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RESEARCH ARTICLE

Comparing between Camel's Milk and Bovine's Milk antioxidant activity using DPPH method

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Abstract

Bovine and camel milk have several health benefits, including high antioxidant and radical scavenging activities. The thermal stability of camel and bovine milk could enhance the antioxidant potential of several natural sources, such as honey, black tea, and Matcha. The current study assessed the antioxidant capacity and radical scavenging potential of different natural sources in camel and bovine milk by using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method and Ferric-Reducing Antioxidant Power (FRAP) assay. To examine the antioxidant capacity and the thermal stability of honey, black tea, and Matcha, each natural source was added to bovine and cow milk and later heated until it reached boiling point temperature for 30 minutes. Subsequently, the antioxidant capacity of these samples was evaluated. The results indicated that Matcha maintained its antioxidant activity with a significant induction of the radical scavenging potential in both camel ($p \leq 0.0001$) and bovine milk ($p \leq 0.05$) after heating. However, Black tea showed a significantly higher radical scavenging potential in camel milk only ($p \leq 0.001$) but not in bovine milk. The results suggest that camel milk has better heat stability in conserving the antioxidant activity of black tea and Matcha sources than bovine milk. In contrast, the radical scavenging activity of honey remained consistent and unchanged in both types of milk, with no significant difference compared to the control.

Keywords: Antioxidants capacity, Oxidative stress, DPPH, FRAP, Camel milk, Bovine milk, Matcha, Honey, Black tea

Introduction

Oxidative stress is disequilibrium between the produced Reactive Oxygen Species (ROS) and antioxidant defense mechanisms. Reactive oxygen species include free radical species such as hydroxyl (•OH) and superoxide ($O_2^{\bullet -}$) and non-radical species such as H_2O_2 (Pizzino et al., 2017; Jomova et al., 2023). The generation of these species disrupts the balanced cellular redox state, causes cell death, and causes macromolecule damage such as DNA, lipids, and protein. Moreover, the uncontrolled disruption of the antioxidant defense mechanism leads to the progression of many diseases, including diabetes, Alzheimer's Disease (AD), cancer, atherosclerosis, Parkinson's Disease (PD), and others (Cioffi et al., 2021; Cioffi et al., 2019; Percário et al., 2020; Hayes et al., 2020; Yang et al., 2017 & Oguntibeju et al., 2019). Many endogenous sources of free radicals are produced as intermediates during aerobic respiration and the generation of ATP in the mitochondria. The antioxidant mechanism will be triggered based on the free radical generation as a defense mechanism to neutralize

the free radical species, usually by donating electrons or H-atoms. Antioxidants can reduce the risk of degenerative disease by neutralizing the excess produced free radicals (Demirci et al., 2022; Betteridge et al., 2000). There are many identified exogenous sources of naturally occurring antioxidants, including ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), carotenoids, and polyphenols (Möller et al., 2006; Lourenço et al., 2019). Several Japanese and Chinese green tea varieties, including Matcha, were found to have antioxidant activity (Olson et al., 2020). Matcha is a powdered Japanese green tea (*Camellia sinensis*) with a high antioxidant capacity (Kochman et al., 2020). Matcha contains great nutritional values and health benefits, including cognitive function enhancement, mental clarity improvement, anti-inflammation properties, and antioxidant capacity.

Moreover, honey is another important natural source of antioxidants and antibacterial activities with high health benefits due to the presence of flavonoids, phenolic compounds, and vitamins (Sant'ana et al., 2014). However, the honey's composition, stability, and efficiency of its nutritional values could be affected by different factors such as its origin, geographical regions, and other factors. Black tea is the third natural source that was selected in the current study due to its high antioxidant capacity. Many studies indicated that the main components in black tea that exhibit high antioxidants values are polyphenols, especially catechins, theaflavins, and flavonoids (Grzesik et al., 2018; Sharma et al., 2020; Wang et al., 2023).

Camel milk, in addition, contains a high antioxidant capacity that could alleviate oxidative stress by reducing free radical production (Behrouz et al., 2022; Khan et al., 2021). Bovine milk contains bioactive peptides and proteins that develop antioxidant properties (Clausen et al., 2009; Stobiecka et al., 2022). Interestingly, (Bourassa et al., 2013). Group showed that milk alpha-casein could reduce antioxidant capacity (Bourassa et al., 2013). A study comparing camel and cow (bovine) milk found some differences regarding protein fractions (Valko et al., 2007). For instance, camel milk caseins are lower than cow (bovine) milk, accounting for 52%-87% and 80%, respectively (Bourassa et al., 2013). Another study showed that heating affects the structure and conformity of proteins in camel milk. However, camel milk is more heat stable than cow (bovine) or buffalo milk (Hamouda et al., 2022). The remaining question to be investigated is whether the increased heat stability of camel milk could preserve the antioxidant activity of many natural antioxidant sources in black tea, honey, and Matcha.

Materials and Methods

Determination of DPPH radical-scavenging activity

The 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) method depends on measuring the antioxidant ability to reduce the free radicals from purple-colored DPPH radical into pale yellow-colored reduced DPPH compound. The assay was conducted according to (Brand-Williams et al., 1995) method (Brand et al., 1995). Lower absorbance indicates higher antioxidant activity.

Ferric Reducing Power Assay (FRAP)

Ferric Reducing Power Assay (FRAP) relies on the reduction of Fe^{3+}–TPTZ (2,4,6-tri (2- pyridyl)-1,3,5-triazine) to produce Fe^{2+}–TPTZ navy blue color that is reflected by a significant increase in the absorption level. Higher absorbance indicates higher antioxidant activity. The assay was conducted according to Benzie and Strain developed method (Benzie et al., 1999).

Results

DPPH radicals scavenging activities of black tea, honey, and matcha in bovine and camel milk

A Comparative study between bovine and camel milk was conducted to estimate the antioxidant capacity of black tea, honey, and Matcha using the DPPH method. The DPPH contains long-lived nitrogen with an odd electron, and the antioxidant activity of black tea, honey, and Matcha would turn the deep blue color of DPPH to yellow color by reduction. The antioxidant activity was evaluated by monitoring the absorption decrease, representing a higher antioxidant capacity. In bovine milk, Matcha showed a significant reduction ($p \leq 0.05$) in absorption, reflecting a higher scavenging activity and

a higher antioxidant level compared to black tea and honey in camel and bovine milk. In camel milk, both black tea and Matcha showed significant antioxidant capacities compared to honey by the DPPH method ($p \leq 0.001$, $p \leq 0.0001$), respectively (Fig. 1). On the other hand, black tea did not preserve the antioxidant activity after heating bovine milk, while it showed significant preserved antioxidant activity in heated camel milk. No significant difference was observed between honey's antioxidant capacity in both camel and bovine milk compared to the control.

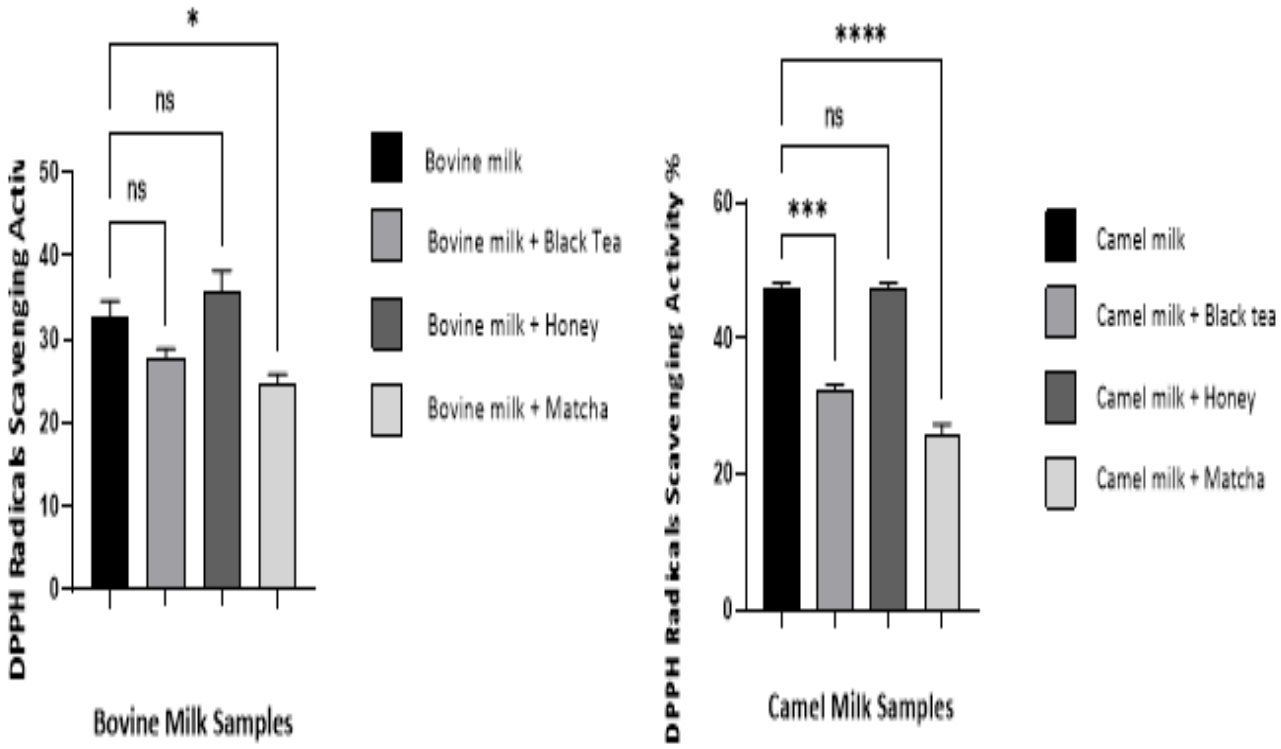

Figure 1. Comparative antioxidant potentials of Black tea, honey, and Matcha in Bovine and Camel milk by DPPH method. Percentage of DPPH inhibition of black tea, Honey, and Matcha indicates a significant difference in the antioxidant capacity of Matcha compared to control in both bovine ($p \leq 0.05$) and camel milk ($p \leq 0.0001$), respectively (Fig. 1). Black tea showed a significant higher antioxidant capacity only in camel milk ($p \leq 0.001$) but not in bovine milk. Honey showed unchanged antioxidant capacity in both bovine and camel milks.

A comparative study between bovine and camel milk was conducted to estimate the antioxidant capacity of black tea, honey, and matcha, which was confirmed by the FRAP method. On the other hand, FRAP assay relies on the reduction of Fe^{3+}–TPTZ to produce a navy-blue color of Fe^{2+}–TPTZ, which is reflected by a significant increase in the absorption level. Consistently with the DPPH method, the FRAP assay showed matcha in bovine milk to contain the highest antioxidant capacity ($p \leq 0.05$) with the deepest navy-blue absorption level compared to black tea and honey (Fig. 2). Non-significant differences were found in honey and black tea compared to the control (bovine milk). In camel milk, on the other hand, both black tea and matcha preserved the antioxidant capacity. They showed a significant increase in the FRAP reduction compared to the control (camel milk) ($p \leq 0.05$, $p \leq 0.01$), respectively. In bovine milk, the effect of heating on diminishing the antioxidant capacity of honey was non-significant, and a non-significant reduction of FRAP reagents was observed compared to the control. In addition, no significant difference was observed between honey's antioxidant capacity in camel and bovine milk compared to the control.

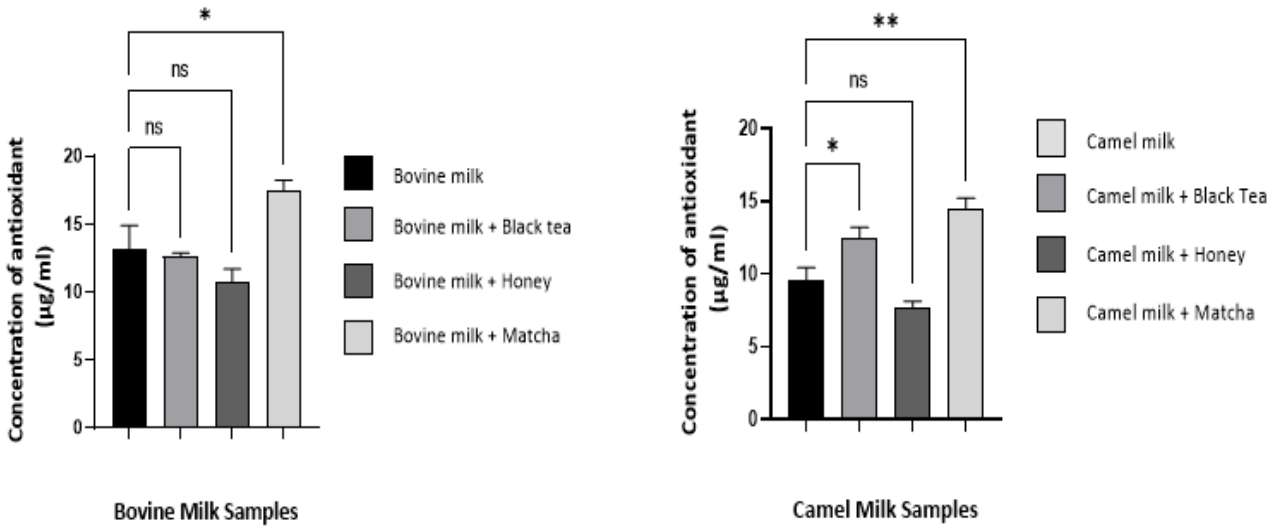

Figure 2. Comparative antioxidant potentials of Black tea, honey, and Matcha in Bovine and Camel milk by FRAP method. Percentage of FRAP of black tea, Honey, and Matcha indicates significant differences of Matcha antioxidant capacity compared to control in both bovine ($p \leq 0.05$) and camel milk ($p \leq 0.01$), respectively (Fig 2). Black tea showed a significant higher antioxidant capacity only in camel milk ($p \leq 0.05$) but not in bovine milk. Honey showed unchanged antioxidant capacity in both bovine and camel milks.

From buffalo, sheep, cow, camel, and goat (Khan et al., 2019). They found the highest capacity in buffalo and sheep, while cow and camel milk showed similar antioxidant capacity levels when examined by the DPPH method (Khan et al., 2019). The antioxidant properties in milk products are ascribed to vitamins A, cysteine, phosphate, and others. Regarding the heat effect on both cow and camel milk sources, a previous study showed that camel milk is more heat stable in preserving its nutritional value than bovine milk. In the current study, we compared the antioxidant capacity of three different natural sources, including black tea, honey, and Matcha, after the heating process. Matcha and black tea preserved their antioxidant value in camel milk more efficiently than in bovine milk. These results indicate that camel milk's higher heating stability could better preserve the antioxidant activity of both match and black tea more significantly than cow milk. In order to assess the radical scavenging activity of different natural antioxidant sources, the DPPH method was chosen in the current study due to its simplicity, accuracy, sensitivity, and speed. The DPPH contains long-lived nitrogen with an odd electron, and the antioxidant source in black tea, honey, and Matcha would be reduced, and the deep blue color of DPPH would turn yellow after reduction (Kedare et al., 2011). The antioxidant activity in this method was evaluated by monitoring the decrease in absorption.

Moreover, the FRAP assay was used as another confirmation method for the obtained results. The FRAP assay relies on reducing Fe^{3+}–TPTZ to produce Fe^{2+}–TPTZ by the added natural source of antioxidants. The antioxidant activity of each source will be equivalent to the reduced Fe^{2+}–TPTZ, which is determined by the formation of a navy-blue color. The antioxidant activity in this method is directly proportional to the absorbance intensity (Benzie et al., 1996). Based on the results obtained by using the previously indicated methods, Matcha was found to be the antioxidant source that persists in activity in both bovine and camel milk even after the heating process.

Interestingly, black tea preserved the antioxidant value only with camel milk, while no measurable antioxidant activity was noticed with bovine milk. This exciting result would add another value for the camel milk previously indicated by Hamouda et al. to have more heat stability compared to other milk sources (Hamouda et al., 2022). In addition to its high heat stability, camel milk would preserve the antioxidant activity of the added natural antioxidant source in the tested milk during the heating process for 20 minutes, which was noticed to recede in bovine milk. Another possible explanation for the results obtained from previous studies is the ability of casein proteins in milk to bind to the antioxidant components and reduce the free radical scavenging capacity of these antioxidant components. The antioxidant contents

(polyphenols) found in black tea have a strong affinity to bind to milk proteins, leading to decreased free radical scavenging capacity of these components (Kilmartin et al., 2003; Dubeau et al., 2010).

Moreover, Bourassa et al. suggested that camel milk's total casein level is lower than cow (bovine) milk, accounting for 52%-87% and 80%, respectively (Bourassa et al., 2013). This previous observation could explain the increased antioxidant capacity of black tea polyphenols in camel milk compared to bovine milk due to the reduced level of casein proteins in camel milk. The lower level of casein proteins in camel milk could be a contributing factor to the increased antioxidant capacity of black tea polyphenols in camel milk compared to bovine milk. However, another comparative study, including comparing the effect of casein proteins in both camel and milk on the different natural antioxidant sources, will be suggested for further investigation.

Contrary results have been found in the literature regarding the unaffected antioxidant activity of honey after thermal treatment. Kowalski et al. and Elamine et al. reported similar results to the current study, with a statistically non-significant change in the antioxidant activity among different honey sources after thermal treatment assessed through the DPPH assay (Kowalski et al., 2013; Elamine et al., 2020). Another consistent study by Šaric et al. showed statistically non-significant changes ($p>0.05$) in different samples of acacia honey and chestnut honey after thermal treatment at 95°C using FRAP and DPPH assays (Šarić et al., 2013). The only significant increase was observed in acacia honey ($p<0.05$) using a DPPH assay. Moreover, eighteen honey samples were subjected to thermal exposure at different temperatures from 45°C to 65°C for one hour and subsequently analyzed with DPPH for antioxidant inhibition, and no significant effect was observed (Ramlan et al., 2021). A study recommended using the 2,20-casino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) method to more accurately evaluate the free radical scavenging activity, especially in honey samples (Magalha et al., 2008). Further confirmation might be needed to evaluate the antioxidant activity of honey after thermal exposure through ABTS assay.

On the contrary, studies by Jahan et al. and Turkmen et al. suggested that the thermal treatment of honey increased its antioxidant activity while the antimicrobial effect was reduced (Jahan et al., 2015; Turkmen et al., 2006). Turkmen et al. group suggested that higher temperatures showed different effects on the antioxidant activity of honey, and they observed higher antioxidant activity at 70°C (Turkmen et al., 2006). Furthermore, another study indicated increased antioxidant activity of honey at 80°C compared to 48°C by monitoring the thermal effect on free radical scavenging using DPPH, beta-carotene bleaching inhibition, and Fe+3 reduction (Gheitanchi et al., 2021). Molaveisi et al. reported increased antioxidant activity in jujube honey samples at temperatures between 45°C and 65°C (Molaveisi et al., 2019). Zarei et al. observed a decrease in the antioxidant capacity of three types of Iranian honey after thermal treatment at 65°C for different time intervals between 10 minutes and 30 minutes using DPPH assay (Zarei et al., 2019). Therefore, the degree of temperature, honey botanic sources, the method used to monitor the antioxidant levels, and the heating duration of honey could be possible explanations for the different contrary results during the antioxidant capacity assessment of honey. Most of the other studies recorded no significant effect of thermal treatment on the antioxidant quality of honey, which may indicate high stability. FRAP assay in our study did not show a significant heating effect on the antioxidant competence.

This method was previously examined on different honey samples for measuring the nutritional values of honey by Hagr et al. and Tuksitha et al. (Hagr et al., 2017; Tuksitha et al., 2018). The FRAP values in some types of honey showed higher values as the heat treatment temperature increased and the heat exposure duration expanded (12 hours) (Jahan et al., 2015). At a temperature of 90°C, a shorter heating time (less than 5 minutes) with some honey samples showed higher FRAP values, while others showed inconsistent results (Braghini et al., 2019). The increased antioxidant capacity after heating treatment was explained by Braghini et al. to be due to the increased level of some phenolic compounds released after heat treatment, which contribute to the antioxidant properties. Insufficient thermal treatment and duration could affect the antioxidant level observed with the FRAP assay (Braghini et al., 2019). Although in the current study, the thermal treatment duration was 30 minutes until it reached boiling temperature, an increase in the antioxidant capacity of honey was not observed.

To our knowledge, no previous study has examined and compared the effect of thermal treatment on the antioxidant activity of honey, black tea, and Matcha in camel and bovine milk. However, from the nutritional perspective, understanding the factors that could affect or decrease the antioxidant capacity is essential in evaluating many antioxidant resources' nutritional and health benefits. Further research is needed to explore the mechanisms underlying the preservation of antioxidant activity in camel milk and to investigate the role of casein proteins in different natural antioxidant sources. Additionally, more studies are needed to explore the effect of thermal exposure on the antioxidant capacity of honey using different methods and conditions.

Conclusions

The current study provides valuable comparative assessments of the antioxidant activity in different natural sources such as black tea, matcha, and honey. It provides a comparative study of the effect of thermal processing on the natural source's antioxidant activity in camel and bovine milk. After the thermal treatment of black tea, honey, and matcha in camel and bovine milk, a comparative study was applied to assess the antioxidant potential of these diverse natural antioxidant sources. The results indicated that matcha and black tea preserved their radical scavenging values more efficiently in camel milk compared to bovine milk, even after thermal treatment. Camel milk might have better thermal stability in conserving the radical scavenging capacity of some natural sources, including black tea and match, than bovine milk. However, honey's free radical scavenging potential remained unaffected after thermal processing in both types of milk compared to the control. Further confirmation is needed to explain the obtained results with different honey floral origins and thermal exposure time. Moreover, further research is required to translate the mechanisms behind the observed results and estimate the influence of distinct factors on the radical scavenging potential of different natural sources of antioxidants.

Scientific responsibility statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all the preparation and scientific review of the contents and approval of the final version of the article.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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