

Effect of High-Pressure Processing (HPP) on composition, lactose and microstructure of goat milk

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Article history:

Received: 22 May 2020

Received in revised form: 2 September 2020

Accepted: 25 January 2021

Available Online: 31 January 2021

Keywords:

Fat globule,
Goat milk,
High pressure processing,
Lactose,
Microstructure

DOI:

[https://doi.org/10.26656/fr.2017.5\(S1\).050](https://doi.org/10.26656/fr.2017.5(S1).050)

Abstract

Thermal pasteurisation is an established method for milk processing. However, the high temperature could affect the micronutrients in the milk. High pressure processing (HPP) is a cold alternative to thermal pasteurisation that can maintain the fresh-like properties of liquid food. However, employing pressure could potentially affect the composition and microstructure of milk and milk products. Therefore, this study focusses on evaluating the effect of high pressure processing (HPP) towards the composition, lactose content and microstructure (in term of fat globules) of goat milk. The goat milk was subjected to HPP at a pressure range of 200 to 600 MPa and process holding time at 5 - 15 mins. There were insignificant differences in terms of fat, protein and carbohydrate, but significant changes observed for lactose content of pressurised goat milk (PGM). The lactose content of PGM was in the range of (2.540 – 2.986 g/mL), while 1.253±0.01 g/100 mL for untreated goat milk (UGM). A higher number of the small size of goat milk fat globules observed at 600 MPa compared to lower processing pressure (200 and 400 MPa) at the same pressure holding time (5 to 15 mins). The mean diameters of fat globules were in the range of 5.215 to 5.651 µm. This size reduction of milk fat globules is an advantage for cheese making or other dairy product making industries, because it can help to possess a smoother and more refined texture of milk products.

1. Introduction

Goat milk is widely used in dairy products because it contains many nutritional values, which protein, fat and carbohydrate are the main components (Haenlein, 2004; Kalyankar *et al.*, 2016; Lou *et al.*, 2018). Goat milk becomes familiar to the consumer due to low lactose content but with high protein and high calcium content (Haenlein, 2004; Kalyankar *et al.*, 2016). Hence, goat milk is an excellent alternative to people with lactose intolerant (Zenebe *et al.*, 2014). However, during the processing of dairy products, milk proteins are likely to flocculate and precipitate, which potentially affects the quality of goat milk products (Li *et al.*, 2020). The stability of the milk protein is determined by its structural properties, which are related to the functional properties of the macroscopic structural changes (Li *et al.*, 2020). Therefore, functional properties, such as viscosity and texture of milk, could be affected.

In the dairy industry, heat treatment such as high-

temperature short time (72°C/15 s), low-temperature long time (63°C/30 min), and ultra-high temperature (135°C/ a few seconds) pasteurisations are standard processing methods used to ensure the safety and providing an extended shelf life of raw milk (Barraquio, 2014). This process eliminates the heat-sensitive raw milk microbiota (Buffa, Trujillo, Pavia *et al.*, 2001). However, this treatment caused damage or changes in the nutrients and altered the structural properties such as the fat globules (Tamime, 2009; Bogahawaththa *et al.*, 2018). Gervilla *et al.* (2001) reported that in fresh milk, the lipids appear as dispersed milk fat globules. These fat globules which contain 98% triglycerides are bounded by a membrane that can prevent flocculation and coalescence of fat globules.

Non-thermal processing is gaining more interest in the food industry since it is possible to minimise the changes in terms of nutrient and textural properties of dairy products. High-pressure processing (HPP) is a non-thermal processing method, which an alternative to

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traditional pasteurisation treatment. It has been shown by other authors that HPP provides several advantages to food products either through improvement or maintaining quality and safety (Sulaiman *et al.*, 2017; Evelyn and Silva, 2017; Razali *et al.*, 2019a; Razali *et al.*, 2019b). HPP is a processing method in which the food product is subjected to very high pressure ranging from 100 to 600 MPa (Naik *et al.*, 2013; Moatsou and Park, 2017; Stratakos *et al.*, 2019; Silva and Evelyn, 2020). It is also known as cold pasteurisation since temperature employed in the process is at the ambient range and proven to provide sufficient inactivation towards spoilage microorganisms (Evelyn and Silva, 2015)

HPP potentially affects the coagulation and structural properties of the pressurised milk (Buffa, Trujillo and Guamis, 2001). Together with these changes, the HPP might also modify the fat globules. The fat droplets particle size that presents in dairy products is essential in defining the flavour release, mouthfeel, and emulsion stability (Stratakos *et al.*, 2019). Therefore, it is necessary to clarify the changes in microstructure that is related to goat milk fat globules after HPP. Since the subsequent process that used goat milk for cheese and yoghurt making might be affected due to changes in the milk microstructure. Previous reports also stated that applying the HPP to goat milk and goat milk products can improve milk and product quality - enhance nutrient, flavour and colour retention (Buffa *et al.*, 2001; García *et al.*, 2014). However, a limited study can be found on the HPP effect on the composition and microstructure of goat milk. Thus, the objectives of this study were to investigate the effects of high-pressure processing towards the composition, lactose content, and microstructure of goat milk. Understanding the changes in the microstructure, particularly on the milk fat globules and lactose content of goat milk, will facilitate the production process that involves milk separation and possible to improve the quality of goat milk products.

2. Materials and methods

2.1 Preparation of sample

Fresh raw goat milk was obtained from a local farm (MFRS Agrofarm) in Selangor, Malaysia. The fresh goat milk was kept in bottles and stored at chilled condition (4°C). Control samples were held in the refrigerator (at 4°C), while the test samples were treated using High Pressure Processing Unit. Before treatment, milk samples were filled in food-grade plastic pouches (Quiware, Malaysia) and were ensured to remove as much as air possible. These plastic pouches composed of

polyethylene and nylon. Each plastic pouch consists of 100 mL of goat milk. Packed milk samples were then treated in HPP unit with the specified processing conditions, and after treatment, the properties of milk were analysed.

2.2 High Pressure Processing (HPP) treatment

The sample pouches with goat milk samples were placed inside the High Pressure Processing Avure 2L-700 HPP Laboratory Food Processing System (Avure, USA) with general make up as in Figure 1. The samples were subjected to HPP treatment at a pressure range of 200, 400 and 600 MPa; and process holding time of 5, 10 and 15 mins. Temperature for each treatment was set up at 25°C (initial temperature). The temperature was indicated by the thermocouple which was equipped with the HPP chamber. The temperature increase due to adiabatic heating caused by pressurisation was noted to be less than 40°C for the higher pressure of 600 MPa. After the pressurisation process completed, the packed were unloaded and kept in the freezer at -20°C (Hazirah *et al.*, 2018) to stop/slow down any microbiological and biochemical activity until further processing.

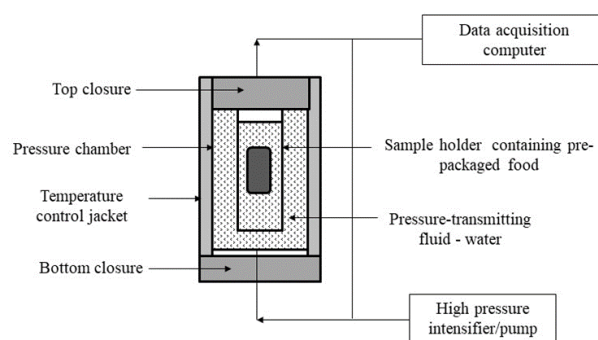


Figure 1. High Pressure Unit configuration set up

2.3 Proximate analysis

Proximate analysis was performed for goat milk powder and based on the AOAC method (AOAC, 2000). Moisture content was determined after oven drying at 105°C for 8 hrs and ash content was carry out in a furnace at 550°C (Muffle Furnace KSL-1700X). The Kjeldahl method was applied to determine protein content with a conversion factor of 6.25. Finally, fat content was carried out by soxhlet extraction (FOSS Soxtec™ 2050) using hexane as the solvent. Carbohydrate was determined from the difference of moisture, ash, protein, and fat content as described by Equation (1).

$$\text{Total carbohydrate} = 100 - (\text{weight in grams} \text{ [protein + fat + water + ash] in 100 g of sample}) \quad (1)$$

2.4 Lactose content

High-performance liquid chromatography (HPLC) was used to determine the lactose content in goat milk. An analysis column (Phenomenex, Rezex RCM Ca - Monosaccharide column) was used in the HPLC system (Agilent Technologies, USA). Pre-degassed distilled water was used as the mobile phase. The samples were injected, and lactose content was detected using an evaporative light scattering detector (ELSD). D-Lactose monohydrate (Sigma-Aldrich, Germany) was used as standard.

2.5 Scanning electron microscopy (SEM)

The morphology of the HPP treated and control goat milk powder (without HPP treatment) were observed using Scanning Electron Microscope (SEM) (S-3400N, Hitachi, Japan). Samples were coated with carbon before the examination to avoid charging of specimens and were mounted on the stub for microscopy analysis. The fat globules present in the goat milk powder was analysed from the image generated from the SEM at 3000x magnification. In scanning electron microscopy, an image of the surface of the specimen was obtained. ImageJ (1.52a, NIH, United States) was used to analyse the geometrical properties of the milk fat globules.

2.6 Statistical Analysis

Each sample was analysed in duplicate and the data were assessed by analysis of variance (ANOVA) by one way using Minitab 18 (Version 18, Minitab Statistical Software, United States). Evaluations were based on a 5% significance level ($P < 0.05$). All the data were presented in mean \pm standard deviation.

3. Results and discussion

3.1 Proximate Analysis

The proximate analysis was performed after the freeze-drying process (Table 1). The majority of the water contents were removed during freeze-drying. Thus the moisture content of the goat milk was lower value (1.33 to 1.43% (weight basis)) and the liquid goat milk was transformed to solid goat milk powder. The composition result of the control sample or unpressurised goat milk (UGM) was observed to have fat (29.35%), protein (30.38%), carbohydrate (33.43%), and ash (5.52%). In comparison with the UGM, the data for fat, protein, ash and carbohydrate of pressurised goat milk (PGM) was slightly changed but statistically insignificant ($p > 0.05$) between pressure treatment ranging from 200 to 600 MPa. In general, these results show that either UGM or PGM, both still have high contents of fat, protein and carbohydrate.

The major carbohydrate of goat milk is lactose (Park, 2017), thus high content carbohydrates indicate high lactose content. Lactose is a disaccharide composed of one glucose and one galactose molecules, which a source of nutrition for the human diet. Table 1 shows the lactose content of UGM was 1.253 ± 0.01 g/100 mL, provide lower value if compared with PGM, in which the differences in lactose content of PGM were significant ($P < 0.05$). The lactose content for all PGM milk (2.540 – 2.986 g/mL) had increased more than double from UGM (1.253 ± 0.01 g/100 mL). This condition could induce more lactose content in goat milk after high-pressure treatment. Ferreira *et al.* (2011) revealed that high pressure (400 MPa) promotes the swelling of cellulose structure which may ease access to glycosidic bonds. The latter shows that high-pressure treatments enhanced the hydrolysis of cellulosic fibre and this could be the reason for the increase of lactose concentration after HPP. The lactose content of PGM that was treated with

Table 1. Composition of Goat Milk Powder

Pressure (MPa)	Time (mins)	Composition (Percentage %)						
		Fat	Protein	Ash	Moisture Content	Total Carbohydrate	pH	Lactose (g/100 mL)
Control		29.35 \pm 0.19 ^a	30.38 \pm 0.19 ^{abcd}	5.52 \pm 0.81 ^a	1.33 \pm 0.03 ^a	33.43 \pm 1.22 ^a	6.61 \pm 0.01 ^b	1.253 \pm 0.01 ^c
200	5	32.78 \pm 0.50 ^a	28.78 \pm 0.67 ^{bcd}	5.55 \pm 0.66 ^a	1.43 \pm 0.02 ^a	31.46 \pm 0.48 ^a	6.67 \pm 0.02 ^a	2.561 \pm 0.01 ^d
	10	32.57 \pm 1.99 ^a	29.14 \pm 0.68 ^{abcd}	6.04 \pm 0.07 ^a	1.38 \pm 0.01 ^a	30.87 \pm 1.22 ^a	6.64 \pm 0.01 ^{ab}	2.779 \pm 0.04 ^b
	15	33.80 \pm 0.93 ^a	28.64 \pm 0.02 ^{cd}	5.88 \pm 0.06 ^a	1.42 \pm 0.11 ^a	30.27 \pm 0.86 ^a	6.67 \pm 0.01 ^a	2.769 \pm 0.03 ^{bc}
400	5	33.57 \pm 1.25 ^a	28.52 \pm 0.59 ^d	6.00 \pm 0.04 ^a	1.42 \pm 0.01 ^a	30.50 \pm 1.88 ^a	6.65 \pm 0.00 ^{ab}	2.540 \pm 0.02 ^d
	10	31.30 \pm 1.79 ^a	30.79 \pm 0.26 ^a	6.06 \pm 0.12 ^a	1.39 \pm 0.04 ^a	30.46 \pm 1.61 ^a	6.68 \pm 0.00 ^a	2.797 \pm 0.00 ^b
	15	30.80 \pm 1.99 ^a	30.57 \pm 0.07 ^a	5.59 \pm 0.69 ^a	1.40 \pm 0.08 ^a	31.63 \pm 1.46 ^a	6.65 \pm 0.01 ^{ab}	2.797 \pm 0.02 ^b
600	5	30.13 \pm 1.88 ^a	31.06 \pm 0.60 ^a	5.74 \pm 0.36 ^a	1.35 \pm 0.03 ^a	31.73 \pm 0.88 ^a	6.66 \pm 0.00 ^a	2.679 \pm 0.02 ^c
	10	30.35 \pm 2.49 ^a	30.56 \pm 0.09 ^{ab}	5.55 \pm 0.77 ^a	1.34 \pm 0.05 ^a	32.19 \pm 1.76 ^a	6.65 \pm 0.01 ^{ab}	2.895 \pm 0.03 ^a
	15	31.98 \pm 2.88 ^a	30.51 \pm 0.78 ^{abc}	5.51 \pm 0.75 ^a	1.36 \pm 0.04 ^a	30.64 \pm 1.39 ^a	6.65 \pm 0.01 ^{ab}	2.986 \pm 0.01 ^a

Values are expressed as mean \pm SD, n = 2. Values with the different superscript within the same column are significantly different among treatments ($P < 0.05$).

three different pressure (200 to 600 MPa) at 5 mins holding time showed significant difference ($P < 0.05$) as compared with a sample treated at the same pressure but at different holding time. For holding time of 10 and 15 mins, the results of lactose content showed no significant difference ($P > 0.05$) at the same pressure. The highest lactose content among PGM samples was at 600 MPa for 15 mins with a value of 2.986 ± 0.01 g/100 mL. While the lowest lactose content (2.561 ± 0.01 g/100 mL) for PGM was at condition treated with 200 MPa for 5 mins.

pH value for UGM was 6.61, which not much different and in the range of pH data of goat milk reported by a previous researcher (Park *et al.*, 2007). The pH value for PGM was slightly higher ($P < 0.05$) as compared with UGM, with the average pH value ranging from 6.64 to 6.68 for all conditions treated by high pressure. However, overall differences in the pH were not significant ($P > 0.05$) for all milk samples treated with high pressure. High pressure treatment might be possible to cause an increase in the concentration of calcium ion in milk due to calcium release from casein micelles immediately after treatment (Zobrist *et al.*, 2005; Cadesky *et al.*, 2017), which causes the increase in the milk pH.

3.2 Analysis of the microstructure

The microstructure of unpressurised goat milk (UGM) and pressurised goat milk (PGM) treated at 200 – 400 MPa are shown in Figures 2 to 5. From the micrographs obtained from SEM, the average geometrical parameter values of goat's milk fat globules were analysed and shown in Table 2. The milk fat globules of UGM were smooth, remained intact, no burst or cleavage had been observed (Figure 2). The average diameter of UGM fat globules was found 6.126 ± 0.609 μm with a surface area was 33.908 ± 4.619 μm^2 . The diameter of UGM fat globules in this study is larger than 3.49 μm reported by Park *et al.* (2007). Raw goat milk's fat globule had a spherical structure with a roundness of 87.7% (0.877 ± 0.099) with some of the fat globules coalesced.

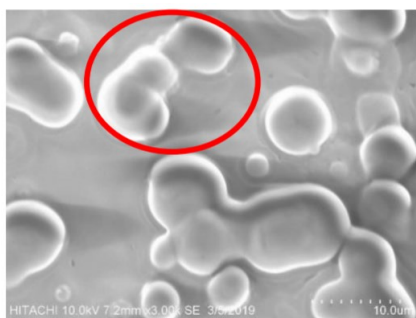


Figure 2. Microstructure of untreated goat milk in a red circle (larger compared to after HPP) at 3000x magnification.

The microstructure of PGM treated with 200 MPa

for 5 – 15 mins are shown in Figure 3. The average diameter of PGM treated at 200 MPa decreased when the holding time increased with values of 7.136 ± 2.175 μm (5 mins.), 6.176 ± 2.255 μm (10 mins), and 5.894 ± 1.624 μm (15 mins). Compared with UGM, the diameter of PGM treated at 200 MPa for 5 and 10 mins were slightly increased. At 5 mins (Figure 3a), the fat globules not fission but tend to aggregates. At 10 mins (Figure 3b), there were distortions of fat globules with smaller fat globules observed and also coalesced between fat globules. Prolonged holding time to 15 mins (Figure 3c), small size fat globules were observed as compared to UGM. At this condition (200 MPa for 15 mins.), might be enough time for fat globules to fission into a smaller size (5.894 ± 1.624 μm) and disaggregate. In term of shape, the PGM's fat globules (treated at 200 MPa for 5 mins) had more towards oval shape with an average roundness of 79.8% (0.798 ± 0.051) as compared to the others pressurised treated condition of 10 min (86.4%); 15 mins (89%) and UGM (87.7%).

Table 2. Average geometrical parameter values of goat's milk fat globules

Pressure (MPa)	Time (mins)	Geometrical Properties		
		Diameter (μm)	Roundness	Area (μm^2)
Control		6.126 ± 0.609	0.877 ± 0.099	33.908
200	5	7.136 ± 2.175	0.798 ± 0.051	49.206
	10	6.176 ± 2.255	0.864 ± 0.090	44.044
	15	5.894 ± 1.634	0.890 ± 0.049	43.512
400	5	8.300 ± 2.830	0.830 ± 0.061	64.181
	10	6.805 ± 1.226	0.814 ± 0.071	43.321
	15	10.066 ± 1.624	0.694 ± 0.071	74.779
600	5	5.397 ± 0.750	0.859 ± 0.091	24.627
	10	5.215 ± 1.070	0.876 ± 0.109	22.310
	15	5.651 ± 2.228	0.879 ± 0.119	25.032



Figure 3. Microstructure of goat milk treated with 200 MPa for (a) 5 mins, (b) 10 mins, (c) 15 mins at 3000x magnification

At high pressure processing of 400 MPa, varying diameter size for fat globules of PGM were observed when the holding time prolonged from 5 -15 mins (Figure 4a – c). The size of fat globules was observed to be larger (8.300 ± 2.830 μm) at holding time of 5 mins, then decreased to 6.805 ± 1.226 μm (10 mins) and finally at longer holding time (15 mins) the size increased to 10.066 ± 1.624 μm . At 10 mins of holding time, smaller globules were observed, which might occur due to the fission of fat globules. However, at longer holding time (15 mins) smaller globule might coalesce (aggregate) together resulted in an increase in the size of fat globules. The fat globules size results also correlated

with the shape (roundness) of fat globules, which varies distributed with 83% (5 mins), 81.4% (10 mins), and 69.4% (15 mins). Compared with UGM, the diameter size for all fat globules treated at 400 MPa with 5 - 15 mins were larger, and similarly with results of surface area that also given highest values ($64.181\pm 34.428 \mu\text{m}^2$ (5 mins); $43.321\pm 14.188 \mu\text{m}^2$ (10 mins); $74.779\pm 21.307 \mu\text{m}^2$ (15 mins)).

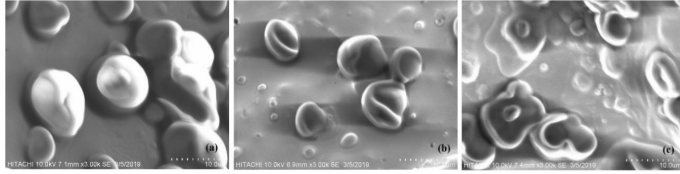


Figure 4. Microstructure of goat milk treated with 400 MPa for (a) 5 mins, (b) 10 mins, (c) 15 mins at 3000x magnification.

Figure 5 (a-c) shows the microstructure of PGM treated at 600 MPa from 5 – 15 mins of holding time. The diameter of fat globules of PGM treated at 600 MPa for all holding time (5 - 15 mins) were similar in size with a diameter of $5.397\pm 0.750 \mu\text{m}$ (5 mins), $5.215\pm 1.070 \mu\text{m}$ (10 mins) and $5.651\pm 2.228 \mu\text{m}$ (15 mins), respectively (Table 1). Stable globular structure of fat globules was observed at 5 mins (Figure 5a), while at 15 mins (Figure 5c) slightly aggregation of fat globules been observed. At the highest pressurised condition (600 MPa), the fat globules fission into smaller sizes and the size were smaller as compared to PGM treated at 200 and 400 MPa. Serra *et al.* (2007) had studied high pressurised treated (100 to 330 MPa) of cow milk, which they also observed fat globules reduction as compared to raw cow milk. Gervilla *et al.* (2001) found slight changes in milk fat globules of ewe's milk which were pressurised between 100 to 500 MPa, with the average diameter size of fat globules of pressurised milk ranges from 4.3 to 5.1 μm and the unpressurised fat globules were 5.0 μm . In general, the use of HPP contributes to the homogenisation of milk products through a reduction of fat globule size, which with smaller globules cannot form large enough clusters for creaming to occur; causing an increase of milk shelf-life (Stratakos *et al.*, 2019).

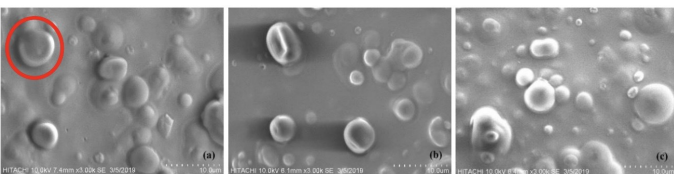


Figure 5. Microstructure of goat milk treated with 600 MPa for (a) 5 mins, (b) 10 mins, (c) 15 mins at 3000x magnification. Smallest compared (red circle) to raw, 200 MPa and 400 MPa.

The results for surface areas of PGM treated at 600 MPa ranging from $24.627\pm 8.168 \mu\text{m}^2$ (5 mins), $22.310\pm 14.084 \mu\text{m}^2$ (10 mins) and $25.032\pm 19.808 \mu\text{m}^2$ (15 mins) were lower as compared with UGM

($33.908\pm 4.619 \mu\text{m}^2$). However, the shape (roundness) of the PGM fat globules were more roundness (85.9-87.9%) and smooth, which not much differences with UGM roundness (87.7%). In general, smaller fat globules will improve the dispersion and more homogeneous mixture of fat in goat milk that would offer lipases with a larger surface area of fat for greater digestive action (Park, 2017).

4. Conclusion

High pressure processing of goat milk caused significant changes to milk's protein, fat, and carbohydrate compositions. Lactose content increased more than double after pressurised treatment as compared to untreated milk. Pressurised goat milk show no significant changes to the pH of treated milk ($P < 0.05$). In term of morphological size of fat globules, two opposing effects were observed. The diameter size of fat globule for goat milk treated below 400 MPa appears to increase as compared with untreated goat milk. While the size of fat globules were seen to decrease after pressurisation at 600 MPa compared to untreated milk showing evidence of fat globules fission. The size reduction of milk fat globules provides advantages in cheese or other dairy product making industries that possible to possess smoother and finer texture for the goat milk products.

Acknowledgements

The authors acknowledge the Universiti Putra Malaysia for the support on research facilities, chemicals and consumables and to the Department of Food Biotechnology, Agro-Biotechnology Institute (ABI), Malaysia for allowing us to use the high pressure unit.

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