

## Research Article

### Physicochemical Properties and Antioxidant Activity of Milk Samples Collected from Five Goat Breeds in Malaysia

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**Abstract:** Five different goat breeds (*Saanen*, *Kacang*, *Jamnapari*, *Boer* and a crossbred of *Jamnapari* and *Saanen*) were obtained from one farm in Bandar Baru Bangi. The aim of this study was to determine the physicochemical properties and antioxidant capacity based on TPC, FRAP and DPPH. Goat milk samples were collected during the same lactation period (middle lactation) and were subsequently compared with cow milk. The results of the study showed that goat milk exhibited a significantly higher ( $p < 0.05$ ) antioxidant capacity than cow milk. *Jamnapari* milk exhibited the highest antioxidant capacity in Total Phenol Content (TPC), Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays with values of 544.08 mg GA/100 g FW, 481.69 mg TE/100 g FW and 67.44%, respectively. By contrast, the milk samples obtained from the *Boer* exhibited the lowest corresponding values of 460.00 mg GA/100 g FW, 386.06 mg TE/100 g FW and 59.68%, respectively. Results showed that physicochemical properties were significantly different ( $p < 0.05$ ) among the milk samples, with some samples more superior compared with others in one or more aspects. *Jamnapari* milk was superior in terms of ash, total soluble solid and protein with values of 0.96%, 13.02 Brix and 5.11%, respectively and this milk also showed the lowest moisture (81.28%) among all milk samples. Cow milk exhibited the highest fat (4.43%) and *Kacang* milk had the lowest titratable acidity (0.06%) than the other milk samples. Thus, goat milk can be considered as an excellence source of antioxidants.

**Keywords:** Antioxidant activity, breed, goat's milk, *Jamnapari*, Malaysia, physicochemical properties

## INTRODUCTION

Goats provide people with various important products such as meat, milk, yoghurt, cheese and other dairy products, skins, mohair, cashmere, draft and pack power and high-quality manure for crops (Chandan *et al.*, 1992; Fahmi *et al.*, 1956). This animal is one of the most versatile domestic animals that can adapt to diverse stressful climatic conditions such as humid, tropical, cold, desert and mountain climates (Chilliard *et al.*, 1984; Mackinlay *et al.*, 1966; Nicol and Davis, 1967). Moreover, goats can also adapt to arid environments where water is insufficient for an appropriate diet. They can survive even when forage is scarce and can feed on rough and inaccessible lands (Hilario *et al.*, 2009).

This study focuses on dairy goats because of the unique characteristics of goat milk compared with cow milk. Goat milk and its dairy products are important in

the human diet, particularly in infants for whom goat milk can be provided as an alternative to cow milk (Hanlein, 2001). Moreover, goat milk possesses low allergenic potential compared with cow milk on the basis of the characteristics of  $\alpha_{S1}$ -casein (Roncada *et al.*, 2002). Therefore, goat milk has been used successfully to relieve allergy caused by cow milk. Goat milk has also been fed to patients with various metabolic and gastrointestinal ailments. In addition, goat milk proteins differ genetically from several cow milk proteins and fat in goat milk typically presents a more nutritious fatty acid profile than fat in cow milk (Park and Haenlein, 2006).

Goat milk is rich in various physiologically functional components, including proteins, vitamins (such as vitamins E and C), flavonoids and carotenoids with antioxidant properties. Therefore, goat milk is considered to possess high antioxidant capacity that resists oxidative stability and highly protects consumers

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from exposure to oxidative stress, which is an important characteristic of numerous acute and chronic diseases (Dalle *et al.*, 2006; Valko *et al.*, 2007). Antioxidants may positively affect human health by protecting the body against damage caused by Reactive Oxygen Species (ROS), which attack membrane lipids, protein and DNA. These compounds are also involved in several important protective functions in many diseases such as cardiovascular diseases, cancer, diabetes mellitus and Alzheimer's (Aruoma, 1998; Valko *et al.*, 2007). Antioxidants protect organisms against free radicals, but a sufficient concentration of antioxidants is necessary to balance the disruption caused by these radicals (Vasundhara *et al.*, 2008). ROS and other free radicals are significantly involved in many degenerative diseases. Li *et al.* (2009) recommended fresh milk intake, particularly breastfeeding, as an important food source of antioxidants to prevent or reduce oxidative damage in various body tissues.

Previous studies have shown that goat and other animal's milk exhibit properties (Chen *et al.*, 2002, 2003; Hilario *et al.*, 2009; Mardalena *et al.*, 2011; Simos *et al.*, 2011). However, different studies evaluated the physicochemical characteristics of goat milk in different countries (Morgan *et al.*, 2003; Drackova *et al.*, 2008; Strzalkowska *et al.*, 2009; Costa *et al.*, 2010; Asif and Sumaira, 2010; Mayer and Fiechter, 2012; Delgado-Pertíñez *et al.*, 2013). In the present study, goat milk from five different local breeds in Malaysia was selected for analysis of antioxidant capacity and physicochemical characteristics. The goat breeds were *Kacang*, *Boer*, *Saanen*, *Jamnapari* and a crossbred of *Saanen* and *Jamnapari*. This study was designed to determine the antioxidant activity and physicochemical properties of goat milk from different breeds in Malaysia and subsequently compare them with cow milk.

## MATERIALS AND METHODS

**Sample collection:** Fresh goat and cow milk were obtained from one farm in Bandar Baru Bangi at the second milk lactation and same field conditions (seasonal, breeding, climate, grazing and feeding staff). The animals were fed regularly with grass and feed consisting of fresh fruits and vegetables with specific supplements, including soya, brown sugar, calcium powder and other chemical food that improve milk quality. All five goat breeds and cow were milked manually and collection was done early morning in the farm. Samples were transferred immediately to University Kebangsaan Malaysia (UKM) Food Science Laboratory in sterile bottles under aseptic technique and cool condition.

**Sample preparation and antioxidant extraction:** Fresh goat milk samples were preserved at -80°C until further use. Antioxidant compounds from the fresh milk

were extracted according to methods of Li *et al.* (2007) with some modification. One normal solution of HCl (1 N)/95% ethanol (v/v, 15/85) was prepared and used as extraction solvent. The extraction procedure involved addition of 1 mL of the fresh milk to 10 mL solvent separately in 50 mL brown bottles and shaking for 1 h at 30°C in a rotary shaker (MaxQ 5000, BI Barnstead/Lab-Line and Dubuque, IA, USA) set at 300 rpm. The mixture of solvent and samples were then centrifuged at 7800×g (SS-34 Rotors, RC5C Sorvall Instruments, DuPont, Wilmington, DE, USA) at 5°C for 15 min. The supernatant fluids were kept at -20 °C in the dark until further analysis for DPPH radical scavenging activity, FRAP and TPC.

**DPPH radical scavenging activity:** The method of Musa *et al.* (2011) with minor modification was used to evaluate antioxidant activity through DPPH scavenging system. To prepare the stock solution, 40 mg was dissolved in 100 mL methanol. The solution was then stored at -20°C until use. By mixing 350 mL of the stock solution with 350 mL methanol, an absorbance of 1.0±0.01 unit was obtained using a spectrophotometer (Epoch, Biotek, USA) at 517 nm wavelengths. Approximately 100 µL of each fresh milk extract with 1 mL methanolic DPPH solution was prepared and kept in the dark for 2 h to allow scavenging reaction to occur. The percentage of DPPH scavenging activity was calculated as:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

where, A is the absorbance

**Ferric Reducing Antioxidant Power (FRAP):** FRAP assay was performed according to Musa *et al.* (2011) with minor modification. FRAP reagent was prepared fresh using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, 16 mL glacial acid made up to 1:1 with distilled water), 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM FeCl<sub>3</sub>•6H<sub>2</sub>O in the ratio of 10:1:1 to give the working reagent. Approximately 100 µL of extracted fresh milk was added to 1 mL FRAP reagent and the absorbance was measured at 595 nm wavelength using a spectrophotometer after 30 min. Calibration curve of Trolox was set up to estimate the activity capacity of samples. Result was expressed as milligram of Trolox equivalents per 100 gram of fresh samples (mg TE/100 g of FW).

**Total Phenol Content (TPC):** Antioxidant activity through TPC was determined according to the method of Musa *et al.* (2011) with minor modification. About 100 µL of extracted fresh goat milk was added to 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent. Samples with the reagent were left

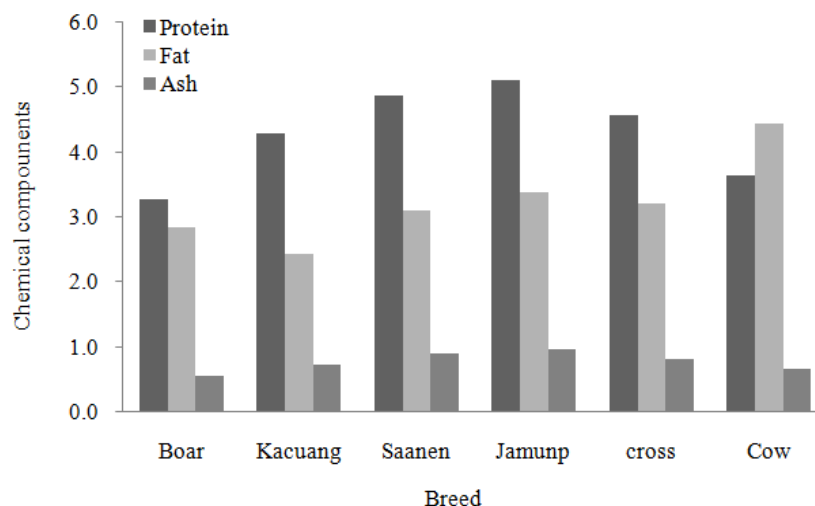


Fig. 1: Chemical composition of fresh goat milk and cow milk samples from one farm in Malaysia

for 5 min and then 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was measured at 765 nm using a spectrophotometer after 2 h. Calibration curve of gallic acid was plotted to evaluate the activity capacity of the samples (Fig. 1). Result was expressed as milligram of gallic acid equivalents per 100 gram of fresh sample (mg GA/100 g of FW).

**Physicochemical properties of milk:** Milk samples were analyzed for physicochemical characteristics according to standard procedures. Data analysis was performed in triplicate. Protein content of the milk was measured by Kjeldhal method No. 920.105 and fat content by Mojonnier method (Anonymous, 2000). Moisture content was evaluated by drying the samples at 105°C overnight in a Memmert Oven (Germany). Gravimetric method was used to determine ash content using a furnace at 550°C as described by AOAC (2000). Titratable Acidity (TA) was determined from 10 mL of sample diluted with 50 mL of water, titrated with 0.1 N NaOH and calculated as percentage of lactic acid as described by AOAC (2000). Total Soluble Solids (TSS) was measured with an abbe refractometer at 20°C and pH of the fresh milk sample was determined using a digital pH meter (AOAC, 1998).

**Statistical analysis:** Data were expressed as the mean of three independent experiments. Statistical comparisons of the results were subjected to one-way ANOVA using SPSS ver.20. Significant differences ( $p < 0.05$ ) among the different breeds of goat were analyzed by Duncan's triplicates range test (Bryman and Cramer, 2012).

## RESULTS AND DISCUSSION

**Antioxidant capacity assays:** Antioxidant compounds react with Folin-Ciocalteu reagent and the reaction can

be performed to measure the concentration of phenolic groups (Nurliyana *et al.*, 2010). Therefore, deep blue coloration in milk samples indicates that high phenolic concentrations are present, whereas light blue coloration in milk samples indicates otherwise. Table 1 shows the antioxidant activity results obtained by TPC, FRAP and DPPH assays of milk samples from different goat and cow breeds used in the present study. Statistical analysis by Duncan's test demonstrated that the difference in breed significantly affected ( $p < 0.05$ ) the TPC of milk samples. At this variance, the milk sample from the *Jamnapari* breed exhibited the highest TPC (544.08 mg GA/100 g FW). By contrast, *Boer* breed exhibited the lowest value of 460.00 mg GA/100 g FW.

FRAP assays depend on the mechanism that involves oxidation and reduction reactions, in which ferric ion is reduced to ferrous ion. This mechanism can be correlated with the redox properties of antioxidant compounds in milk samples. From Table 1, results demonstrate that the *Boer* breed sample had the lowest antioxidant activity through FRAP assay (386.06 mg TE/100 g) followed by the cow sample (398.88 mg TE/100 g), whereas the *Jamnapari* milk sample was superior among the samples (481.69 mg TE/100 g). Thus, the TPC and FRAP assay results are comparable in this study, but they differ from DPPH assay results.

DPPH assays are often used to determine the capacity of primary antioxidants in samples, in which these primary antioxidants react to scavenge free radicals from DPPH solution. Hence, the formation of the initiation chain of free radicals is inhibited and the propagation chain is destroyed through the donation of a hydrogen atom or an electron. Consequently, free radicals can be modified to a more stable form of products (Nurliyana *et al.*, 2010; Yan *et al.*, 2006). This result explains the discoloration of a milk sample from purple to yellow in DPPH solution. Similar to TPC and

Table 1: Average of the antioxidant activity assays of fresh goat milk breeds and cow milk samples obtained from one farm in Malaysia

Breed	TPC (mg/100g FW)	FRAP (mg/100g FW)	DPPH (%)
<i>Boer</i>	460.00±2.14 <sup>f</sup>	386.06±1.03 <sup>f</sup>	59.68±0.59 <sup>f</sup>
<i>Kacang</i>	494.23±1.71 <sup>d</sup>	416.97±1.52 <sup>d</sup>	63.06±0.46 <sup>d</sup>
<i>Saanen</i>	529.94±1.24 <sup>b</sup>	464.95±1.02 <sup>b</sup>	65.67±0.40 <sup>b</sup>
<i>Jamnapari</i>	544.08±1.83 <sup>a</sup>	481.69±1.56 <sup>a</sup>	67.44±0.47 <sup>a</sup>
Crossbred	503.51±1.98 <sup>c</sup>	431.62±1.00 <sup>c</sup>	64.26±0.31 <sup>c</sup>
Cow	477.68±1.20 <sup>e</sup>	398.88±1.54 <sup>e</sup>	60.81±0.43 <sup>e</sup>

<sup>a-f</sup>: Mean with different letters within the same column indicate significant difference (p<0.05)

Table 2: Correlation coefficients of the antioxidant activities among fresh milk using different assays

	TPC	FRAP	DPPH
TPC	*	*	*
FRAP	0.98	*	*
DPPH	0.94	0.92	*

Table 3: Physical properties of fresh goat milk from different breeds and cow milk samples obtained from one farm in Malaysia

	Moisture (%)	pH	Titrateable acidity (%)	TSS (Brix)
<i>Boer</i>	89.68±0.19 <sup>a</sup>	6.63±0.03 <sup>c</sup>	0.17±0.01 <sup>d</sup>	9.80±0.10 <sup>e</sup>
<i>Kacang</i>	85.82±0.17 <sup>c</sup>	6.78±0.02 <sup>a</sup>	0.06±0.02 <sup>f</sup>	10.43±0.06 <sup>d</sup>
<i>Saanen</i>	82.15±0.25 <sup>e</sup>	6.44±0.02 <sup>f</sup>	0.29±0.01 <sup>a</sup>	11.63±0.06 <sup>b</sup>
<i>Jamnapari</i>	81.28±0.12 <sup>f</sup>	6.70±0.01 <sup>b</sup>	0.12±0.02 <sup>e</sup>	13.02±0.03 <sup>a</sup>
Crossbred	84.43±0.17 <sup>d</sup>	6.58±0.02 <sup>d</sup>	0.21±0.02 <sup>c</sup>	11.00±0.20 <sup>f</sup>
Cow	87.81±0.15 <sup>b</sup>	6.52±0.01 <sup>e</sup>	0.26±0.02 <sup>b</sup>	10.17±0.29 <sup>d</sup>

<sup>a-f</sup>: Mean with different letters within the same column indicate significant difference (p<0.05)

FRAP, a similar result was observed in the free radical scavenging percentage: *Jamnapari*, 67.44%; *Saanen*, 65.67%; crossbred (*Jamnapari*+*Saanen*), 64.26% *Kacang*, 63.06%; and *Boer*, 59.68%. Antioxidant activities of goat milk samples from different breeds were significantly different (p<0.05).

Milk samples from some goat breeds exhibited higher antioxidant activities than cow milk samples. *Jamnapari*, *Saanen*, crossbred and *Kacang* exhibited higher antioxidant activities through all the assays than cows and *Boer* showed the lowest antioxidant activity. These results were consistent with the assessment of Simos *et al.* (2011), who demonstrated the total antioxidant capacity of different breeds, including cow and goat breeds. *Jamnapari* goat is the most majestic large goat breed of India and its breeding is advantageous because of its high adaptability to difficult climate (Alibaba, 2014), such as the hot and humid climate observed in Malaysia. Furthermore, the *Jamnapari* goat also has a longer lactation period compared with other goats because of genetic factors. The superiority of the breed in terms of exhibiting the highest level of antioxidant activity compared with other goat breeds may be attributed to the aforementioned traits.

**Correlation between the antioxidant assays:** The correlation between the three antioxidant assays was performed using the function CORREL in the Microsoft Excel program. TPC, FRAP and DPPH

results exhibited positive correlation (Table 2). TPC and FRAP assays showed the highest correlation among the fresh milk samples ( $R^2 = 0.98$ ). This result is consistent with that discussed in a previous section. However, the correlation between DPPH and FRAP assays were lower than that between DPPH and TPC. Li *et al.* (2009) evaluated the TPC and DPPH activities of breast milk and found the same result. The same correlation between FRAP and TPC has also been revealed in other previous studies (Akowuah *et al.*, 2005; Sulaiman *et al.*, 2011; Tan *et al.*, 2012). Several studies have also shown a highly positive correlation between FRAP, TPC and DPPH assays (Thaipong *et al.*, 2006; Mahattanatawee *et al.*, 2006; Corral-Aguayo *et al.*, 2008; Maizura *et al.*, 2011; Siow and Hui, 2013; Zuhair *et al.*, 2013).

**Physicochemical properties of the milk:**

Consequently, the influences of the differences between breeds on the physicochemical characteristics of goat milk in Malaysia have been investigated. Testing physical properties of goat milk, such as pH, TA, moisture and TSS are important in studying the physicochemical composition as nutritional value attributes. Table 3 shows the mean values of the physical parameters of milk samples obtained from different breeds at the same conditions. The pH and TA of the milk samples from all the breeds were measured at the same sampling day. The results showed that pH values ranged from 6.4 to 6.74 and *Kacang* breed had the highest pH value and *Saanen* had the lowest. These results demonstrated that the pH values of the goat milk were significantly different among the breeds and higher than the cow milk, with the exception of *Saanen* (p<0.05). This range of pH values of goat milk were in accordance with the finding of previous investigations reported by Sawaya *et al.* (1984), Rehman and Salaria (2005) and Asif and Sumaira (2010). TA of goat milk was also significantly different among the goat breeds and ranged from 0.06 to 0.29 (p<0.05). *Saanen* breed had the highest acidity among the samples and *Kacang* had the lowest percentage of lactic acid.

The concentration range of TSS was from 9.80±0.10 to 13.02±0.03, as shown in Table 1. *Boer* breeds had the lowest amount of TSS (9.80±0.10) followed by the cow milk sample (10.17±0.29), whereas the highest amount of TSS was found in the *Jamnapari* breed (13.02±0.03). These values of concentration were significantly different (p<0.05) between all samples except for the cow and *Kacang*. Significant difference (p<0.05) was also found in moisture content among all the samples. Milk sample from goat breeds were found to have lower moisture content than the cow milk except for *Boer* breed (89.68±0.19).

The goat milk samples were also subjected to different chemical tests. The protein, fat and ash

contents were determined and are presented in Fig. 1. Chemical compositions of milk samples revealed variation among samples, with some samples showing superiority over others in one or more aspects. As shown in Fig. 1, goat milk samples showed variance in total protein content, with *Jamnapari* as the superior breed with 5.11%. Cow milk sample had lower protein content at 3.62% compared with goat milk except for the *Boer* breed with 3.26%. This result demonstrates that in fresh natural milk, goat milk is a rich source of protein. Fat content in the collected milk samples ranged from 2.43 to 4.43%. Cow milk sample had significantly higher fat breeds ( $p < 0.05$ ) content than the milk samples from goat. Among all tested milk samples, local breed *Kacang* had the lowest fat content. Significant difference ( $p < 0.05$ ) was also found in the amounts of ash among all of the milk samples. *Jamnapari* milk had the highest ash content value ( $0.96 \pm 0.03$ ), whereas *Boer* had the lowest ( $0.54 \pm 0.04$ ).

Although the cow and five goat breeds chosen in this study were kept and fed under identical conditions at the same farm, the milk samples showed varying results in terms of physical properties and chemical composition as shown in Table 2 and Fig. 1. This result is in contrast to the study of Mayer and Fiechter (2012), in which no significant differences were observed between physicochemical characteristics of milk samples from six goat breeds that were collected from the one farm under the same care conditions. This result may have been caused by the difference in the origin and purpose of the goat breed: *Kacang* breed is of Malaysian origin and used for meat and milk; *Jamnapari* is from India and used for meat and milk; *Saanen* is from the United States and used for milk only; and *Boer* is from South Africa and used for meat only (Wikipedia, 2014). Results also suggest that the lowest property values were obtained from the milk sample from the *Boer* breed. Nonetheless, the present study's results are still within the broad range that has been reviewed recently, although previous studies have also reported quite significantly varying levels of chemical composition of goat milk (Jandal, 1996; Souci *et al.*, 2000; Morgan *et al.*, 2003; Park *et al.*, 2007; Raynal-Ljutovac *et al.*, 2008). Several studies have reported variations in physical properties and chemical composition of milk samples from different goat breeds in different countries (Alichanidis and Polychroniadou, 1996; Trancoso *et al.*, 2010).

## CONCLUSION

The present study provides preliminary data on the physicochemical properties and antioxidant activity of goat milk from different breeds in Malaysia. Results showed that goat milk exhibits a high antioxidant capacity with significant differences among the chosen

goat breeds. In particular, *Jamnapari* breed exhibited the highest antioxidant capacity, in contrast to the *Boer* breed, which exhibited the lowest. *Jamnapari* breed is also superior in different physicochemical properties such as protein, TSS and ash content and also contained the lowest moisture. These findings suggest that goat milk could be considered as a promising food component that could prevent oxidative damage and reduce the risk of degenerative diseases. Further studies should be conducted to investigate the physicochemical composition, nutritional value, rheology, color and other properties of goat milk from different breeds not covered in the present study.

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