

Physicochemical composition, microbiological quality and consumers' acceptability of raw and pasteurized locally produced goat milk

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Abstract

The present study aimed to compare the raw and pasteurized goat milks in aspects of physicochemical composition, microbiological quality, and stability of lactic acid bacteria. Consumers' acceptability to locally produced goat milk was also determined. Raw and pasteurized (LTLT) Saanen goat milks were analyzed while commercial (HTST and homogenized) goat milk was used as control. While pasteurization (LTLT) showed no significant effect on fat, protein, lactose, and ash content of raw goat milk ($p > 0.05$). LTLT pasteurization kept the physicochemical properties of goat milk such as pH and viscosity similar to raw goat milk ($p > 0.05$), there was a significant difference of titratable acidity ($p < 0.05$). After LTLT and HTST pasteurizations, goat milk showed an increase in lightness with a reduction in yellowness, as well as significant reduction in microbial load. Survivability of lactic acid bacteria (anaerobic) was not affected by both pasteurization process ($p > 0.05$). All of the studied goat milks contain lactic acid bacteria, primarily *Enterococcus* sp. via molecular identification by using 16S rRNA primer. Thus, LTLT pasteurization process at 63°C, 30 minutes might be applied to develop fermented dairy products such as probiotic goat drinks. According to sensory evaluation, consumer showed acceptability to locally produced goat milk with preference to commercial goat milk samples. In conclusion, lactation stage of goats and thermal processing conditions applied need to be considered since it might affect the quality of goat milk produced. To sum, the current information of locally produced goat milk would be useful for the development and manufacture of goat milk products especially in local dairy industry.

Keywords: Pasteurized goat milk, physicochemical composition, microbiological qualities, lactic acid bacteria, sensory evaluation

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INTRODUCTION

Pasteurized milk is the milk that has been heated properly with operated equipment, at a specific temperature, and held continuously at specified time while raw milk is obtained straight from the ruminant livestock such as cows and goats. Milk and dairy products are widely consumed worldwide and it has become an essential part of human dietary. Due to the rapid increasing human population, demand for food including milk and milk products are on the rise. The rising demand can be met by growing other ruminant livestock population for their meat and milk. In Malaysia, the National Dairy Industry Development Programme had been implemented since 2017 which intend to increase the production of fresh milk by adding another 20 million liters through the importation of 10,000 pregnant dairy cows. The government of Malaysia is aiming for self-sufficiency level (SSL) in local fresh milk production within the next five years (Anonymous, 2019).

Goat which is universally named as poor man's cow is a good alternative when there is an insufficiency of cow and cow milk. According to Food and Agricultural Organization (2018), goat shows a flattering lactation curve with higher persistency compared to dairy cow. Moreover, goat has better adaptability to diverse environment such as harsh climate and provides food security than cow (Devendra, 2007). Hence, dairy goat farming is suitable for landless farmers or small farm system. In addition, goat milk production plays a significant role in economy and health of many countries especially developing countries such as Asia, Africa, the Middle East, and Mediterranean countries (Yangilar & Filiz, 2013). In rural and developing countries, goat milk is the main milk producer and provides the nutrient to the poor and undernourished population in order to cope with malnutrition problem (Devendra, 2007). There are a lot of proven health benefits of goat milk. Goat milk showed better buffer capacity, digestibility, and its particular therapeutic value in

medicine and human nutrition compared to cow milk (Park, 2007). Despite its potential, goat milk production in Malaysia is not commercialized widely. Besides, it is found that the local market needs of milk in Malaysia may not be fully supported by total milk production of small-scale dairy farms (Lye et al., 2013). Furthermore, the number of researches conducted on locally produced goat milk is limited. Quality standard of goat milk is highly depending on sources of other countries such as China, Thailand, New Zealand, Indonesia, and European standard (Lai et al., 2016).

Information on physicochemical properties of goat milk is useful to improve goat milk industry in Malaysia as compositions of local goat milk may different to the outsource information. The details regarding physicochemical attributes of goat milk might be used in a food industry as a functional diet for human health since goat milk has similarity to human milk (Hayam et al., 2014). Composition of goat milk is easily influenced by locations, weather and breeds (Lai et al., 2016). Besides, outbreak of foodborne infections associated with raw milk consumption due to the presence of pathogenic bacteria has been reported (Eglezos et al., 2008). Although the consumption of raw milk is a health threat, there are consumers obtain raw milk via direct purchase from farmers (Oliver et al., 2009). Consumption of raw milk is also common among farm families (Lejeune & Schultz, 2008).

A good understanding of physicochemical properties of goat milk can help to improve the quality of locally produced goat milk so that it can become a viable alternative to cow milk in Malaysia. According to Lai et al. (2016), proximate analysis determines the constituents of milk which give effect on the nutritional and sensory properties that contribute to the good quality of milk (Armstrong, 1995). It is believed that well-handled goat milk can provide a delicious, slightly sweet taste with a salty tint which is similar to the taste and odor of cow milk (Wanjekeche et al., 2016).

Therefore, the objectives of the study were to determine composition and physicochemical properties, enumerate the common microbial load (aerobic bacteria, coliform, *Escherichia coli*, *Staphylococcus aureus*), identify the stability and species of lactic acid bacteria in raw and pasteurized (LTLT and HTST) goat milk, as well as to determine consumer's acceptability to locally produced goat milk.

EXPERIMENTAL

Materials

Raw goat milk samples from Saanen-type goats (90 % pure breed) were collected at UniSZA community farm in Besut, Terengganu. Those Saanen goats were fed with napier grass combine with concentrate containing 18 % of crude protein and 20 % crude fibers, obtained from local feed supplier. Those goats weighed from 29 kg to 60 kg. Since this study was done in a farmer's farm, of the age of these goats were varied. This experimental design limitation was overcome through allocation of the most uniform available does in the same replication thus reducing the experimental error. The lactation period is the period between parturition (giving birth) to cessation of milk. The standard lactation period in Saanen goats for this study were 280 days. There were three (3) cycles of goat milk collection involved in this study during lactation period. In the first cycle, goat milk was collected on day 91, 93, and 95. In the second cycle goat milk were collected on day 125, 127, and 129 while in the third cycle goat milk were collected on day 159, 161, and 163.

Raw goat milk received from UniSZA community farm was quickly filled into small, sterilized plastic bottles of 200 mL capacity each and was stored at temperature ≤ -18 °C before further analysis. Eight (8) liters of the raw goat milk received from dairy goat farm were treated with low-temperature-long-time (LTLT) pasteurization at 63 °C for 30 minutes. Pasteurized goat milk samples were divided into aliquots for the microbiological analysis which was performed on the same day of pasteurization process. The rest of the pasteurized goat milk samples were frozen at storage temperature ≤ -18 °C until analysis. Proximate, physicochemical, and microbiological analyses were performed on raw and pasteurized Saanen goat milk, with commercial goat milk as a control. The commercial goat milk used in

this study was purchased from market which was locally produced goat milk, and was treated with homogenization and high-temperature-short-time (HTST) pasteurization at ≥ 90 °C for 3 seconds.

Proximate analysis

Proximate analysis of goat milk samples was conducted to determine the moisture content (water), protein, fat, carbohydrates, and ash contents according to AOAC (2000). Ash content was determined by furnace drying, moisture content was determined by oven-drying method, crude protein (N=6.38) by Kjeldahl method, and crude fat by Soxhlet method. Carbohydrate was determined by calculation which the total amount of protein, fat, moisture, and ash was deducted from 100 %. The analysis of each sample was performed in triplicates. The proximate result was expressed in wet basis.

pH analysis

The strength of acid in milk was indicated by pH value. The pH of sample was determined using Thermo Scientific pH Electrode (Orion Star, USA). The pH meter was calibrated with buffer solution of pH 4.0, 7.0, and 10.0 prior to analysis.

Titrate acidity analysis

Acidity of milk was measured by method of neutralization with alkali as described by Connor (1995). A milk sample of 10 g was measured into a conical flask and was titrated with 0.1N sodium hydroxide with phenolphthalein indicator. The end point of titration was reached when a faint pink color appeared. Titratable acidity of milk was expressed as lactic acid percent as milk had natural acidity or developed acidity due to the bacterial action on lactose in milk. Lactic acid percentage was calculated using the equation below as described in GEA (2006).

$$\% \text{ Lactic acid (wt/wt)} = \frac{9 \times N \times V}{W}$$

where: N= Normality of NaOH
V= Volume of titrant (mL)
W= Weight of sample (g)

Viscosity analysis

Viscosity of milk was measured by using Brookfield viscometer (Model LVDV-II+ Pro, USA) at given shear rate under ambient temperature of 25 °C. The viscometer was leveled and auto zeroed before taking measurement. Spindle LV1 used was set at 100 rpm. Viscosity measurements of milk samples was taken after 40 s of spindle rotation and recorded in unit of centipoise (cP).

Color analysis

The color measurement of goat milk samples was measured by using Chroma Meter CR-400 (Konica Minolta, Japan). The color readings are according to L* [Lightness (L = 100; White and L = 0; Black)], Chroma a* [Green chromaticity (-60) to red (+60)] and chroma b* [blue chromaticity (-60) to yellow (+60) space value. The chroma meter was calibrated by using calibration plate.

Lactose content analysis

Lactose content of milk was determined by using lactose test kit (K-LOLAC) introduced by Megazyme with spectrophotometer (Thermo Spectronic 4001/4, USA). Sample clarification, pre-incubation of samples, removal of free D-glucose, and measurement of lactose content through sequential enzymatic reaction were conducted according to the manual instruction. Absorbance for blank and samples were read at 365 nm. The lactose content of sample was calculated by using the following equations:

$$C_{\text{lactose}} = [0.3233 \times F \times \Delta A_{\text{lactose}}] \times 1.8529$$

where: F = dilution factor, 250 (sample preparation for 'regular' dairy product)

$$\text{Lactose content} = \frac{C_{\text{lactose}} [\text{g/L sample solution}]}{[\text{g/100 g}]} \times 100$$

Microbiological analysis

Serial dilutions of 10^{-1} , 10^{-2} and 10^{-3} were prepared in sterile peptone water for enumeration of total aerobic bacteria, coliforms, *E. coli*, and *Staphylococcus aureus* while serial dilutions of 10^{-1} , 10^{-2} and 10^{-3} of MRS broth used for enumeration of lactic acid bacteria, respectively. A volume of 0.1 mL from each dilution was pipette and spread plated in duplicate onto agar plates while 1 mL of each dilution was used in petrifilm. The total plate count of aerobic bacteria was enumerated by the plate count technique by using plate count agar (Merk) medium incubated at 37 ± 1 °C for 48 hours. Coliforms and *E. coli* were enumerated by the plate count technique by using Eosin-Methylene Blue (EMB) agar (Merk) medium incubated at 37 ± 1 °C for 48 hours. Blue-dark purple colonies were counted as coliforms. Dark colonies with green metallic sheen were counted as *E. coli*. *Staphylococcus aureus* was enumerated by using 3MTM Petrifilm Staph Express Count Plate which contains modified Baird-Parker medium incubated at 37 ± 1 °C for 48 hours. Red violet colonies were counted as *Staphylococcus aureus*.

Lactic acid bacteria (LAB) are facultative bacteria that are capable of growing in the presence or absence of oxygen. Thus, in this study, we intended to identify the concentration of lactic acid bacteria in goat milk samples for both aerobic and anaerobic conditions prior to be proceed with molecular identification in order to determine the species. In this study, lactic acid bacteria were enumerated under aerobic condition by using MRS agar as well as under anaerobic condition by using 3MTM PetrifilmTM. A comparison study by Nero *et al.* (2000) reported that there was no significant difference ($p > 0.05$) when compared PetrifilmTM AC and Agar MRS (Man-Rugosa-Sharpe) for the enumeration of lactic acid bacteria in fermented milk. This comparison study also in agreement with the study done by Satomi *et al.* (2018) where all colonies growing on Petrifilm LAB Count Plates were confirmed to be LAB. In addition, Maria *et al.* (2007) stated that there was the possibility of using Petrifilm AC plates for enumeration of LAB in milk, even with the use of selective supplements which the results showed excellent correlation indexes between both methodologies using three culture media for LAB.

After incubation at 37 ± 1 °C for 48 hours, pale straw colonies on MRS agar and red colonies with or without entrapped gas on petrifilm were counted as lactic acid bacteria. The bacterial colonies were counted as equation shown and expressed in log CFU/ml.

$$\text{CFU/ml} = \text{count} \times \frac{1}{\text{dilution}} \times \frac{1}{\text{inoculum}}$$

Molecular identification of lactic acid bacteria

Lactic acid bacteria isolated on 3MTM PetrifilmTM Lactic Acid Bacteria Count Plates was inoculated aseptically into deMan, Rogosa, and Sharpe (MRS) broth. A layer of mineral oils was added on the top of MRS broth to create anaerobic condition that favored the growth of lactic acid bacteria. The cultures in MRS broth was grown overnight at 37 °C in incubator shaker.

DNA extraction

Genomic DNA of isolated lactic acid bacteria was extracted by using Wizard® Genomic DNA Purification Kit according to the instruction use. Five main processes involved in DNA extraction were lysis of cell and nuclei by 50 mM EDTA, 10 mg/mL lysozyme and Nuclei Lysis Solution, digestion by RNase Solution, removal of protein by Protein Precipitation Solution, DNA precipitation using ethanol and isopropanol and DNA rehydration by DNA Rehydration Solution.

Polymerase chain reaction (PCR) and gel electrophoresis

Genomic DNA from lactic acid bacteria isolates were amplified in targeted 16S rRNA gene sequence via polymerase chain reaction (Tilahun *et al.*, 2018). The reaction was done in Eppendorf Thermal Cycle (Applied Biosystem, USA) by using PF3 (5'-CTAAGAGAAGCAAGTGTAATTGGCTAGCG-3') as forward primer and PR3 (5'-GTCATGGTCTTATAGTCCTTGGGAATG-3') as reverse primer. The PCR conditions were standardized as follows: initial denaturation at 95 °C (5 minutes), denaturation at 94 °C (1 minute), annealing at 60 °C (1 minute) and extension at 72 °C (2 minutes). The cycle was repeated for 30 times and final extension at 72 °C for 5 minutes. The PCR products were analyzed by 1 % agarose gel electrophoresis with ethidium bromide together with 1 kb DNA marker (Promega, USA). The presence of the PCR product was visualized by using Gel-doc system.

DNA Sequencing

All purified PCR products were sent for sequencing and alignment of DNA sequences were done by using Basic Local Alignment Search Tool (BLAST). The sequence was compared with the bacteria database available in the National Center for Biotechnology Information (NCBI) for bacteria identification.

Sensory analysis

Sensory evaluation with pasteurized goat milk and commercial goat milk was conducted to determine consumer acceptability to goat milk. A total of 65 panelists evaluated the attributes of goat milk (color, thickness, odor, taste and overall acceptability) by using 7-point hedonic scales (Islam *et al.*, 2012).

Statistical analysis

Data of analysis was analyzed by using SPSS Statistics version 17.0 software. One-way ANOVA followed by Tukey Kramer procedure were used to test whether there was a significant difference between samples at 95 % confidence level.

RESULTS AND DISCUSSION

Proximate analysis and lactose content

Proximate composition and lactose content of all goat milk samples are presented in Table 1. The effect of pasteurization on moisture content of goat milk was significant ($p < 0.05$). Raw goat milk showed the higher ($p < 0.05$) moisture content while compared to pasteurized (LTLT and HTST) goat milk showed the lowest moisture content. In contrast, pasteurized goat milk contained higher ($p < 0.05$) total solid than raw goat milk. Lower moisture in pasteurized goat milk may be attributed to the evaporation of water during heat treatment. Al-Hilphy and Ali (2013) also reported that moisture of milk decreased after flash pasteurization at 100 °C for 0.01 seconds might be attributed to the evaporation of water at high temperature.

Protein content of goat milk ranged from 3.26 ± 0.16 % to 3.43 ± 0.04 %. The current result was in line with previous study of Denis *et al.* (2016) which reported that the protein content of goat milk ranged from 2.79 % to 3.76 %. The protein content of goat milk showed no significant difference ($p > 0.05$) after pasteurization process. This might due to approximately 75 % of milk protein was heat-stable casein. Mild heat treatment may induce the interaction of denatured whey protein with casein micelles (Huppertz & Kelly, 2009). However, the protein nutritional quality in milk was not affected by protein denaturation (MacDonald *et al.*, 2001). Fat content of goat milk samples ranged from 1.34 ± 0.20 % to 2.75 ± 0.15 %. The result showed that raw and pasteurized (LTLT) goat milk showed no significant difference in fat content ($p > 0.05$) while they were significantly higher to commercial goat milk samples ($p < 0.05$). This indicates that pasteurization gives no impact on milk fat content while the fat content varies with type of breeds. According to Al-Hilphy and Ali (2013), fat content in goat milk remained similar after pasteurization process. It is further supported by Huppertz and Kelly (2009) mentioned that the temperature required for non-oxidative thermal degradation of lipid in milk is more than 200 °C. Moreover,

the variation of total fat content in goat milk was due to breed, feed, and seasonal factors (Lai et al., 2016).

The proximate carbohydrate measurement of all goat milk samples was significantly different ($p < 0.05$) and ranged from 4.62 ± 0.25 % to 6.68 ± 0.21 %. The current result was higher than the reference values of 4.17 % from USDA (2018) but was in the agreement to the findings of Hassan et al. (2010) that carbohydrate compound in goat milk ranged from 5.4 % to 6.4 % throughout lactation month. In this study, goat milk was collected on days 91, 93, 95, 125, 127, 129, 159, 161, and 163 of lactation for quantity and quality evaluation.

According to Mourad et al. (2014), lactose is the major carbohydrate in milk and is made up of one molecule of D- galactose and one molecule of D-glucose. Lactose content of the current study ranged from 4.35 % to 4.65 % and showed no significant difference ($p > 0.05$) between raw, pasteurized (LTLT), and commercial sample (HTST) (Table 1). The current lactose result was in line to the lactose present in goat milk reported by Mourad et al. (2014) compiled from previous study which was in range of 4.4 % to 4.7 %. According to Forsback et al. (2010), lactose was a stable compound in osmotic regulation of milk and was found to have a little variation. However, from current study, the lactose and carbohydrate contents of pasteurized goat milk samples (LTLT and HTST) were different considerably, ($p < 0.05$). This might be due to the presence of other types of carbohydrates in significant amount in both pasteurized samples. According to Zenebe (2014), oligosaccharides, glycopeptides, glycoproteins, and nucleotides were also found in goat milk in small amounts. Moreover, goat milk is significantly rich in lactose-derived oligosaccharides compared to cow milk (Sachin et al., 2017). The ash content of goat milk samples ranged from 0.69 ± 0.10 % to 0.74 ± 0.20 %. The obtained result was in agreement to the previous study of Hassan et al. (2010) where ash content of goat milk showed similar trend throughout the lactation period which was approximately 0.7 %. The effect of heat treatment during pasteurization process both LTLT and HTST (commercial) on the ash content of goat milk was no significant ($p > 0.05$). This indicates that ash was stable under high temperature. Ash is the remained residue after incineration and represents the total minerals in milk (Lai et al., 2016). Ash comprised of carbonates, oxides and phosphates of mineral element due to chemical changes during ashing process (Kanwal et al., 2004).

Table 1 Proximate composition and lactose content of goat milk samples.

Composition (%)	Raw	Pasteurized (63 °C, 30 minutes, LTLT)	Commercial (≥ 90 °C, 3 seconds, HTST)
Moisture	$88.66^a \pm 0.51$	$87.54^b \pm 0.14$	$87.94^{ab} \pm 0.02$
Total solid	$11.34^b \pm 0.51$	$12.46^a \pm 0.14$	$12.06^{ab} \pm 0.02$
Protein	$3.43^a \pm 0.04$	$3.26^a \pm 0.16$	$3.35^a \pm 0.29$
Fat	$2.54^a \pm 0.06$	$2.75^a \pm 0.15$	$1.34^b \pm 0.20$
Carbohydrate	$4.62^c \pm 0.25$	$5.71^b \pm 0.17$	$6.68^a \pm 0.21$
Lactose	$4.65^a \pm 0.30$	$4.35^a \pm 0.16$	$4.48^a \pm 0.12$
Ash	$0.74^a \pm 0.20$	$0.74^a \pm 0.10$	$0.69^a \pm 0.10$

The numbers represent mean \pm sd of triplicate. Mean values in the same row with different superscripts are significantly different ($p < 0.05$).

Physicochemical Properties

Physicochemical properties of goat milk samples which include pH, titratable acidity and viscosity are shown in Table 2. There was a significant difference of titratable acidity ($p < 0.05$) between raw goat milk and pasteurized goat milk (LTLT). However, the increased titratable acidity in pasteurized goat milk (LTLT) did not reflect on a significant pH change. This might due to goat milk has a better buffer capacity that could resist the pH changes as stated by Park (2007). The titratable acidity and pH of raw, pasteurized (LTLT) and commercial (HTST) goat milk samples obtained in current study ranged from 0.16 ± 0.01 % to 0.17 ± 0.01 % and 6.50 ± 0.01 to 6.53 ± 0.01 respectively. The obtained result was in line with the study of Fandialan and Davide (2001) where the titratable acidity and pH of goat milk were in range of 0.126 % to 0.195 % and 6.20 to 6.55 respectively. This result indicates that there was lactic acid produced by lactic acid bacteria in goat milk virtually. In addition, the acidity of goat milk might be due to the presence of citrates, carbon dioxide, phosphates, whey proteins and casein (Lai et al., 2016). The normal pH range of current study showed that there was no sign of mastitis infection in all samples (Ogola et al., 2007). Mastitis increases milk pH due to the blood and extracellular fluid components in inflamed quarters enter into milk during secretion (Kandeel et al., 2018).

The viscosity result of current study was in accordance to the findings of Roman et al. (2015) that goat milk samples showed viscosity values in a range from 1.63 cP to 1.85 cP at 20 °C. Commercial goat milk (HTST) sample showed higher viscosity and was significant different ($p < 0.05$) to raw and pasteurized (LTLT) goat milk. This might due to commercial goat milk (HTST) is type of homogenized milk. According to Bakshi and Smith (1984), homogenized milk has higher viscosity than unhomogenized milk as the fat was fine and well dispersed. In addition, high-temperature-short-time (HTST) pasteurization might also confer commercial goat milk higher viscosity. According to Roman et al. (2015), content of fat, dry matter and proteins showed significant effect on viscosity of goat milk. Anema and Li (2003) reported that heat treatment induced association of denatured whey protein with casein micelles which contributed to an increase in viscosity of skim milk.

Table 2 Physicochemical properties of goat milk samples.

Analysis	Raw	Pasteurized (63 °C, 30 minutes, LTLT)	Commercial (≥ 90 °C, 3 seconds, HTST)
Titratable Acidity (%)	$0.16^b \pm 0.01$	$0.17^a \pm 0.01$	$0.16^b \pm 0.01$
pH	$6.50^a \pm 0.01$	$6.52^a \pm 0.02$	$6.53^a \pm 0.01$
Viscosity (cP)	$1.54^b \pm 0.04$	$1.48^b \pm 0.07$	$2.06^a \pm 0.04$

The numbers represent mean \pm sd of triplicate. Mean values in the same row with different superscripts are significantly different ($p < 0.05$).

Color analysis

The color results of raw, pasteurized, and commercial goat milk are presented in Table 3. There was a significant effect ($p < 0.05$) of pasteurization process both LTLT and HTST on the color of goat milk in terms of L^* (lightness), a^* (redness) and b^* (yellowness). Raw goat milk showed higher L^* and a^* value but a lower b^* value compared to pasteurized and commercial goat milk. The current result was in agreement to the study of Chugh et al. (2014) that raw skim milk increased in L^* value but reduced in b^* value after processing with high-temperature- short-time (HTST) pasteurization. This indicates that goat milk gained lightness and lost yellowness, achieving its typical whitish color after pasteurization. Denaturation of soluble whey protein in milk during high temperature processing

might cause an increase in lightness (Browning *et al.*, 2001). Based on obtained result, commercial (HTST) goat milk showed the highest L* value among goat milk samples and this might due to commercial (HTST) goat milk was homogenous and no significant difference ($p > 0.05$) in a*(redness) and b*(yellowness). Well dispersed and fine fat particles with no fat clusters confer more even light reflection. However, according to Solah *et al.* (2007), natural color of milk is also attributed to the reflectance of light by dispersed milk fat globules, proteins, and other milk pigments such as riboflavin and carotenoids. From the result, significant differences ($p < 0.05$) were observed in the redness and yellowness value for raw as well as pasteurized goat milk both LTLT and HTST (commercial). Thus, it might be said that, the heat and homogenization process applied to the milk might have effect on color attributes.

Table 3 Color value of goat milk.

Sample of milk	L (lightness)	a* (redness)	b* (yellowness)
Raw	63.99 ^c ± 2.08	-1.92 ^a ± 0.24	6.11 ^a ± 0.41
Pasteurized (63°C, 30 minutes, LTLT)	70.34 ^b ± 0.92	-2.93 ^c ± 0.05	3.36 ^c ± 0.14
Commercial (≥ 90 °C, 3 seconds, HTST)	75.93 ^a ± 0.72	-2.29 ^b ± 0.02	4.33 ^b ± 0.04

The numbers represent mean ± sd of triplicate. Mean values in the same column with different superscripts are significantly different ($p < 0.05$).

Microbiological analysis

The bacteria load of goat milk samples is shown in Table 4. There was a significant effect of pasteurization (LTLT and HTST) on the total aerobic bacteria, coliform and *Staphylococcus aureus* in goat milk ($p < 0.05$). Total aerobic count can be an indicator of milk quality. Total aerobic bacteria count was the highest in raw goat milk and aerobic bacteria concentration was reduced significantly ($p < 0.05$) in pasteurized (LTLT) and commercial (HTST) goat milk. The total aerobic bacteria in raw goat milk was 5.99 ± 0.11 log CFU/mL and exceeded the microbiological standard set by Food Regulations (1985) which was 5.0 log CFU/mL for safe consumption of pasteurized milk. The aerobic bacteria count of raw goat milk also represented the level of contamination during milking process that might cause by several factors such as cleanliness of equipment, milk handlings and health of the goat's udder. However, pasteurized (LTLT) and commercial (HTST) goat milk showed acceptable total aerobic bacteria load which were in accordance to Food Regulations (1985). According to Murphy (2007), aerobic bacteria survive in pasteurized milk are the gram- positive thermophilic bacteria present in raw milk such as lactic acid bacteria and spore-forming bacteria.

Presence of coliform organisms in milk is an indication of unsanitary production or improper handling during milking. Pasteurization process (LTLT and HTST) showed significant effect on the coliform load of goat milk ($p < 0.05$). The current study showed that only raw goat milk contained coliforms and *Escherichia coli* (one of the coliforms) was not detected in all goat milk samples. Coliform in raw goat milk was higher than the level set by Food Regulations (1985), where pasteurized milk should not contain coliform more than 1.7 log CFU/mL. This indicates that pasteurization process of raw milk is necessary in order to reduce coliform load for safe consumption. The absence of coliforms in goat milk after pasteurization in current study was in accordance to Banik *et al.* (2014) who reported that there was no coliform present in milk samples treated with proper pasteurization process. This was further supported by Murphy (2007) who stated that gram-negative bacteria generally do not survive under pasteurization process condition.

Table 4 shows that there was a significant effect ($p < 0.05$) of pasteurization (LTLT and HTST) on *Staphylococcus aureus* count in

goat milk. Current result showed that *Staphylococcus aureus* in raw milk was unable to survive after processing with pasteurization (LTLT and HTST). The presence of *Staphylococcus aureus* in raw goat milk indicated that there was contamination during milking, most probably from humans. The absence of *Staphylococcus aureus* in pasteurized goat milk (LTLT and HTST) was in agreement to study reported by Leite *et al.* (2002) that there was no *Staphylococcus aureus* being detected in milk treated with pasteurization process. According to Oliveira *et al.* (2011), *Staphylococcus aureus* count with more than 3.0 log CFU/mL increased the risk of staphylococcal toxin production which was resistant under thermal processing.

Table 4 Bacterial count of goat milk samples (log CFU/mL).

Sample of milk	Aerobic bacteria	<i>E. coli</i>	Other coliforms	<i>Staphylococcus aureus</i>
Raw	5.99 ^a ± 0.11	0.00 ± 0.00	3.01 ^a ± 0.75	2.87 ^a ± 0.24
Pasteurized (63°C, 30 minutes, LTLT)	4.68 ^b ± 0.06	0.00 ± 0.00	0.00 ^b ± 0.00	0.00 ^b ± 0.00
Commercial (≥ 90 °C, 3 seconds, HTST)	4.01 ^c ± 0.11	0.00 ± 0.00	0.00 ^b ± 0.00	0.00 ^b ± 0.00

The numbers represent mean ± sd of duplicates. Mean values in the same column with different superscripts are significantly different ($p < 0.05$).

Stability of lactic acid bacteria (LAB)

The result shown in Table 5 indicates that there was lactic acid bacteria present naturally in goat milk. The current result showed that lactic acid bacteria load was higher under anaerobic condition than that under aerobic condition. This indicates that absence of oxygen favored the growth of lactic acid bacteria. According to Michaela *et al.* (2009), lactic acid bacteria are aerotolerant anaerobes. Lactic acid bacteria grow favorably under anaerobic conditions, but they can also grow in presence of oxygen as they contain peroxidases to protect themselves from oxygen by products such as H₂O₂ (Khalid, 2011). The effect of pasteurization process (LTLT and HTST) was no significant ($p > 0.05$) when lactic acid bacteria isolated from goat milk was incubated under anaerobic condition. This indicates that lactic acid bacteria in goat milk was thermophile and could survive under pasteurization temperatures (LTLT and HTST). Perez-Chabela *et al.* (2008) reported that four strains of lactic acid bacteria including *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Pediococcus pentosaceus* and *Pediococcus acidilacti* are able to survive under thermal treatment at 70 °C for 60 minutes. Macronutrient in pasteurized milk might be the reason for the lactic acid bacteria to survive against pasteurization temperature (Malik *et al.*, 2018).

Table 5 Lactic acid bacteria load log CFU/mL in goat milk.

Sample of milk	Aerobic	Anaerobic
Raw	3.48 ^a ± 0.11	4.21 ^a ± 0.72
Pasteurized (63°C, 30 minutes, LTLT)	2.89 ^b ± 0.16	4.04 ^a ± 0.03
Commercial (≥ 90 °C, 3 seconds, HTST)	0.00 ^c ± 0.00	4.30 ^a ± 0.75

The numbers represent mean ± sd of duplicates. Mean values in the same column with different superscripts are significantly different ($p < 0.05$).

Molecular identification of lactic acid bacteria (LAB)

Microorganism in goat milk primarily consisted of *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Enterococcus* species which known with probiotic properties (Oscar et al., 2016). Lactic acid bacteria within the goat milk samples isolated on selective MRS agar formed colony with similar morphology and this can be assumed that there was one dominant lactic acid bacteria present in goat milk samples. Based on Table 6, by sequencing the 16S rRNA gene from lactic acid bacteria isolated from goat milk samples, it was identified that *Enterococcus* sp. was the predominant lactic acid bacteria present in all goat milk samples with identity percentage higher than 95 %. This indicates that *Enterococcus* sp. in goat milk is resistant to pasteurization temperatures. According to Mirtha (2005), enterococci are recognized as the most thermo-resistant among the non-sporulated bacteria. Ismail et al. (2018) reported that lactic acid bacteria isolated from fermented, pasteurized milk drinks were identified as *Enterococcus* genus. They also are able to survive during milk refrigeration and pasteurization temperatures due to their psychotropic nature, heat resistance and adaptability to different substrates and growth conditions (Bhardwaj et al., 2008).

According to Yang et al. (2014), several *Enterococcus* strains produce antimicrobial compounds including bacteriocins which has been applied in food preservation and is now being considered as a probiotic trait. Enterococci are important in dairy industry and its presence among other lactic acid bacteria in raw milk can act as natural starter (Hanchi et al., 2018). *Enterococcus* strains are able to survive, compete and adhere to host cells in the GIT. These features are important for a successful use as probiotics (Laukova et al., 2017). *Enterococcus* also suitable as starter cultures as they may give the typical organoleptic characteristics of various fermented foods, including dairy products. Some *Enterococcus* strains have been proved to be safe and effective and are used as food supplements in several probiotic preparations such as *E. faecium* SF-68 and *E. faecium* M74 (Serio et al., 2010). In addition, the use of administered *Enterococcus* strains as probiotics has been studied to treat irritable bowel syndrome, diarrhea or antibiotic-associated diarrhea, to lower the cholesterol levels as well as to improve host immunity (Araujo & Ferreira, 2013). For instance, *E. durans* and *E. faecalis* have been reported in raw and fermented milk (Hanchi et al., 2014). Thus, based on result obtained in Table 6, goat milk might also being process into fermented food such as probiotic drink since *Enterococcus* sp. present in all goat milk samples.

Table 6 Lactic acid bacteria (LAB) isolated from goat. milk

Sample of milk	Bacteria Identified	Identity Percentage (%)
Raw	<i>Enterococcus</i> sp.	95.23
Pasteurized (63°C, 30 minutes, LTLT)	<i>Enterococcus</i> sp.	95.23
Commercial (≥ 90 °C, 3 seconds, HTST)	<i>Enterococcus</i> sp.	96.58

Amplification of 16S rRNA gene region via polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a technique used to amplify or make many copies of particular targeted region of DNA *in vitro*. In this study, PCR was completed by using primers encoded for bacteria 16S rRNA gene sequence. Fig. 1(b) shows that the size of PCR amplified products which were approximately 1500 base pairs (bp) based on gene marker of 1 kb DNA ladder (Promega, USA) shown in Fig. 1(a). It is supported by the previous study of Hidayat (2017) that the DNA of isolated lactic acid bacteria amplified 16S rRNA gene by universal primer via PCR showed product size of 1500 base pairs. 16S rRNA gene sequence was used in this study as it is the most common genetic marker to study bacterial phylogeny and taxonomy (Janda & Abbott, 2007). In addition, 16S rRNA gene sequences present in most of the bacteria and function of the 16S rRNA gene over time remain

unchanged (Patel, 2001). Moreover, the 16S rRNA gene consists of approximately 1,500 base pairs which are large enough for informatics purposes (Patel, 2001).

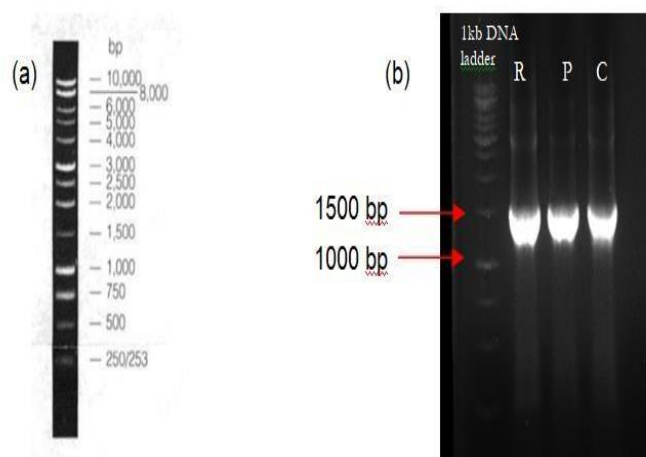


Fig. 1(a) 1kb DNA ladder (Promega, USA) (Stevens, 2011). (b) PCR product from raw (R), pasteurized (P), commercial (C) goat milk.

Sensory analysis

Based on Table 7, there was significant difference ($p < 0.05$) between goat milk samples (LTLT and HTST) in terms of color, thickness, taste and overall acceptability. Commercial goat milk (HTST) showed a higher score ($p < 0.05$) in color than pasteurized milk (LTLT). This indicates that panelist preferred goat milk that was more whitish in color. Commercial goat milk (HTST) showed a higher L^* value than pasteurized goat milk (LTLT) (Table 3). Commercial goat milk (HTST) also scored higher than pasteurized goat milk (LTLT) ($p < 0.05$) in thickness. Thickness of commercial goat milk (HTST) was more favored by panelist due to its higher viscosity (Table 2) with well dispersed fine fat particles. In terms of parameter in taste, panelist rated the commercial goat milk (HTST) as 'slightly like' while rated pasteurized goat milk (LTLT) as 'neither like or dislike'. This was attributed to the lower fat content of commercial goat milk (HTST). Fatty acids such as capric, caprylic and caproic acids contribute to the specific 'goaty' flavor of goat milk (Kompan & Komprij, 2012). Based on the overall acceptability, commercial goat milk (HTST) showed a higher acceptability than pasteurized goat milk (LTLT). This indicates that homogenization and lower fat content may probably increase the consumers' acceptability to goat milk.

Table 7 Average results (n=65) of sensory acceptance evaluation of goat milk samples in 7-point hedonic scales.

Attributes	Pasteurized (63 °C, 30 minutes, LTLT)	Commercial (≥ 90 °C, 3 seconds, HTST)
Color	5.20 ^b ± 1.64	6.26 ^a ± 0.69
Thickness	5.02 ^b ± 1.46	5.88 ^a ± 0.84
Odor	5.09 ^a ± 1.37	5.42 ^a ± 1.25
Taste	4.35 ^b ± 1.54	5.22 ^a ± 1.45
Overall acceptability	4.74 ^b ± 1.19	5.55 ^a ± 1.28

Mean values in the same row with different superscripts are significantly different ($p < 0.05$).

CONCLUSION

Low-temperature-long-time (LTLT) pasteurization process used in this study altered the proximate of goat milk by reducing significantly ($p < 0.05$) its moisture content and thus increasing significantly its total solid content. Pasteurization at 63 °C, 30 minutes (LTLT) kept other proximate component such as fat, protein, and ash content similar to raw goat milk. Increased total solid after LTLT pasteurization contributed to a higher proximate carbohydrate content in goat milk. However, both pasteurization process (LTLT and HTST) showed no significant effect ($p > 0.05$) on lactose content. Proximate carbohydrate by difference was not a good estimation of lactose content in milk because it may contain other types of carbohydrates. Significant changes ($p < 0.05$) in titratable acidity of goat milk due to pasteurization (LTLT) did not reflect on the pH of goat milk might due to its buffer capacity. Viscosity of goat milk did not change significantly ($p > 0.05$) due to LTLT pasteurization but viscosity of goat milk might be increased due to homogenization and HTST pasteurization. Both pasteurization process (LTLT and HTST) increased the lightness and reduced yellowness of goat milk and made it more favored by consumers. Pasteurization at (63 °C, 30 minutes) improved the microbiological quality of goat milk by reducing the total aerobic bacteria, total coliforms and *Staphylococcus aureus* to an acceptable level and significant ($p < 0.05$). Lactic acid bacteria (anaerobic) showed good stability even under pasteurization temperature and did not reduce in concentration after both pasteurization process (LTLT and HTST ($p > 0.05$)). *Enterococcus* sp. was the main population of lactic acid bacteria present naturally in all goat milk sample tested. Thus, it might be assumed that goat milk has potential to be developed into fermented dairy products such as probiotic goat milk drinks. Sensory evaluation data showed that consumers accepted locally produced goat milk. Lowering fat content and homogenization might increase the consumers' acceptability to goat milk. Lactation stage of goats and thermal processing conditions applied might affect the quality of goat's milk produced. Since there is insufficient current data and standard for goat milk in Malaysia, more study about locally produced goat milk is recommended in order to improve the commercialization of goat milk in dairy industry as well as local farms. Moreover, platform test such as clot on boiling test and alcohol test should be performed upon sample collection to ensure good stability of milk protein under heat treatment.

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