



ORIGINAL ARTICLE

Milk Composition and Antioxidant Activity of Butterfly Pea (*Clitoria ternatea* L.) Goat Milk

Nur Mukhliliana Mukhnizan^a, Nurfaizatul Addila Abdullah¹, Nasuha Mohamad Hussain¹, Hanis Syazwani Mat Ghan², Noor Syaheera Ibrahim^{1*}

¹School of Animal Science, Aquatic Science and Environment, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Malaysia

²School of Food Industry, Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Malaysia

*Corresponding author: syaheeraibrahim@unisza.edu.my

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Abstract

In Malaysia, the popularity of goat milk has increased due to its health benefits, therapeutic properties, and religious significance. The extract of butterfly pea is rich in antioxidants, which can neutralize free radicals. The goat milk combined with butterfly pea flowers enhances the nutritional profile of the dairy product. The varied concentrations used were as follows: G1 (0%) as a control, G2 (1%), G3 (2%), G4 (3%), G5 (4%), and G6 (5%). The study has two main objectives: to determine the composition of butterfly pea goat milk and to evaluate the antioxidant properties of the milk at various concentrations. The milk samples were collected from Saanen goats at Pasir Akar Farm, UniSZA and recorded to be approximately 1.28 litres per goat (n=7). The milk production was subjected to vat pasteurization at a temperature of 60°C for a duration of 30 minutes. The milk samples were analyzed at UniSZA, Besut, Terengganu. The assessment of antioxidant activity was conducted by the utilization of Total Phenolic Content (TPC), Ferric Reducing Ability of Plasma (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The study revealed that the highest composition including 5% butterfly pea milk had increased levels of protein, solid-non-fat, and lactose content ($P<0.05$) respectively. The antioxidant activities showed that 5% had the highest antioxidant ($p<0.05$) compared to other concentrations of butterfly pea goat milk. Flavoured milk exhibited a superior nutritional profile and demonstrated the highest level of antioxidant activity, hence providing a multitude of health advantages for human consumption.

Keywords: Butterfly pea, Milk Composition, Antioxidant Activity, Goat milk

Introduction

The current growth in dairy goat production in Malaysia can be linked to a growing demand for goat milk, driven mostly by the traditional belief in its health advantages. The dairy goat breeds

that are most prevalent in Malaysia are Saanen, Anglo-Nubian, and Jamnapari (Liang & Paengkoum, 2019). Milk and dairy products are globally eaten and have become a fundamental component of the human diet (Zakaria et al., 2020). Flavoured milk is a dairy beverage that undergoes sweetening and colouring, either through artificial or natural means, to improve its flavour and visual appeal, particularly among young individuals (Praveen & Shakeel, 2017).

Clitoria ternatea L., also known as butterfly pea, is a perennial herbaceous creeping plant belonging to the Fabaceae family. It is distributed across various nations (Suarna & Wijaya, 2021). *Clitoria ternatea* L. has visually appealing petal colours as a result of the presence of its bioactive chemicals. The plant has been assessed for its potential as a therapeutic source due to its antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, and antimicrobial properties (Jamil et al., 2018).

Pasteurization is a prevalent procedure in the dairy business, involving the heating of a liquid to a temperature below its boiling point to eliminate germs (Pathak, 2019). Pasteurization is a process that can decrease the number of bacteria in raw milk, which is crucial for prolonging the milk's storage time. Conversely, the method has no impact on the composition of milk or the profile of fatty acids (Nasir et al., 2018). The pasteurization process consists of four methods: high-temperature short time (HTST), low-temperature long time (LTLT), flash pasteurization, and ultra-high temperature (UHT) pasteurization. The LTLT is frequently known as batch pasteurization or vat pasteurization. The procedure entails increasing the temperature of the milk to a minimum of 63°C and sustaining it at that level for a period of at least 30 minutes (Dhotre, 2014).

Butterfly pea, a widely grown plant in Southern Asia, is used in herbal beverages and cooking. In Malaysia, it is primarily used in food preparation as a natural colourant. As people become more aware of herbal components, they are turning to herbal food products for health benefits. However, the nutritional values and antioxidant activity of butterfly pea milk are still unknown in Malaysia. The two main objectives of the research are to determine the composition of butterfly pea goat milk and to examine the antioxidant properties of the milk at different doses

Materials and Methods

Ethics Approval

Animal ethics application through this study has been informed through the UniSZA Animal and Plant Research Ethics Committee (UAPREC) form and it has been submitted before the experiment begins. The permit number has been successfully applied with the registered no (UAPREC/008/002).

Milk Collection and Pasteurization

Seven (n=7) healthy Saanen goats from UniSZA Pasir Akar Farm in Terengganu, Malaysia were selected for the milking procedure at 9.00 a.m. The chosen goats were between 3 and 4 years of age and had a body condition score (BCS) ranging from 2 to 3. These goats had symmetrical udder characteristics. The goat milk samples were manually collected at a rate of approximately 1.28 litres per goat through hand milking. Next, the milk that has been collected is subjected to vat pasteurization at a temperature of 63°C for a duration of 30 minutes, as described by Dhotre (2014) with some modifications.

Butterfly pea powder

The butterfly pea flowers were collected around the UniSZA Tembila Campus. The butterfly pea petals were dried in the dryer at 60°C for 90 minutes following the method that has been prepared by Tri et al. (2019) with modifications

Butterfly pea goat milk flavored and extraction

The butterfly pea goat milk flavour was prepared (duplicates) following the procedure described by Kashid et al. (2007) with a few alterations. There were six groups of samples formulated with butterfly pea and goat milk flavours. G1 (Control) had a 0% concentration of butterfly pea powder. The remaining five groups were formulated using varying ratios of butterfly pea extract to pasteurized and goat milk: G2 (1%), G3 (2%), G4 (3%), G5 (4%), and G6 (5%) accordingly.

Following Gheisari et al. (2020), the experiment involved treating butterfly pea goat milk flavoured extract with modifications and triplicate. One gram of methanolic extract was combined with five ml of methanol and placed in an incubator shaker at a temperature of 15°C for a duration of 12 hours. The mixture was then filtered and transferred to a sterilized 15 ml centrifuge tube, which was subsequently stored in the laboratory freezer at a temperature of -23°C. The extracted substance was thereafter placed in the freezer before conducting the antioxidant analysis.

Milk composition and Antioxidant analysis

The six groups of the butterfly pea goat milk samples including the control were analysed for their composition by using Milkotester Ultrasonic Milk Analyzer (Milkotester, Master Eco, Bulgaria) with duplication. The milk composition procedure was followed by Ibrahim and Jalil (2022). It contributes to the fat, protein, lactose and solid-non-fat content.

Antioxidant analysis

Total Phenolic Compound (TPC)

The six groups of milk samples with different formulations were evaluated for the antioxidant content by Total Phenolic Compound (TPC). Total phenol contents of butterfly pea goat milk flavoured were determined in triplicate using the Folin-Ciocalteu method analysis by Shirazi et al. (2014) with modifications. The total phenolic content of each formulation for the Gallic acid standard curve was expressed as a percentage (%) of absorbance.

The same procedure was repeated with the prepared butterfly pea goat milk flavoured methanolic extract formulation including the control by replacing the Gallic acid solution. The total phenolic content of each formulation was expressed as Gallic acid equivalent (mg GAE/ml).

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The six formulations of the milk samples were analyzed for antioxidant content by DPPH radical scavenging activity by Hwang et al. (2009) with modification. A standard Trolox curve was constructed by preparing the dilution of (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) in methanol from the standard solution of Trolox. 0.3 ml of each dilution was added to 1.2 ml methanol and 1.5 ml of 0.5 mM DPPH solution in the 15 ml centrifuge tube. The centrifuge tubes were covered with the aluminium foil to avoid the environmental contact.

Then, the mixture kept in the dark at room temperature 31 for 90 minutes. The DPPH result of the standard curve was then measured at 517 nm by using UV-VIS spectrophotometer (UVmini-

1240, Shimadzu, Japan). The DPPH radical scavenging activity of Trolox in the standard curve was expressed as a percentage (%) of absorbance. The same procedure was repeated with the prepared butterfly pea goat milk flavoured methanolic extract formulation by replacing the Trolox solution. Each formulation analysis was performed in triplicate.

The control was assayed with pasteurized goat milk extract. The following equation was employed to calculate the percentage of inhibition for each extract by Chandru et al. (2018). The DPPH radical scavenging activity of Trolox of each formulation was expressed as inhibition (%).

$$\text{DPPH free radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

Where A_c is the absorbance of control (DPPH free radical without the addition of test solution), and A_s is sample absorbance (absorbance of DPPH free radical after the addition of test solutions).

Ferric reducing antioxidant power (FRAP)

The antioxidant content in all the milk formulations was determined with FRAP assay by Bertoncelj et al. (2007) with modification. The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+} -TPTZ) to its ferrous, coloured form (Fe^{2+} -TPTZ) in the presence of antioxidants. The stock solution was prepared which included 300mM acetate buffer (pH 3.6), 10 mM 2,3,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid (HCl) and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution. The FRAP reagent was freshly prepared by mixing acetate buffer, TPTZ solution and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution with a ratio of 10:1:1 in ml and then incubated at 37°C in the water bath for 10 minutes.

A standard of an aqueous solution of ferrous sulphate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was constructed by preparing the dilution of (10, 20, 20, 40, 50 and 60 mg/ml) in distilled water from the standard solution of ferrous sulphate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution. 0.2 ml of each dilution was mixed with FRAP reagent in the 15 ml centrifuge tube. The centrifuge tubes were covered with the aluminium foil to avoid the environmental contact. Then, warm the mixture at 37°C in the water bath for 10 minutes. The FRAP result of the standard curve was measured at 593 nm by using a UV-VIS spectrometer (UVmini-1240, Shimadzu, Japan).

The FRAP standard curve of each formulation was expressed as a percentage (%) of absorbance. The same procedure was repeated with the prepared butterfly pea goat milk flavoured methanolic extract formulation by replacing the distilled water solution. The FRAP result of each formulation was expressed as distilled water equivalent (mg/ml). Each formulation analysis was performed in triplicate.

Statistical Analysis

In this study, the collected data from six formulations of butterfly pea goat milk were analyzed for their milk composition and antioxidant analysis by using mean \pm standard deviation (SD) from Microsoft Excel. Then, the results were interpreted using one-way ANOVA with ($p < 0.05$) is considered a significant difference.

Result and Discussion

Milk Composition of Pasteurized Butterfly Pea Goat Milk

Fat

The result showed the addition of butterfly pea in the milk had no significant differences ($p > 0.05$) in the fat content of 0% concentration of butterfly pea compared to the other five formulations. Nevertheless, the level of fat did not escalate with the increase of butterfly pea concentration. G1 had the lowest fat amount at 3.8%, while G5 had the highest fat content at 4.1%.

The fat content exhibited an increase from plain goat milk to G5, with G1 demonstrating the lowest percentage at 3.8%. Subsequently, sustain at a rate of 4.0% for subsequent formulations. The previous investigation yielded a comparable outcome, indicating that the inclusion of butterfly pea extract in the cannabis tuber snack bar did not have any noticeable impact on its fat level. This can be attributed to the fact that butterfly pea extract has a low-fat content (Neda et al., 2013).

Protein

Table 1 displays the protein composition of five different formulations of butterfly goat milk, including ordinary goat milk as the control. The inclusion of butterfly pea flower resulted in significant increases ($p < 0.05$) in the protein content. The study analyzed the protein composition of five variations of goat milk infused with butterfly pea, including plain goat milk.

The results demonstrated a considerable increase in protein content with the addition of butterfly pea flowers. Incorporating butterfly pea flower resulted in a rise in protein content, with a concentration of 5% in G5 yielding a protein content of 3.8%. Consistent with prior studies, this discovery indicates that increasing the concentration of butterfly pea flower extract in snack bars leads to a corresponding rise in protein levels (Neda et al., 2013).

Solid non-fat (SNF)

According to the findings in Table 1, the solid-non-fat (SNF) content of G1 (Control), which was 7.8%, exhibited statistically significant differences ($p < 0.05$) when compared to all five formulations. G6 exhibits the greatest Solids-Not-Fat (SNF) content, amounting to 10.5% when 5% butterfly pea is added.

These findings indicate a positive correlation between the concentration of butterfly pea and the SNF content, meaning that when the concentration of butterfly pea increases, the SNF content also increases. As stated by the Mourad et al. (2014), the solid-non-fat component contains minerals such as calcium and phosphorus. The nutritional study of *C. ternatea* L. flowers have revealed the presence of minerals such as calcium (2%), phosphorus (0.48%), potassium (2.08%), and magnesium (0.29%) (Turnos, 2021). The elevated mineral content in butterfly pea may result in an increased level of SNF (solids-not-fat) content.

Lactose

Table 1 demonstrates a statistically significant distinction ($p < 0.05$) between the 0% concentration of butterfly pea and the other five formulations, which exhibit an increase in butterfly pea content up to 5%. The lactose content exhibited a gradual increase in proportion to the concentration of butterfly pea, although not at a rapid rate. Plant-based milk carbohydrates were quantified after lactose in a prior investigation (Walther et al., 2022).

In a study conducted by Susilo et al. (2023), it was shown that the low carbohydrate content in butterfly pea blossoms did not have any noticeable impact on the carbohydrate content of the snack bar. This study did not see any notable disparity in lactose content (ranging from 1mg to 2mg of butterfly pea) in snack bars (Susilo et al., 2023). However, the present investigation identified substantial variations in lactose levels. This can be attributed to the varying composition

of butterfly pea and main samples, particularly in this investigation where the use of goat milk may yield divergent outcomes.

Table 1. Mean and standard deviation of different concentrations of Butterfly Pea goat milk

Percentage of Butterfly Pea	Milk composition			
	Fat (%)	Protein (%)	SNF (%)	Lactose (%)
0	3.8 ^a ± 0.0	2.9 ^b ± 0.0	7.8 ^b ± 0.0	4.3 ^b ± 0.0
1	4.0 ^a ± 0.14	3.5 ^a ± 0.14	9.7 ^a ± 0.42	5.3 ^a ± 0.28
2	4.1 ^a ± 0.14	3.6 ^a ± 0.14	9.9 ^a ± 0.40	5.5 ^a ± 0.21
3	4.0 ^a ± 0.0	3.5 ^a ± 0.14	9.7 ^a ± 0.01	5.3 ^a ± 0.0
4	4.1 ^a ± 0.07	3.8 ^a ± 0.14	10.4 ^a ± 0.37	5.7 ^a ± 0.14
5	4.0 ^a ± 0.14	3.8 ^a ± 0.14	10.5 ^a ± 0.42	5.75 ^a ± 0.21

*Data are mean ± standard deviation (SD) values of duplicate results within a column with different superscripts of "a" and "b" representing the significant differences. The same alphabet indicates no significant differences in parameters. SNF: solid-non-fat

Antioxidant Activities of Butterfly Pea Goat Milk

Total Phenolic Content (TPC)

The total phenolic content (TPC) of butterfly pea goat milk flavoured formulations was determined to identify phenolic compounds in the milk (as shown in Figure 1). Among the formulations, the addition of 1% butterfly pea resulted in the lowest total phenolic content of 0.37 mg GAE/ml, while the formulation with 5% butterfly pea exhibited the highest total phenolic content of 0.43 mg GAE/ml. According to Vuong & Hongsprabhas (2021), butterfly pea flowers are rich in phenolic compounds (30 mg Gallic acid equivalents/g dry matter). The antioxidant activities of products are enhanced when plant materials with high phenolic content are utilized (Stobieck et al., 2022).

Tungmunningthum et al. (2018) reported that the quantity of phenolic compounds in butterfly pea is influenced by factors such as harvest season, cultivars, and plant varieties, necessitating consideration in general phytochemical analysis. The extraction process, including the solvent temperature, also affects the yield of bioactive components. However, in this study, the extraction technique applied to butterfly pea flower parts exhibited no impact on total phenolic levels. This finding is corroborated by Singh et al. (2022), who observed a significant increase ($p < 0.05$) in TPC content with the addition of butterfly pea to blend formulations, underscoring the potential of butterfly pea in producing health-enhancing puffed snacks and breakfast cereals.

DPPH

The DPPH Radical scavenging assay is a commonly employed method for assessing the radical scavenging ability of plant extracts (Aparadh et al., 2012). This technique is favoured due to its rapidity and sensitivity, as well as its reliance on basic laboratory equipment. Figure 2 showed the analysis of p-values revealed that the DPPH scavenging activity in the six formulations (G1, G2, G3, G4, G5, and G6) exhibited a significant increase. Specifically, the DPPH results indicated a noteworthy ($p < 0.05$) rise in the percentage inhibition of the butterfly pea goat milk-flavoured product. The inhibition percentage refers to the minimum concentration of antioxidant chemicals required to completely suppress the activity of free radicals. Chen et al. (2018) found that the

DPPH free radical scavenging activity of the cold butterfly pea flower extract reached 63.25% when the concentration was raised to 100 mg/ml.

Wulansari & Chairul (2011) reported that the butterfly pea kombucha exhibited antioxidant activity ranging from 60% to 70%, indicating a significant quantity of antioxidants. The figure 2 demonstrates that the percentage of DPPH scavenging activity for all plant extracts rises as the concentration of the plant extract increases. Notably, the 1% concentration of butterfly pea flower exhibits the lowest percentage inhibition at 16.56%. However, when 5% of butterfly pea flower is added, there is a significant increase in the percentage inhibition to 68.39%, indicating a higher concentration of antioxidant compounds required to inhibit free radicals. This claim is substantiated by Ramaswamy et al. (2011), who found that when the concentrations of extracts increased, the percentage of free radicals scavenged by the extracts also increased.

FRAP

The study analyzed the ferric reducing antioxidant power (FRAP) activity of different butterfly pea goat milk flavoured formulations (Figure 3). With the addition of 1 to 5% of the concentration, the results demonstrated an increase in FRAP activity ($p < 0.05$). The highest FRAP value was observed at 5% of butterfly pea flowers (0.54 mg/ml). The highest FRAP value was observed at 1% of butterfly pea flowers (0.27 mg/ml). The *Clitoria ternatea* L. species (butterfly pea) extract showed the highest reducing power with the concentration. Other study found similar results obtained in *Cleome speciosa*, which showed the highest antioxidant activity as it had the highest reducing power at all concentrations of its extract (Aparadh et al., 2012). The study also found that the highest FRAP value was observed at 5% of butterfly pea flowers (0.54 mg/ml). This indicates that the FRAP activity of butterfly pea goat milk flavoured is significantly increased with the addition of butterfly pea flower. The findings suggest that the addition of butterfly pea flowers to milk can enhance the antioxidant activity of the milk.

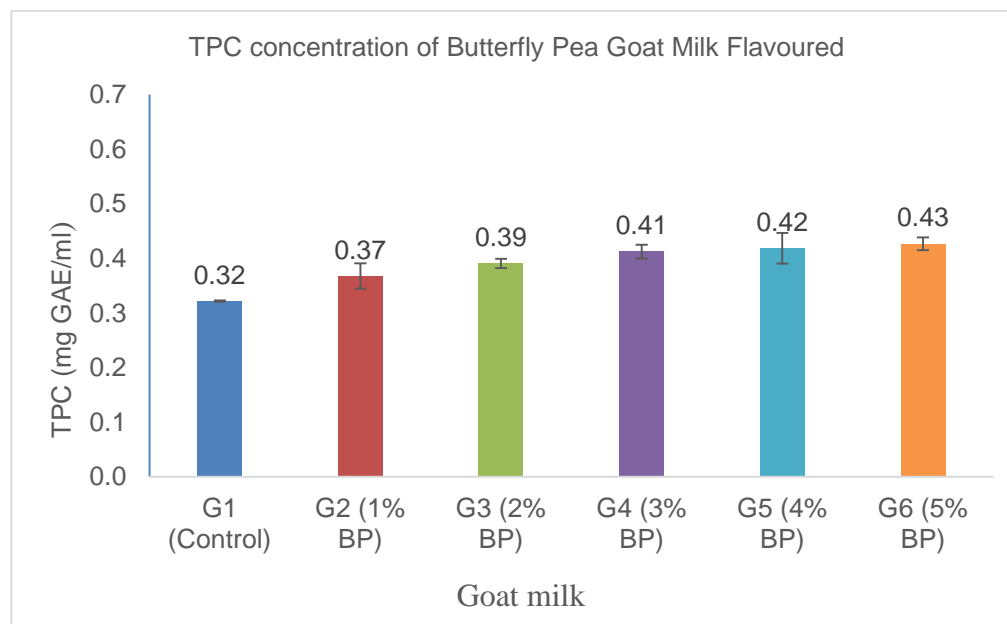


Figure 1. Total Phenolic Content (mean \pm standard deviation) of butterfly pea goat milk extract with different concentrations ($P < 0.05$). BP refers to butterfly pea

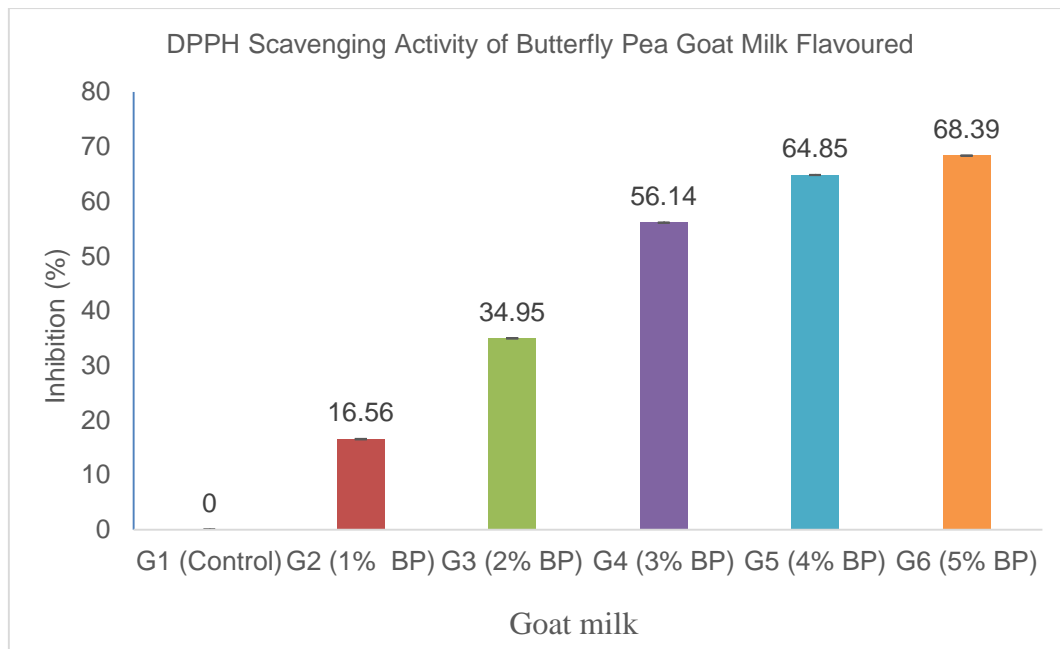


Figure 2. Percentage Inhibition of 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging of butterfly pea goat milk ($P < 0.05$). BP indicates butterfly pea

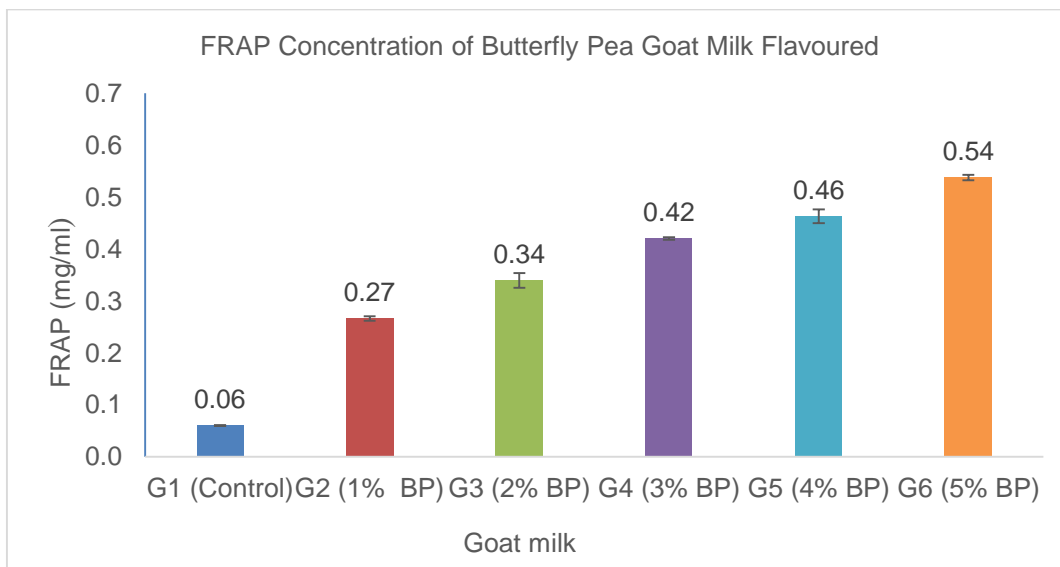


Figure 3. Ferric Reducing Antioxidant Power (FRAP) value of butterfly pea goat milk flavoured extract ($P < 0.05$). BP indicates butterfly pea

Conclusion

In summary, the goat milk with 5% BP had the highest concentration of antioxidant activity and the highest milk composition value. It has the highest levels of fat, protein, lactose, SNF, and antioxidant activity compared to other concentrations. This study indicates butterfly pea goat milk contains high protein, lactose and SNF and is high in antioxidants that are beneficial and nutritious for consumption.

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