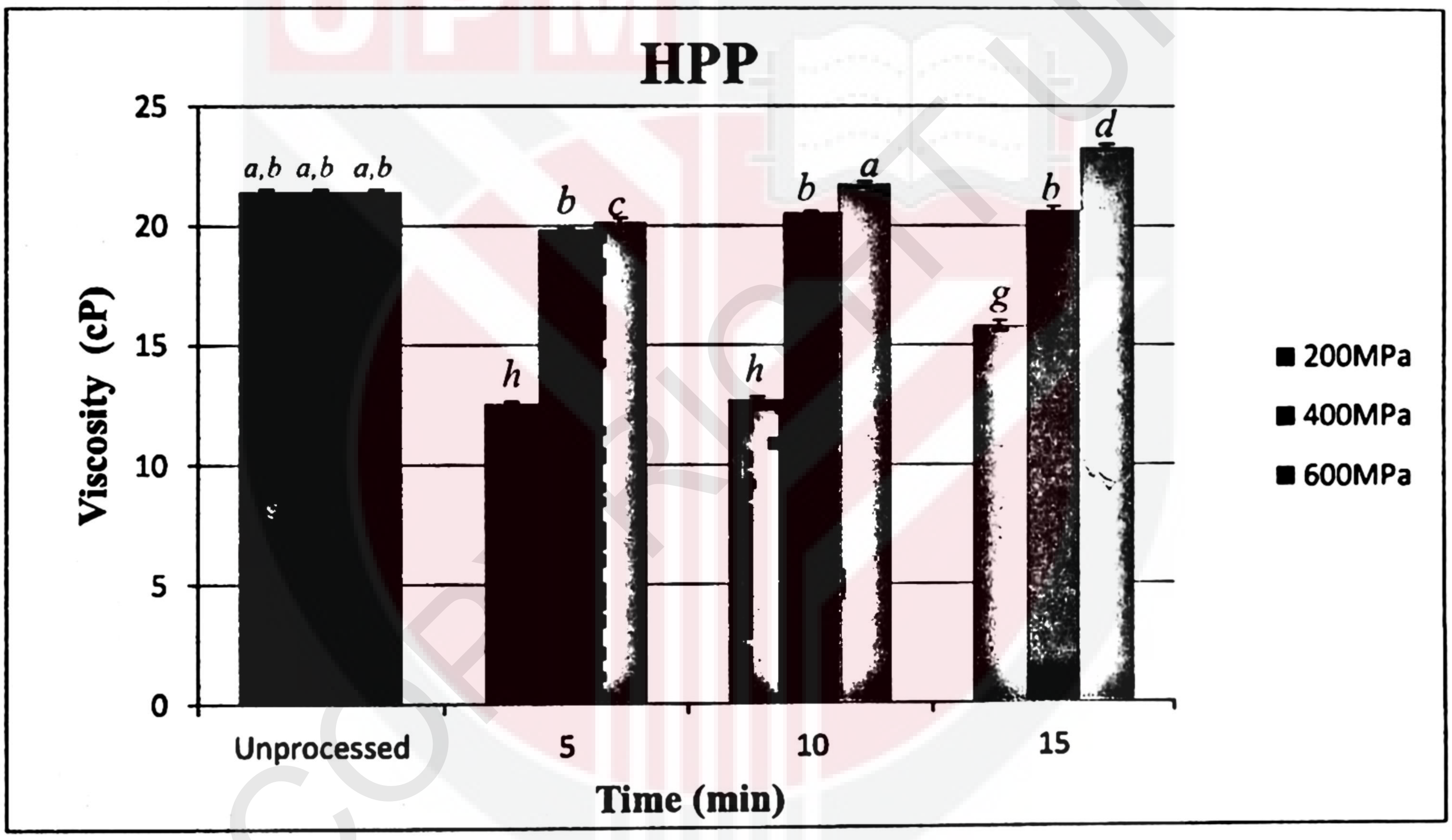


Figure 4.3-2: Viscosity of goat milk after HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.4. Effect of different processing methods on goat milk physicochemical parameters

4.4.1. Physicochemical parameters of thermal processed goat milk

4.4.1.1. pH of thermal processed goat milk

The thermal processed goat milk samples showed slight changes in pH based on Figure 4.4-1. For milk samples of thermal process at 60°C for 5 min, pH remained constant at 6.76 similar as the pH of raw unprocessed milk. However, pH of goat milk declined as milk samples processed at excessive processing time. For pasteurized goat milk at thermal (70°C, 5 min), pH was 6.85; which was higher than milk samples processed at (70°C, 15 min) that recorded pH reading of 6.80. According to Mediwaththe et al. (2018), reduction in pH was observed as general trend in comparison with the control since pH is directly proportional to the viscosity. Thus, since the viscosity of milk decreases initially due to the reduction in calcium phosphate solubility, pH of the milk decreases as well initially after applied heat treatment. The pH of thermal treated milk samples dropped from normal milk pH at 6.7 to acidic values which was directly influenced by the severity of processing conditions because of distribution of whey proteins that got disrupted due to heat treatment (McAuley et al., 2016). As for increase in temperature of goat milk samples being thermally processed, pH increases in corresponding with the temperature. Thermal pasteurized goat milk at 80°C showed distinct increase of pH compared to milk processed at 70°C. This significant changes was corresponding with viscosity and temperature of pre-heating process; where pH of concentration was observed higher when viscosity was increased and temperature was higher (Sutariya et al., 2017).

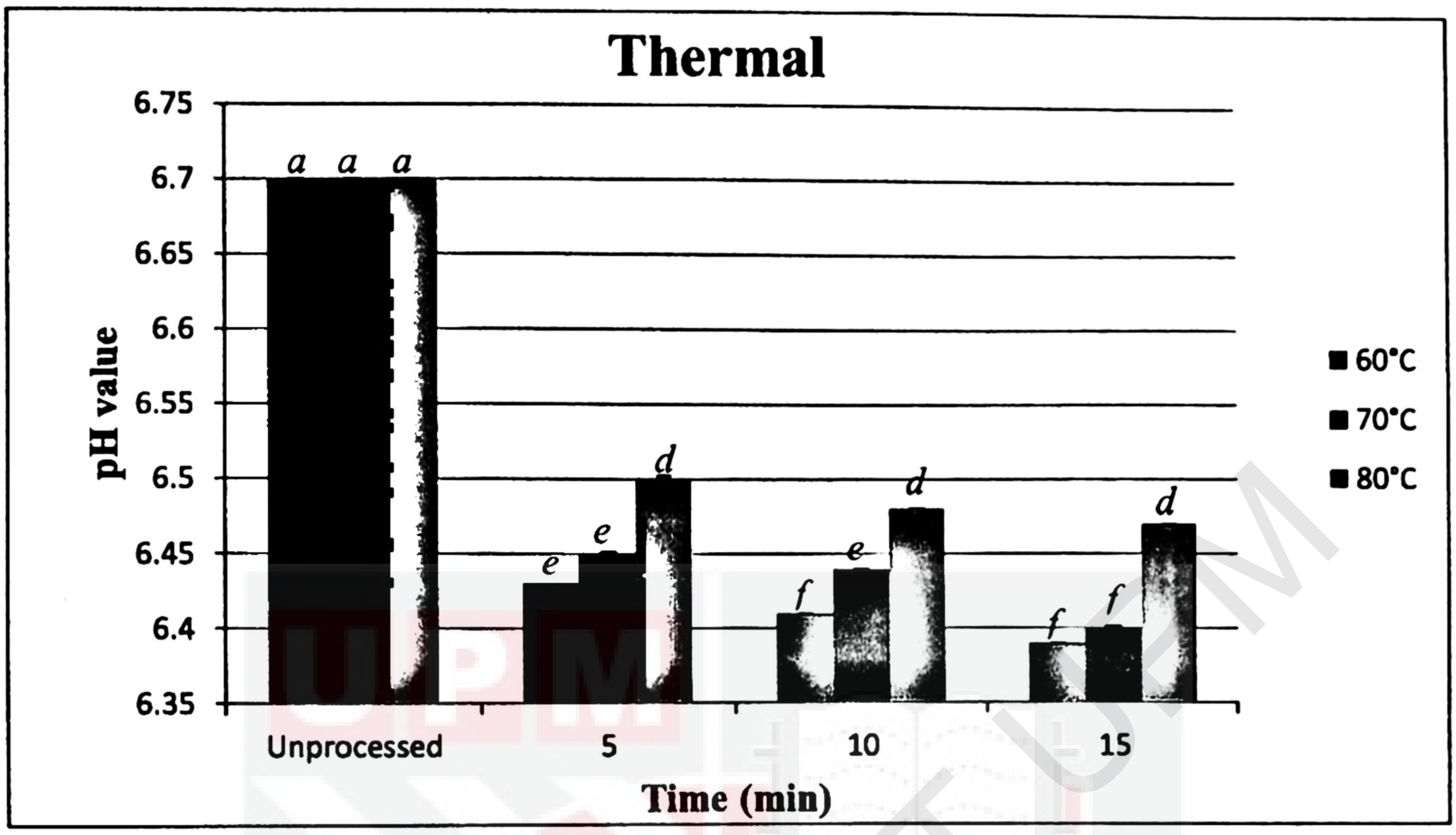


Figure 4.4-1: pH values of goat milk after thermal processing (60, 70, 80°C - 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.4.1.2. Total soluble solid of thermal processed goat milk

Total soluble solid was observed showing significant increase as compared to raw untreated goat milk when samples undergone thermal treatment. °Brix of goat milk at 60°C for 5 min was 10.6; which resulted in increase of 5% compared to untreated milk. Steady increase of total soluble solids was observed as temperature of thermal treatment being increased; where °brix was 10.9 for milk processed at 80°C for the same processing time as shown in Figure 4.4-2. According to McAuley et al. (2016), milk samples thermal treated at (72°C, 15 s) showed higher °brix value of 13.08% compared to the one pasteurized at (63°C, 15s) that reported total solids of 13.01%. For goat milk samples pasteurized at 80°C for 15 min, °brix value was increased to 11.3 as compared with one treated at (80°C, 5 min) as shown in Appendix C. This difference in results may be due to the breakdown of milk proteins into soluble ones by heat treatment (Usha Bajwa and Shikha Mittal, 2013).

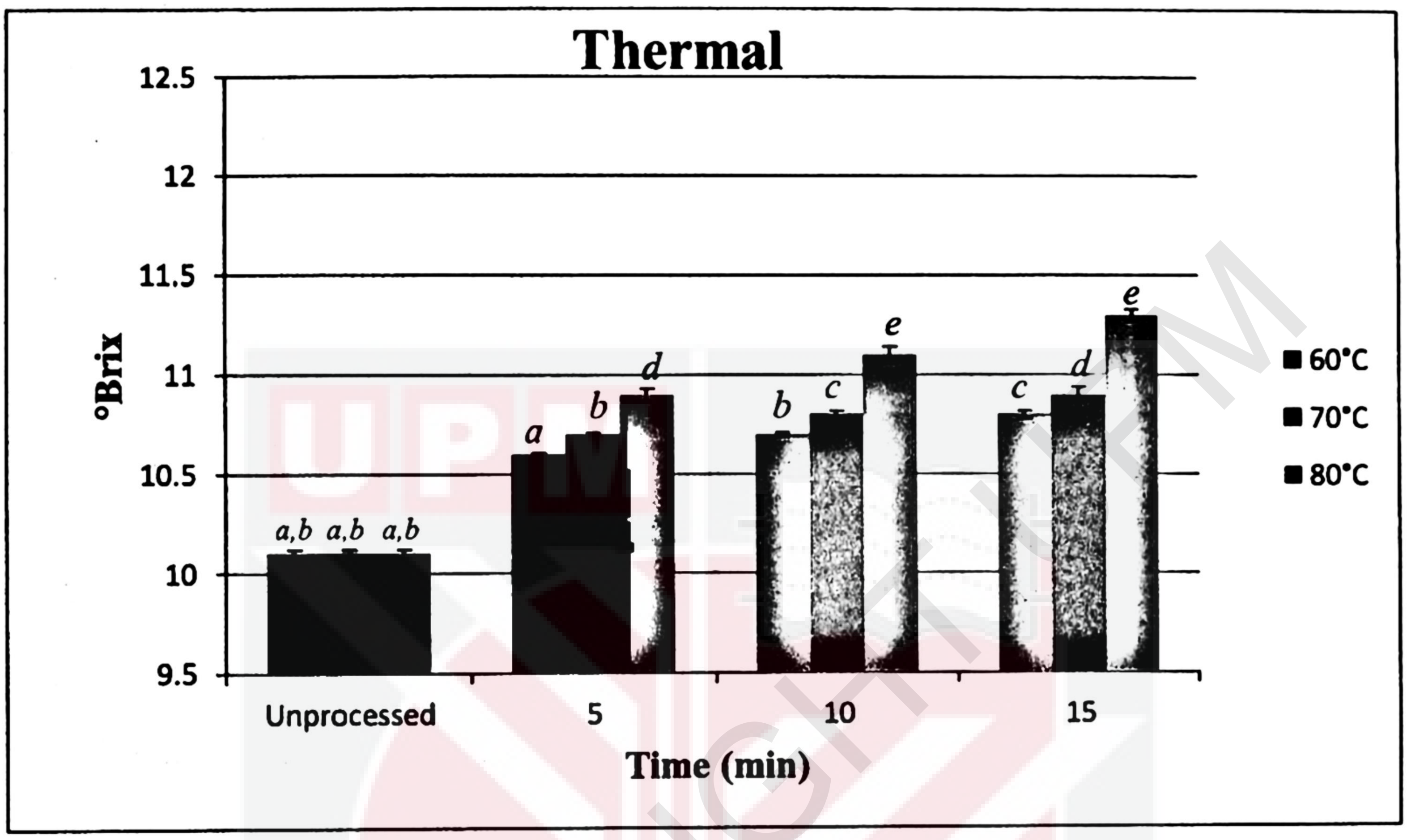


Figure 4.4-2: Total soluble solid in goat milk after thermal processing (60, 70, 80°C - 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.4.2. Physicochemical parameters of HPP processed goat milk

4.4.2.1. pH of HPP processed goat milk

Regarding the effect of pH on goat milk samples of a HPP process, pH of raw unprocessed milk showed reduction when exerted pressure treatments. This can be observed on Figure 4.4-3 when milk samples processed by high pressure at (200MPa, 5min) resulted in pH of 6.59 that was significantly differed from 6.70 (raw unprocessed milk). According to Schrader and Buchheim (1988), pressure-induced partial dissociation of casein micelles which causes coacervation between protein and polysaccharides that results in initial pH drop compared with untreated milk. Increase in pH however was higher when milk was treated at higher pressure compared to pasteurized milk; in which pH of milk at (600MPa, 15 min) was 6.52 than milk thermal treated at (80°C, 15 min) that resulted in pH of 6.47. Similarly, Messens (2003) and De La Fuente et al. (1999) stated that pH of pasteurized milk was lower than one exerted higher pressure treatment. As for the effect of pH on goat milk processed by high pressure under different processing time, pH decreased as processing time increased by referring to Appendix C. For milk samples of a HPP process (600MPa, 10 min), pH was 6.55 compared to one processed at high pressure of 600MPa for 5 min that recorded pH value of 6.57. Besides, pH shift to acidity phase was caused by extensive pressure treatment which also increased the rennet coagulation properties of milk due to size reduction of casein micelles (Messens, 2003; Trujillo et al., 2002).

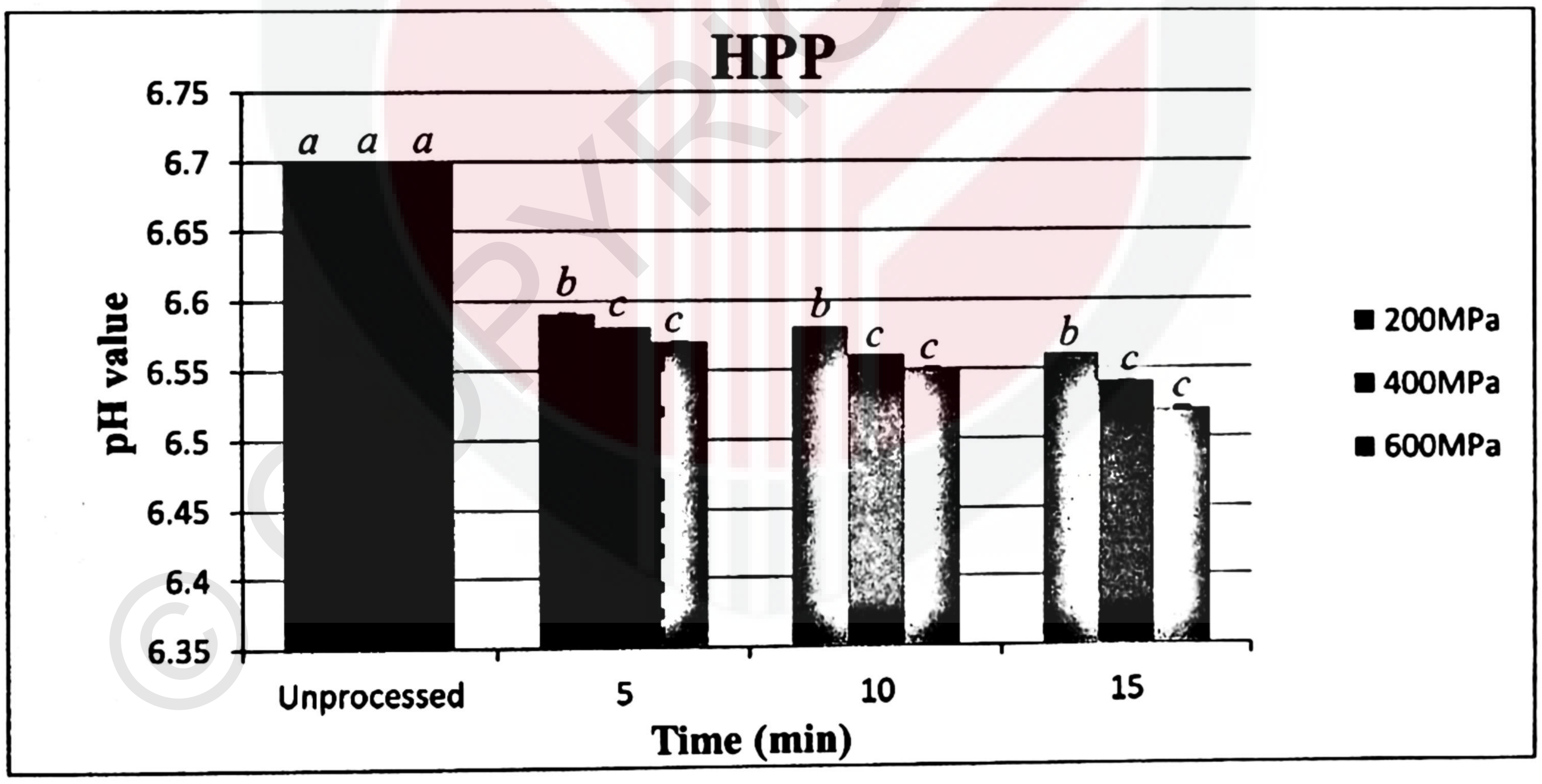


Figure 4.4-3: pH values of goat milk after HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.4.2.2. Total soluble solid of HPP processed goat milk

As for total soluble solids, high pressure processed goat milk showed distinct increase when °brix of milk samples at (200MPa, 5 min) was 12.2 compared to raw unprocessed milk based on Figure 4.4-4. This is due to the disruption of casein micelles because of pressure treatment (Huppertz, 2010; Huppertz and De Kruif, 2006). However, increasing pressure further reduced the total soluble solids content in goat milk; where °brix of milk samples at 400MPa for 5 min was 12.1. Similarly, it has been also reported where milk total soluble solids content decreased with increasing pressure due to disruption of casein micelles under high pressure (Huppertz, 2010; Huppertz and De Kruif, 2006; Kromkamp et al., 1996; Huppertz et al., 2006c; Orlien et al., 2006; Gebhart et al., 2005). For goat milk processed by high pressure at increasing processing time, °brix value of milk of a HPP process at (600MPa, 15 min) declined to 11.1 compared to milk processed at (600MPa, 5 min) that was 11.5 as shown in Appendix C. According to Huppertz (2010), increasing processing time decreases milk total soluble solids content due to greater disruption of casein micelles under high pressure.

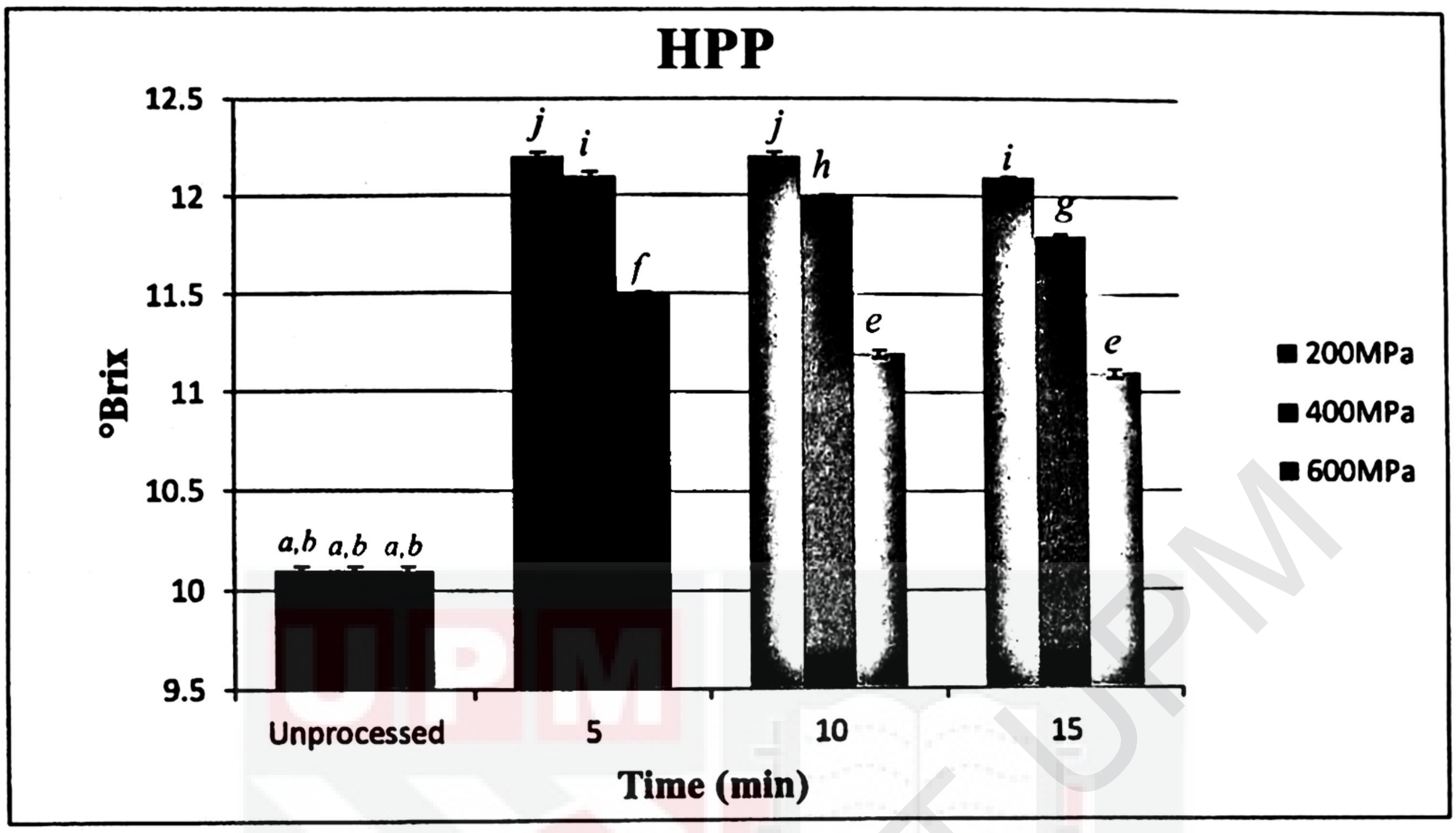


Figure 4.4-4: Total soluble solid in goat milk after HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

4.5. Storage study on different processing methods

4.5.1. Effect of storage on microbial quality

The growth of microorganisms on the processed goat milk sample during refrigerated and room temperature storages are shown in Figure 4.5-1. The data is compared together with standard microbiological count for milk; which is 5 log CFU/ml to identify the acceptable storage time for consumption that that was below the standard range (Food Act 1983). For the two processing methods, the microorganisms' reductions were more stable when storing the goat milk at 4°C rather than room temperature, which were expected. For thermal treated goat milk samples, it showed stability until Day 10, where after Day 10 onwards growth of microorganisms tends to be found. The same result was observed for high pressure processed milk samples that showed presence of microbe after Day 10. However, microbial counts for thermal treated goat milk samples was observed showing higher number of microorganisms compared with high pressure processed milk. This showed that high pressure processing of milk gives more stability compared with thermal treatment since inactivation of bacteria is more effective that causes reduction of microorganisms' number. From the figure itself, it has been observed that the acceptable milk condition suitable for consumption is only until Day 14 according to the microbiological standard limit. After Day 15 onwards, even though the milk condition still does not coagulate that shows spoilage of milk, but the number of plate count that exceeded the standard microbiological count is not safe for consumption. Table 4.5-1 shows the microbial counts in the beginning and at the end of storage period for both processes.

Table 4.5-1: Microbial counts in goat milk after thermal and HPP during 30 days of storage at 4.0±0.1°C and room temperature (25.0±0.1°C)*.

Treatment	Storage temperature (°C)	Microbial counts $_0$ ±SD (log CFU/ml)	Microbial counts $_f$ ±SD (log CFU/ml)
Thermal (15 min, 70°C)	4	ND	9.23±0.21*c*
	25		5.19±0.02*a*
HPP (10 min, 600MPa, 30°C)	4	ND	8.17±0.20*b*
	25		13.33±0.04*d*

*Microbial counts $_0$: initial bacterial count after processing and before storage; Microbial counts $_f$: bacterial count at the end of storage time. ND: Bacterial colonies not detected on plate with different letters are significantly different ($p < 0.05$).

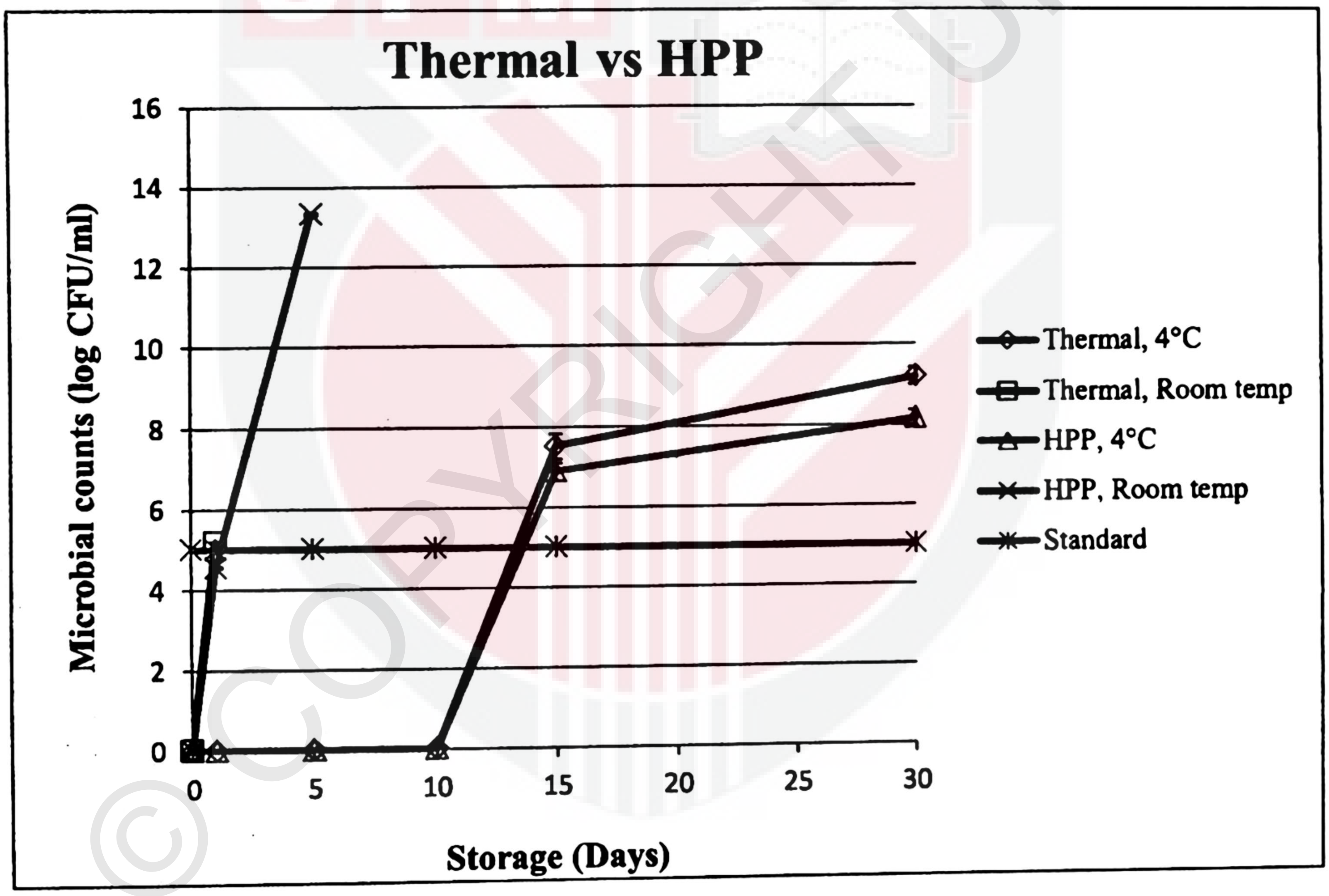


Figure 4.5-1: Microbial counts in goat milk after thermal (70°C /15 min) and HPP (600MPa/30°C/10 min) at storage 4°C and room temperature (25°C) with respect to standard allowable microbial count.

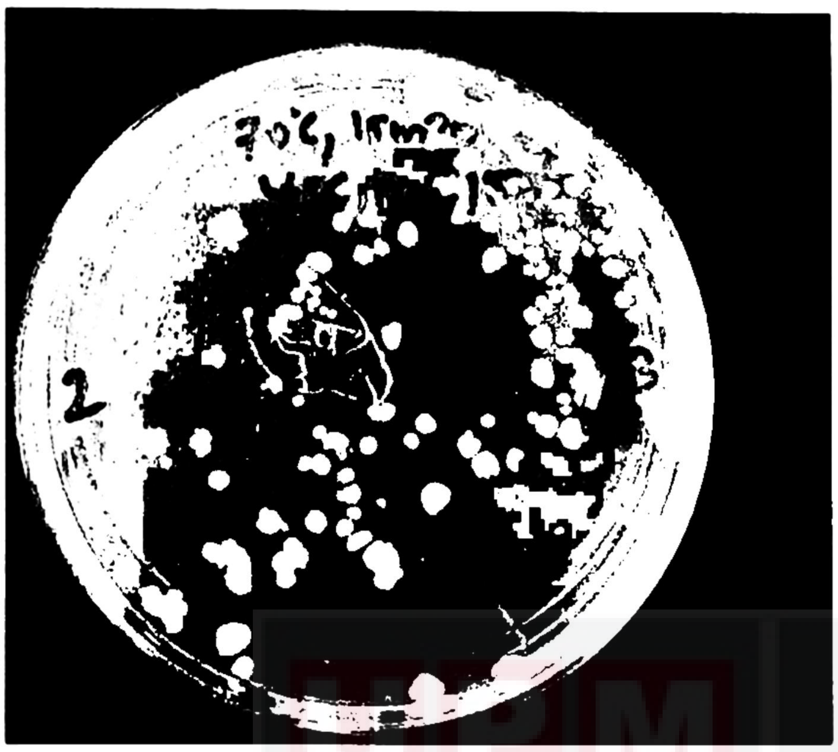


Figure 4.5-2: Microbial plate count of thermal process at 70°C for 15 min on Day 15 for goat milk stored at refrigerated condition.

Figure 4.5-3: Microbial plate count of HPP process at 600MPa for 10 min on Day 15 for goat milk stored at refrigerated condition.

4.5.2. Effect of storage on colour properties

The changes in colour properties of the processed goat milk sample during refrigerated and room temperature storages are shown in Figure 4.5-4. The total colour difference (TCD) gradually increased over the 30 days of storage for goat milk samples processed by the two methods. For the two processing methods, the colour properties were better retained when storing the goat milk at 4°C rather than room temperature, which were expected. HPP processed milk showed higher TCD values compared with thermal treated milk due to the effect of lightness (L*) on milk samples. In result of this, milk samples processed by thermal to be brighter in colour throughout storage period whereas milk samples processed by a HPP process tends to be more yellow in colour since the colour approaches to positive (+) b* value which indicates yellow colour. According to Okpala et al. (2009), effect of high pressure that cause reduction in size of casein micelles influenced milk samples to approach yellowness. Table 4.5-2 shows the TCD value in the beginning and end of storage period for both processes.

Table 4.5-2: Total colour difference (TCD) in goat milk after thermal and HPP during 30 days of storage at 4.0±0.1°C and room temperature (25.0±0.1°C)*.

Treatment	**Storage temperature (°C)**	**TCD_0 ±SD**	**TCD_f ±SD**
Thermal (15 min, 70°C)	4	0.0±0.*0a,b*	7.17±0.23*c*
	25		6.54±1.91*b*
HPP (10 min, 600MPa, 30°C)	4		11.02±0.15*d*
	25		5.46±0.23*a*

*TCD_0: initial total colour difference after processing and before storage; TCD_f: total colour difference at the end of storage time with different letters is significantly different ($p< 0.05$).

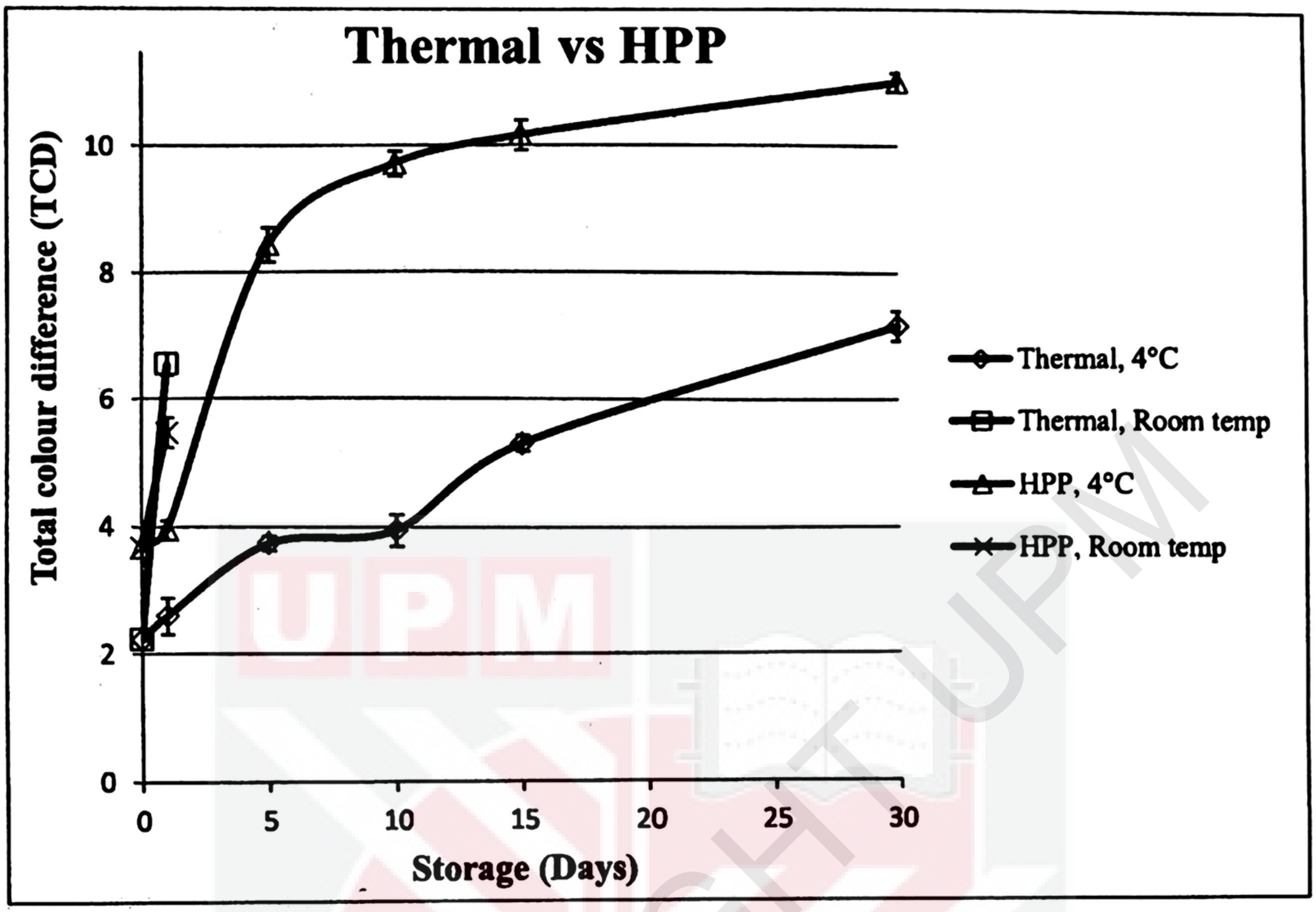


Figure 4.5-4: Total colour difference in goat milk after thermal (70°C /15 min) and HPP (600MPa/30°C/10 min) at storage 4°C and room temperature (25°C).

4.5.3. Effect of storage on physicochemical parameters

The changes in physicochemical parameters (pH and brix) of the processed goat milk sample during refrigerated and room temperature storages are shown in Figure 4.5-5. For the two processing methods, the pH and brix were better retained when storing the goat milk at 4°C rather than room temperature, which were expected as shown in Table 4.5-3. The pH value gradually increased over the 30 days of storage for goat milk samples processed by thermal treatment that were stored at refrigerated condition compared to goat milk stored at room temperature which showed significantly declined value of pH from Day 1 onwards. However, pH value of goat milk samples of a HPP process steadily decline over the 30 days of storage for both refrigerated and room temperature condition. HPP processed goat milk samples showed lower values of pH which tends to shift to more acidity phase compared with thermal treated milk that showed increasing pH values during storage period. According to Messens (2003), lower pH of milk samples reduced the growth of microorganisms which extends the shelf life of milk.

°Brix value whereas gradually declined over the 30 days of storage for goat milk samples processed by the two methods. The total soluble solid (°brix) of goat milk stored at refrigerated condition was observed slightly declined compared to milk stored at room temperature which showed steep decline. From Figure 4.5-6, total soluble solids of goat milk samples processed by high pressure was higher compared with thermal treated milk. This showed that high pressure processed milk has less significant effect on °brix of milk compared with effect of high temperature that cause lower total soluble solids in milk. During storage period, total soluble solids of milk sample decrease slightly due to changes in casein micelle composition because of lactic acid production by casein-phosphate that result in milk acidification (Bashir, 1991; Gastaldi et al., 1997).

Table 4.5-3: Physicochemical parameters (pH and brix) of goat milk after thermal and HPP during 30 days of storage at 4.0±0.1°C and room temperature (25.0±0.1°C)*.

Treatment	**Storage temperature (°C)**	**pH$_0$±SD**	**pH$_f$±SD**	**°Brix$_0$ ±SD**	**°Brix$_f$±SD**
Thermal (15 min, 70°C)	4	6.40±0.01*b*	6.75±0.0*a*	10.9±0.4*g*	9.07±0.13*f*
	25		6.0±0.0c		9.6±0.1*e*
HPP (10 min, 600MPa, 30°C)	4	6.55±0.006*a*	6.40±0.0*b*	11.2±0.2*g*	10.33±0.17*g*
	25		5.5±0.0*d*		8.73±0.32*h*

*pH$_0$: initial pH after processing and before storage; pH$_f$: pH at the end of storage time; Brix$_0$: initial total soluble solids after processing and before storage; Brix$_f$: total soluble solids at the end of storage time with different letters are significantly different ($p < 0.05$).

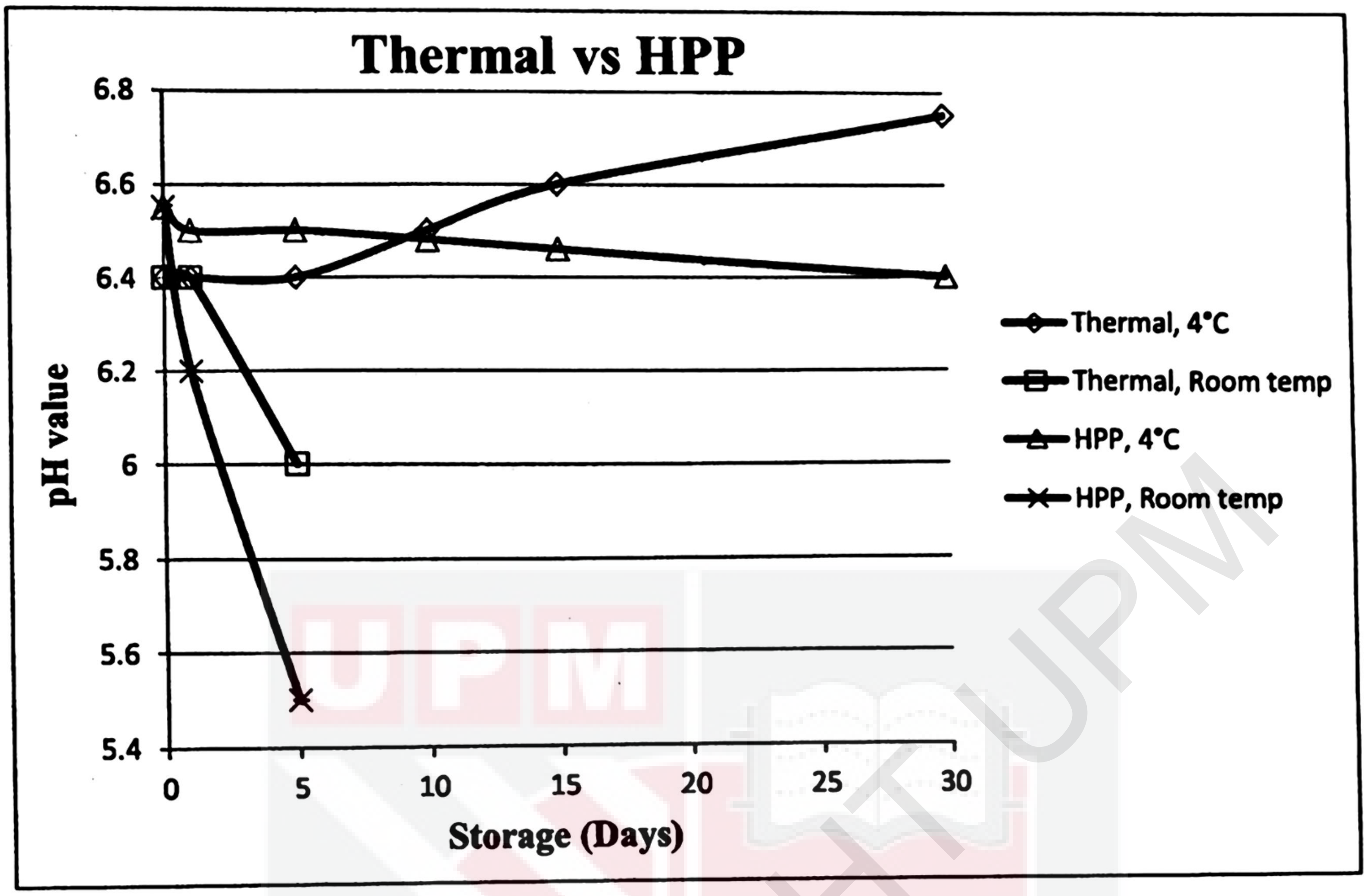


Figure 4.5-5: pH value in goat milk after thermal (70°C /15 min) and HPP (600MPa/30°C/10 min) at storage 4°C and room temperature (25°C).

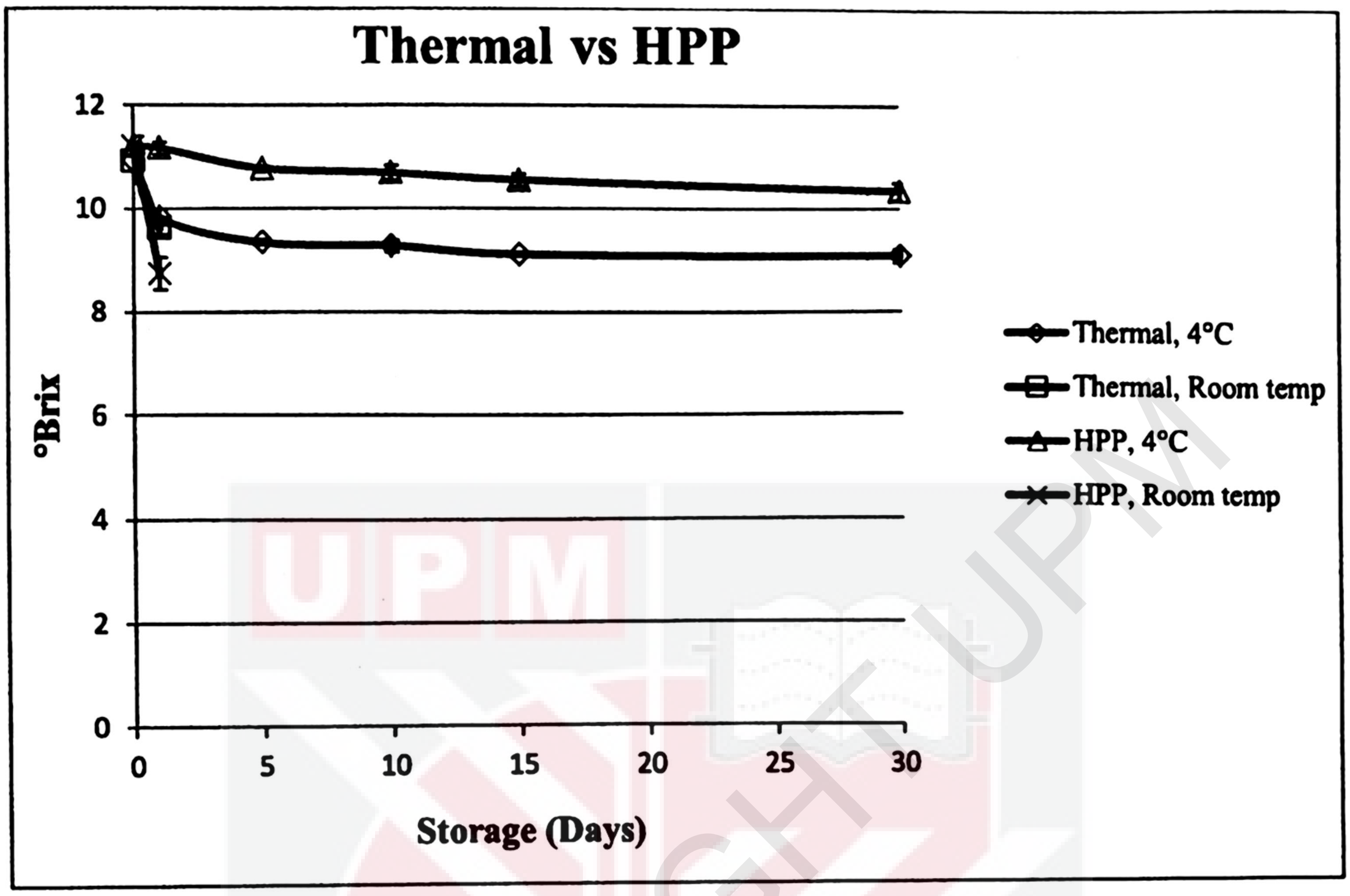


Figure 4.5-6: Total soluble solid in goat milk after thermal (70°C /15 min) and HPP (600MPa/30°C/10 min) at storage 4°C and room temperature (25°C).

4.5.4. Effect of storage on rheological properties

The changes in rheological properties of the processed goat milk sample during refrigerated and room temperature storages are shown in Figure 4.5-7. For thermal treated goat milk samples, viscosity of milk stored at room temperature decreased whereas milk samples stored at refrigerated condition increased until Day 10 and started to decline from Day 10 onwards. Similar results pattern as observed for milk processed by a HPP process which showed decrease in viscosity of milk after Day 10 onwards. A decrease in viscosity of milk occurred when pH of the concentrations decreased (Sutariya et al., 2017).

For the two processing methods, the viscosity was better retained when storing the goat milk at 4°C rather than room temperature, which was expected. Viscosity of goat milk samples of HPP process showed more consistent viscosity reading compared with thermal treated milk that was showed sharp decline of viscosity after Day 10 which shows milk processed by HPP was more stable in viscosity. Table 4.5-4 shows the viscosity of goat milk in the beginning and end of storage period for both processes.

Table 4.5-4: Viscosity of goat milk after thermal and HPP during 30 days of storage at 4.0±0.1°C and room temperature (25.0±0.1°C)*.

Treatment	**Storage temperature (°C)**	**Viscosity$_0$ ±SD (cP)**	**Viscosity$_f$ ±SD (cP)**
Thermal (15 min, 70°C)	4	20.1±0.1*a*	12.26±0.29*b*
	25		16.77±0.12*a*
HPP (10 min, 600MPa, 30°C)	4	21.7±0.1*c*	25.34±0.45*c*
	25		50.0±0.50*d*

*Viscosity$_0$: initial viscosity after processing and before storage; Viscosity$_f$: viscosity at the end of storage time with different letters is significantly different ($p < 0.05$).

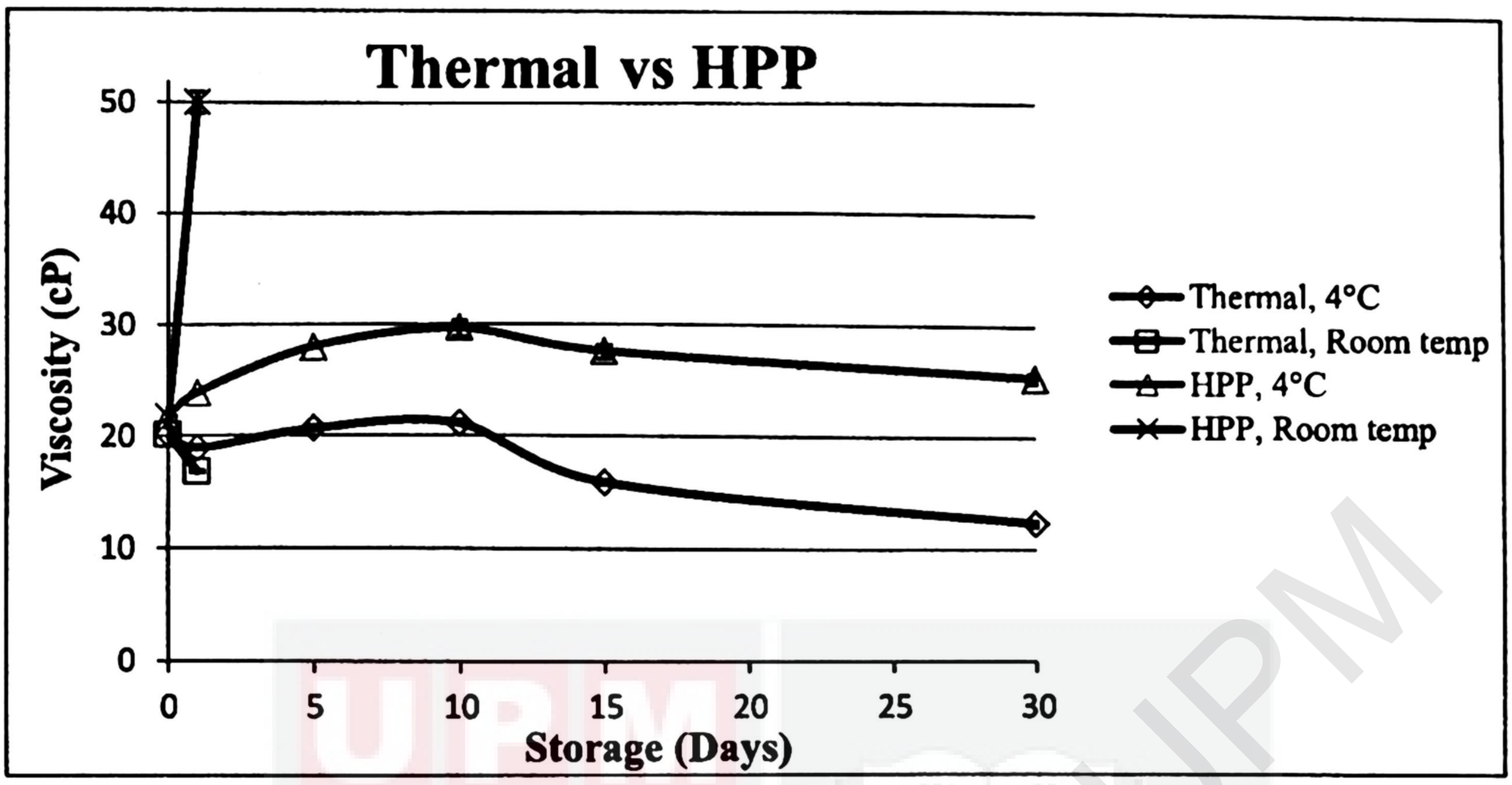


Figure 4.5-7: Viscosity of goat milk after thermal (70°C /15 min) and HPP (600MPa/30°C/10 min) at storage 4°C and room temperature (25°C).

Table 4.5-5: Summarize results comparison of thermal treatment and HPP process on goat milk samples

Parameters	**HPP (600MPa/30°C/10 min)**	**Thermal processing (70°C /15 min)**
Total colour difference (TCD)	Slight difference	Greater difference at higher temperature
Viscosity	High viscous	Low viscous
pH	Higher pH	Lower pH
Total soluble solids (°Brix)	Higher brix	Lower brix
No. of microorganism	Better reduction of bacterial counts	Slight reduction of bacterial counts

HPP processed milk was observed having slight total colour difference compared with thermal treated milk due to reduction in lightness and approaching yellowness. Viscosity of milk samples for high pressurized milk samples was higher than thermal processed milk which showed it has less significant change and higher stability towards pH change and microbial effect. HPP processed milk also showed higher total soluble solids than thermal processed goat milk due to disintegration of casein micelles.

CHAPTER 5

Conclusion

Chapter 5: CONCLUSION

5.1. Summary

In conclusion, the objective of this research has been achieved which is the quality of HPP and thermal processed milk have been evaluated in terms of physical, chemical and microbiological properties. Through the analyses conducted, it has been observed that goat milk of a HPP process retains more nutritional value and gives a better quality of milk with higher viscosity, higher total soluble solids, higher pH, lower total colour difference and better reduction of microorganisms. Objective of this research to study the effect of treated milk on quality evaluation during storage were also achieved which showed HPP processed goat milk with prolonged shelf life than thermal processed goat milk. Other than that, HPP process milk also gives stable physical and chemical properties than thermal processed milk during storage period. Thus, it has been concluded that HPP process goat milk can be further commercialized for better quality retention than conventional thermal processing which can be beneficial to the consumers and also the dairy industries.

5.2. Recommendations/ Suggestions for Future Research

This research topic can be further developed for future improvement. Since the storage study for goat milk in this research was conducted at high pressure of 600MPa for longer treatment time of 10 min, disruption of casein micelles in milk occurred extensively that changes some of the molecular structure in milk. Further checking on the mechanisms of casein micelles disruption should be done in order to verify the statement that colour changes in milk was due to this factor. Other than that, High performance liquid chromatography (HPLC) analysis to determine the total sugar content in milk that was unable to be conducted due to the lacking of time and insufficient equipment has to be done in future research to identify the relation between the total soluble solid (°brix) and total sugar content. This analysis can further verify the effect of different treatments on changes in particles present in goat milk. Moreover, in future the high pressure processing should be applied at higher pressure but at shorter treatment time in order to preserve most of the protein structures in milk. Apart of this, HPP treatment itself was not sufficient to prolong the shelf life of goat milk even though some nutrients are preserved. Therefore, combination of HPP treatment with addition of mild temperature should be applied for better quality and preservation of goat milk. Through this, inactivation of bacteria also will be more effective by application of heat and pressure. Lastly, sensory evaluation on high pressure processed goat milk may also be conducted in order to evaluate the consumer acceptance on taste and flavor properties of milk to support more the statements of HPP processed milk can be commercialized more compared to thermal treated goat milk. Thus, all these suggestions can be implemented in future study to improve findings obtained in this research.

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Appendix A

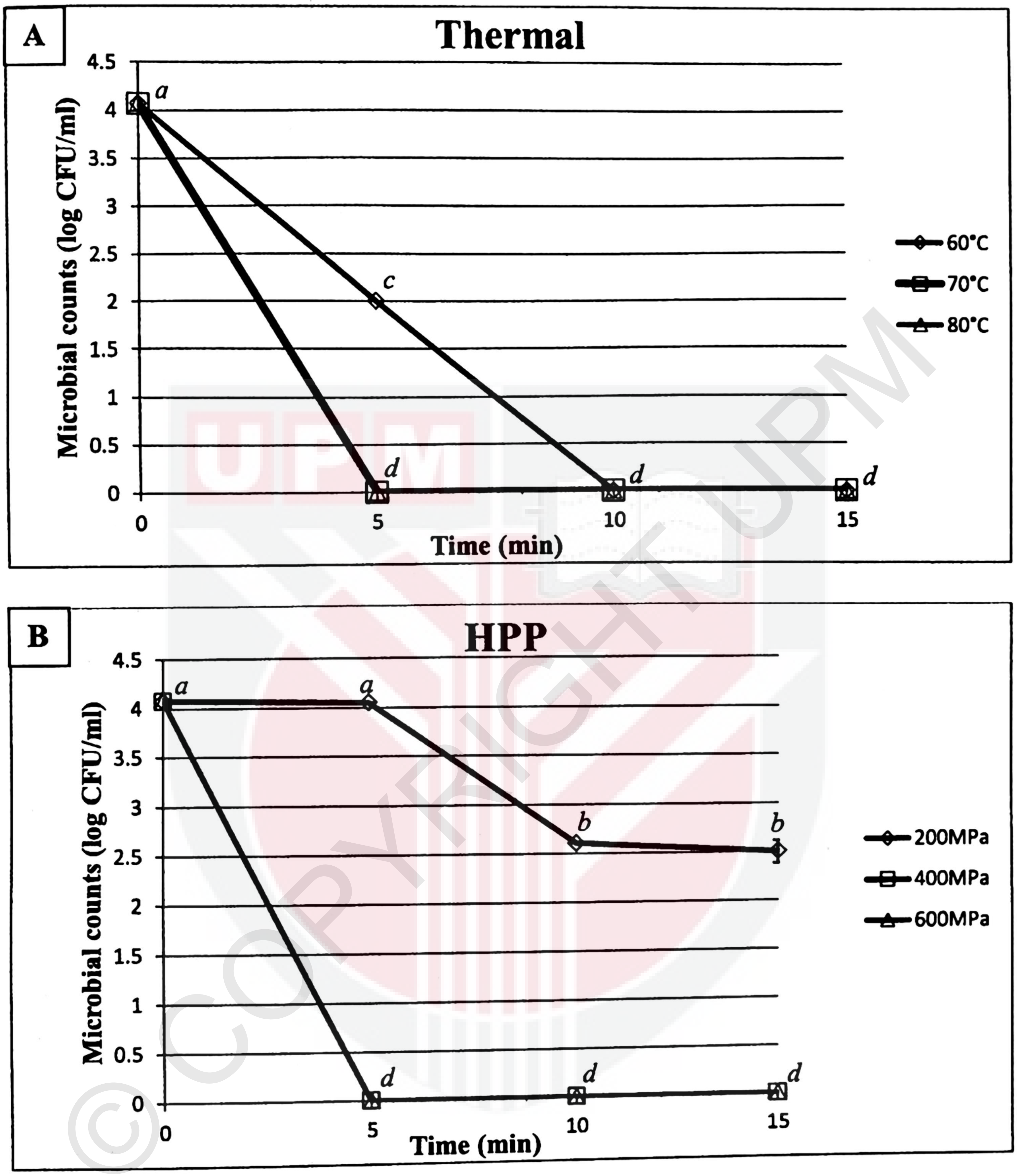

Figure 1: Microbial counts in goat milk after A – thermal processing (60, 70, 80°C - 5, 10, 15 min) and B – HPP (200MPa, 400MPa, 600MPa – 5, 10, 15 min) with different letters are significantly different ($p < 0.05$).

Appendix B

Table 1: Total colour difference (TCD), lightness (L*) and (b*) value in goat milk after thermal and HPP processing*.

Treatment	**Processing conditions**	**TCD ±SD**		**L* ±SD**		**b* ±SD**	
		Raw unprocessed	**After processing**	**Raw unprocessed**	**After processing**	**Raw unprocessed**	**After processing**
Thermal	5 min, 60°C	0.0±0.0*a,b*	1.20±0.006*a*	19.90±0.05*a*	20.07±0.01*a*	10.23±0.15*a,b*	10.11±0.17*a*
	10 min, 60°C		1.29±0.02*a*		21.80±0.12*b*		9.89±0.14*b*
	15 min, 60°C		1.31±0.03*a*		23.40±0.03*d*		9.75±0.20*b*
	5 min, 70°C		2.17±0.10*c*		22.10±0.04*c*		9.97±0.13*b*
	10 min, 70°C		2.20±0.03*c*		23.17±0.01*d*		9.72±0.23*b*
	15 min, 70°C		2.22±0.01*c*		24.04±0.11*e*		9.53±0.20*b*
	5 min, 80°C		3.67±0.27*f*		23.15±0.13*d*		9.84±0.11*b*
	10 min, 80°C		3.75±0.25*f*		24.22±0.30*e*		9.63±0.25*b*
	15 min, 80°C		3.82±0.31*f*		25.01±0.27*f*		9.32±0.17*b*
HPP	5 min, 200MPa		3.59±0.03*f*		21.83±0.12*b*		15.43±0.22*e*
	10 min, 200MPa		2.77±0.18*d*		21.33±0.01*b*		15.87±0.15*e*
	15 min, 200MPa		1.97±0.17*b*		21.03±0.13*b*		16.63±0.24*f*
	5 min, 400MPa		6.99±0.13*g*		21.64±0.05*b*		13.57±0.13*d*
	10 min, 400MPa		3.45±0.16*e*		21.29±0.02*b*		15.40±0.21*e*
	15 min, 400MPa		3.42±0.19*e*		20.29±0.12*a*		16.23±0.10*f*
	5 min, 600MPa		7.02±0.12*h*		21.53±0.15*b*		12.37±0.27*c*
	10 min, 600MPa		3.67±0.13*f*		21.17±0.11*b*		15.03±0.30*e*
	15 min, 600MPa		3.57±0.13*f*		20.24±0.14*a*		16.03±0.18*f*

* Three replicates of the same processing conditions were carried out and TCD was calculated according to Equation (2.5) which is expressed as the average reading together with L* and b* value with different letters are significantly different ($p < 0.05$).

Appendix C

Table 2: Physicochemical parameters (pH and brix) in goat milk after thermal and HPP processing*.

Treatment	Processing conditions	pH ±SD		°Brix ±SD	
		Raw unprocessed	**After processing**	**Raw unprocessed**	**After processing**
Thermal	5 min, 60°C	6.70±0.0	6.43±0.0	10.1±0.12	10.6±0.01
	10 min, 60°C		6.41±0.0		10.7±0.01
	15 min, 60°C		6.39±0.0		10.8±0.02
	5 min, 70°C		6.45±0.001		10.7±0.01
	10 min, 70°C		6.44±0.0		10.8±0.02
	15 min, 70°C		6.40±0.001		10.9±0.04
	5 min, 80°C		6.50±0.002		10.9±0.03
	10 min, 80°C		6.48±0.0		11.1±0.04
	15 min, 80°C		6.47±0.0		11.3±0.03
HPP	5 min, 200MPa		6.59±0.001		12.2±0.02
	10 min, 200MPa		6.58±0.0		12.2±0.02
	15 min, 200MPa		6.56±0.0		12.1±0.0
	5 min, 400MPa		6.58±0.0		12.1±0.02
	10 min, 400MPa		6.56±0.0		12.0±0.01
	15 min, 400MPa		6.54±0.0		11.8±0.01
	5 min, 600MPa		6.57±0.0		11.5±0.01
	10 min, 600MPa		6.55±0.001		11.2±0.02
	15 min, 600MPa		6.52±0.001		11.1±0.02

* Three replicates of the same processing conditions were carried out and pH, brix values are expressed as the average reading.

Appendix D

Table 3: Viscosity of goat milk after thermal and HPP processing*.

Treatment	Processing conditions	Viscosity ±SD (cP)	
		Raw unprocessed	After processing
Thermal	5 min, 60°C	21.4±0.1	17.6±0.1
	10 min, 60°C		18.2±0.1
	15 min, 60°C		18.5±0.1
	5 min, 70°C		17.8±0.1
	10 min, 70°C		18.6±0.1
	15 min, 70°C		20.1±0.1
	5 min, 80°C		19.8±0.2
	10 min, 80°C		20.6±0.1
	15 min, 80°C		21.4±0.1
HPP	5 min, 200MPa		12.5±0.1
	10 min, 200MPa		12.7±0.1
	15 min, 200MPa		15.8±0.2
	5 min, 400MPa		20.1±0.1
	10 min, 400MPa		20.5±0.1
	15 min, 400MPa		20.6±0.2
	5 min, 600MPa		19.8±0.2
	10 min, 600MPa		21.7±0.1
	15 min, 600MPa		23.3±0.1

* Three replicates of the same processing conditions were carried out and viscosity value is expressed as the average reading.