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# Effect of Different Factors on Goat Milk Antioxidant Activity

**Saif Alyaqoubi[1-3]*, Aminah Abdullah[1], Muhamad Samudi[2], Norrakiah Abdullah[1], Zuhair Radhi[1]Addai and Maryam Al-ghazali[4]**

**School of Chemical Science and Food Technology[1], School of Applied Physics[2], Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia.**

**Ministry of Regional Municipalities and Water Resource[3], Sultan Qaboos University, Oman.[4]**

***Corres. author: saifalyaqoubi@yahoo.com, +60169923332**

**Abstract:** Goat milk is an excellent source of antioxidants. This study aims to investigate the effect of different factors, such as milk pasteurization treatment, milk lactation stage, and geographic location, on the antioxidant activity of goat milk. Results showed that these factors significantly affected ($P < 0.05$) the antioxidant capacity of goat milk. The first lactation and unpasteurized milk exhibited higher antioxidant activity in total phenol content (TPC), ferric reducing antioxidant power(FRAP), and 2,2-diphenyl-1-picryl hydrazyl(DPPH) assays than the middle and pasteurized milk. Among the geographic locations, Bander Baru Bangi possessed the highest antioxidant capacity, with 523.80 mg GA/100 g FW of TPC, 456.65 mg TE/100 g FW of FRAP, and 65.85% of DPPH. The effects ofthe three factors on the antioxidant activity of goat milk should be considered.
**Keywords:** Goat's milk, Antioxidant capacity, Lactation stage, Pasteurization treatment, Geographic location, Malaysia.

## Introduction

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 activity that resists oxidative stability and highly protects consumers from exposure to oxidative stress, which is an important characteristic of numerous acute and chronic diseases[1-2]. 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[2-3]. Antioxidants also protect organisms against free radicals, but a sufficient concentration of antioxidants is necessary to balance the disruption caused by these radicals[4].

ROS and other free radicals are significantly involved in many degenerative diseases. [5] recommended fresh milk intake, particularly breastfeeding, as an important food source of antioxidants to prevent or reduce oxidative damage in various body tissues. Moreover, studies have shown that goat and other animal's milk exhibit antioxidant properties[6-10]. However, these antioxidant properties of milk may be affected by different factors such as: milk pasteurization treatment, level stage of milk lactation and geographic location of farm. Therefore, this study was designed to investigate the effect of these factors on the antioxidant activity of goat's milk.

## Materials and Methods

### Sample collection

Fresh goat and cow milk samples were obtained from three local farms inJohor Bahru, Semenyih, and Bander Baru Bangi in Malaysia. A total of 42 milk samples were collected for this study; 18 samples were obtained from two goat species from Johor, 6 goat samples from Semenyih, and 12 goat and 6 cow samples from Bangi. Farm location, milk period lactation, and pasteurization treatment were considered effective factors in this study. All samples were collected at early morning, and frozen until analysis in the Food Science Laboratory of University Kebangsaan Malaysia.

### 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[11] with some modification. One normal solution of HC1 (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, 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 [12] 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 wavelength. 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-

$$\text{DPPH scavenging activity (\%)} = [(\text{A blank} - \text{A sample}) / \text{A blank}] \times 100$$

where A is the absorbance.

### Ferric reducing antioxidant power (FRAP)

FRAP assay was performed according to [12] 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 mMHCl, and 20 mM $FeCl_3 \bullet 6H_2O$ 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 [12]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 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. Result was expressed as milligram of gallic acid equivalents per 100 gram of fresh sample (mg GA/100 g of FW).

### 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' triplicates range test[13].

## Results and Discussion

### Lactation Stage

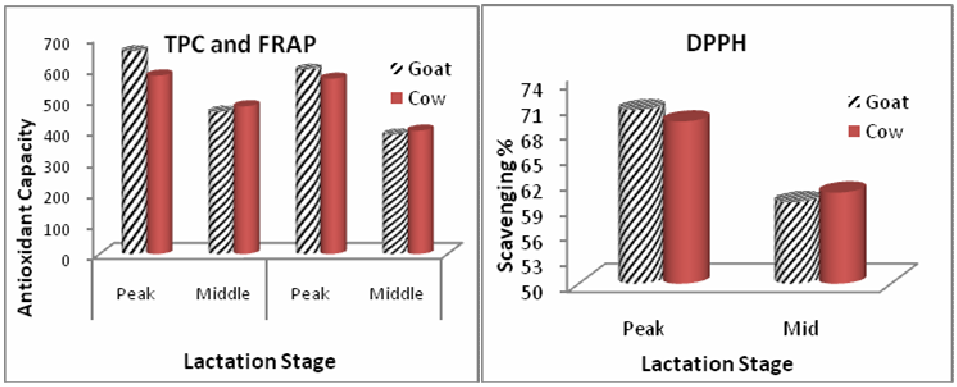


**Fig. 1:Average antioxidant activities of goat and cow milk samples collected on the first and second lactations.**

Two periods of milk lactation (first and second lactations) were considered to study the antioxidant activity through the different stages of milk lactation. As shown in Fig. 1, the goat milk samples collected at the peak stage (first lactation) had higher antioxidant [i.e., gallic acid (GA) content in total phenol content (TPC), trolox equivalent (TE) in ferric-reducing antioxidant power(FRAP), and scavenging percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH) were655.32 mg GA/100g FW, 596.03 mg TE/100 g FW, and 70.60%, respectively] than those collected atthe middle stage. Similarly, the cow milk samples collected at the same lactation stage had higher antioxidant activity (577.12 mg GA/100 g FW, 567.17 mg TE/100 g FW, and 69.24 %, respectively) than those collected at the middle stage. Significant differences ($P < 0.05$) were detected between the two stages of milk lactation. The superiority of the first lactation in antioxidant activity assays to the middle lactation demonstrates the sufficient amounts of total phenol and flavonoid compounds in the milk samples, as characterized by the color change from yellow to deep blue. In addition, lactation has superior ability to scavenge free radicals from DPPH solution, inhibit their chain formation, and destroy both initiation and propagation chains that lead to cancer and other diseases.

### Pasteurization Treatment

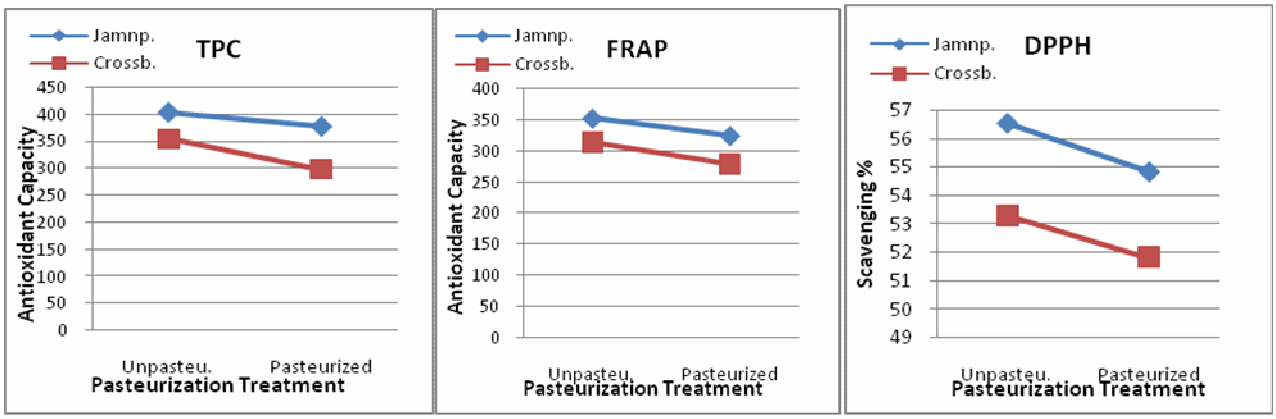


**Fig. 2: Average antioxidant activities of pasteurized and unpasteurized milk samples from *Jamnapari* and crossbred goat species.**

The effects of pasteurization treatment on the antioxidant capacity of the milk samples from *Jamnapari* and crossbred goat species are presented in Fig. 2. Significant differences ($P < 0.05$) in antioxidant activity were detected between the pasteurized and unpasteurized goat milk. Based on TPC, FRAP, and DPPH assays (377.52 mg GA/100 g FW, 324.02 mg TE/100 g FW, and 54.85%, respectively), pasteurized *Jamnapari* milk had lower antioxidant activity than the same unpasteurized breed (403.33 mg GA/100 g FW, 352.81 mg TE/100 g FW, and 56.55%, respectively). Similarly, the pasteurized milk samples from the crossbred of *Saanen* and *Jamnapari* had lower antioxidant activity than their unpasteurized counterparts (354.14 mg GA/100 g FW,

313.58 mg TE/100 g FW, and 53.29%, respectively). These results are consistent with the findings of a previous study,[8]where pasteurization significantly affected ($P < 0.05$) the antioxidant activity and diminished the total polyphenol concentration of goat milk samples.

The lower antioxidant activity of the pasteurized samples than the unpasteurized samples illustrates the diminution of the phenol and flavonoid compounds in the pasteurized milk samples. Furthermore, the antioxidant compounds of oxidation and reduction reaction were reduced in the pasteurized milk samples. The capability of the pasteurized milk samples to scavenge and destroy the free radical chain was weaker than that of the unpasteurized fresh samples. A previous study[14]reported that pasteurization negatively affects the total polyphenol concentration because of the possible denaturation of phenol content and amino acid catabolism. These differences in antioxidant power can be attributed to the higher serum proteins and less albumin of pasteurized milk than its unpasteurized counterpart duringheat stress-induced denaturation. This finding was confirmed in a previous research[15] that determined the total antioxidant activity of commercial milk samples in Italian markets. However, the researcherfound no significant differences between the mean of antioxidant activity values of ultra-heat treated and pasteurized milk. By contrast, the mean antioxidant activity values of raw milk were significantly lower than those of the treated milk samples. The researcherinterpreted that the antioxidant activity values of treated milk are significantly lower than those of raw milk because of the great sensitivity of albumin to heat treatment.

## Geographic Location

**Table 1**: Average antioxidant activity assay values of fresh milk of *Jamnapar*i and crossbredgoat species from three locations: Semenyih, Johor, and Bangi.

| Location | Breed | TPC mg/100g F.W | FRAP mg/100g F.W | DPPH % |
|---|---|---|---|---|
| Johor | *Jamnapari* | 403.33 ± 1.59 [e] | 352.81 ± 1.06 [e] | 56.55 ± 0.46 [e] |
| | Crossbred | 354.14 ± 2.20 [f] | 313.58 ± 1.06 [f] | 53.29 ± 0.20 [f] |
| Semenyih | *Jamnapari* | 469.21 ± 1.71 [c] | 385.08 ± 1.14 [c] | 62.07 ± 0.15 [c] |
| | Crossbred | 439.28 ± 1.56 [d] | 362.25 ± 1.56 [d] | 57.85 ± 0.24 [d] |
| Bangi | *Jamnapari* | 544.08 ± 1.83 [a] | 481.69 ± 1.56 [a] | 67.44 ± 0.47 [a] |
| | Crossbred | 503.51 ± 1.98 [b] | 431.62 ± 1.00 [b] | 64.26 ± 0.31 [b] |

[a–d] Different letters within the same column indicate significant differences ($P < 0.05$).

Three farm locations in Malaysia (Johor Baharu, Bandar BaruBangi, and Semenyih) were chosen to represent the geographic locations of this study. Six milk samples were collected from two goat breeds (*Jamnapari* and the crossbred) in three intervals from each farm. As shown inTable 1, the *Jamnapari* goat milk samples from Bangi exhibited the highest antioxidant activity,with TPC, FRAP, and DPPH values of 544.08 mg GA/100 g FW, 481.69 mg TE/100 g FW, and 67.44%, respectively. By contrast, the crossbred species from Johor Baharu had the lowest values (354.14 mg GA/100 g FW, 313.58 mg TE/100 g FW, and 53.29%, respectively). *Jamnapari* milk had higher antioxidant capacity than crossbred milkin all sites. Duncan analysis revealed thatBangihad the highest antioxidant activities among the locations, with TPC, FRAP, and DPPH values of 523.80 mg GA/100 g FW, 456.65 mg TE/100 g FW, and 65.85%, respectively. Bangiwas followed by Semenyih farm (454.25 mg GA/100 g FW, 373.67 mg TE/100 g FW, and 59.96%, respectively) and then Johor farm (378.74 mg GA/100 g FW, 333.20 mg TE/100 g FW, and 54.92%, respectively). Significant differences($P < 0.05$) in antioxidant activity were detected among the three geographic locations.

Antioxidant activity is affected by different factors, particularly the type of feeding. Antioxidant activity can be enhanced by providing food supplement as a source of antioxidant components.In this study, the goats from Bangi were fed with pineapple rind, watermelon, and other mixed fruits with specific supplements, including soya, brown sugar, calcium powder, and other chemical food. A previous study[16] reported that the sources of antioxidant supplements might include vegetables, fruits, herbs, and spices. In addition, the use of these supplements may help improve lipid metabolism and oxidative status. [17]reported that these supplements improve the oxidative balance and performance of lactating cows by enhancing rumen metabolism. However, a previous research[14]showed that the antioxidant compounds in goat milk are slightly related to their forage intakes, particularly several flavonoids, such as quercetin and rutin. Another researcher[18] reported that quercetin is an effective antioxidant among flavonoids; specifically, quercetin is five times more powerful than vitamins A and C. A previous study[8]also evaluated the presence of bioactive polyphenolic compounds in milk of goats

grazing on shrubby rangeland vegetation and full-indoor confinement. In a previous study, TPC was affected by animal feeding, and its value was higher in the milk of grazing goat than in the milk of indoor goat. Overproduction of animal milk is another factor that may reduce antioxidant activity. A research[19]elucidated that a high milk-producing dairy animal causes oxidative stress, and this situation can be exacerbated under certain environmental, physiological, and dietary conditions. Thus, the low antioxidant activity of the milk samples from Johor can be attributed to the high milk yield in this modern farm.

**Correlation between antioxidant assays**

**Table 2:** Correlation coefficients of the antioxidant activities among all milk samples using different assays

| | **TPC** | **FRAP** | **DPPH** |
|---|---|---|---|
| TPC | * | * | * |
| FRAP | 0.97 | * | * |
| DPPH | 0.94 | 0.91 | * |

The correlation between the three antioxidant assays was evaluated 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 ($R^2$ = 0.97). However, the correlation between DPPH and FRAP assays was lower than that between DPPH and TPC. A previous experiment[5]evaluated the TPC and DPPH activities of milk samples, and found the same results. The same correlation between FRAP and TPC has been revealed in other research.[20–21-22]Several studies have also shown highly positive correlation among FRAP, TPC, and DPPH assays.[23–24-25-26-27-28]

## Conclusion

The antioxidant activity of goat milk is significantly affected ($P < 0.05$) by pasteurization treatment, milk lactation stages, andgeographic location. The first milk lactation and unpasteurized milk exhibited higher antioxidant activity than the middle lactation and pasteurized milk. The goat milk samples from Bander Baru Bangi exhibited higher antioxidant activity than those from the other two sites. These findings illustrate that goat milk has high antioxidant activity; however, the effects of different factors must be considered.

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