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RESEARCH ARTICLE

Harnessing antioxidants: Investigating plant and animal milk variants in matcha tea's health benefits

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Abstract

Matcha, a finely ground green tea powder, is known for its exceptionally high antioxidant content, particularly catechins like epigallocatechin gallate. The addition of milk to tea is a common practice that may affect their antioxidant properties. This study investigated the effects of various milk types on matcha tea's antioxidant activity. Using total phenolic content, total flavonoid content, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging, hydrogen peroxide scavenging, metal chelating activity, and ferric reducing power assays, we examined how cow's milk and plant-based alterna-tives (soy, almond, coconut, and oat milk) impact matcha's antioxidant capacity. Results showed that all milk types reduced matcha's antioxidant activity to varying degrees, with cow's milk having the most pronounced effect, followed by soy milk. Almond, oat, and coconut milk demonstrated less interference with matcha's antioxidant properties, respectively. The reduction in antioxidant activity was attributed to protein-polyphenol interactions, with the extent of reduction correlating with the protein content of the milk. These findings suggest that consumers seeking to maximize the health benefits of matcha may prefer consuming it without milk or choosing plant-based alternatives with lower protein content. This study con-tributes to our understanding of how dietary choices can influence the potential health benefits of antioxidant-rich beverages.

Keywords: Matcha; Antioxidant activity; Milk alternative, Dietary choices, Antioxidant activity

Introduction

Matcha, a finely ground powder derived from specially cultivated Camellia sinensis leaves, has garnered significant attention in both culinary and scientific circles due to its unique flavor profile and purported health benefits. Unlike conventional green tea, matcha is prepared by consuming the entire leaf, resulting in a substantially higher concentration of bioactive compounds. The distinctive cultivation process, which involves shading the tea plants for several weeks prior to harvest, enhances the production of chlorophyll and amino acids, contributing to matcha's characteristic vibrant green hue and umami flavor (Khan & Mukhtar, 2013; Kochman et al., 2020).

The health-promoting properties of matcha are primarily attributed to its exceptional antioxidant content, particularly its high concentration of polyphenolic compounds known as catechins. Matcha contains four major catechins: Epicatechin (EC), Epicate-Chin-3-Gallate (ECG), Epigallocatechin (EGC), and Epigallocatechin-3-Gallate (EGCG), with EGCG being the most abundant and biologically active (Jakubczyk et al., 2020). Research indicates that matcha can

contain up to ten times more poly-phenols than regular green tea, a characteristic largely attributed to the unique shading technique employed during cultivation (Kochman et al., 2020). This elevated polyphenol content is crucial for combating oxidative stress and inflammation, processes implicated in the pathogenesis of numerous chronic diseases.

The interplay between free radicals and antioxidants is fundamental to maintaining cellular homeostasis and overall health. Free radicals, characterized by unpaired electrons in their outer shell, are highly reactive molecules capable of inducing oxidative damage to cellular components such as lipids, proteins, and DNA (Du et al., 2012). These reactive species can originate from both endogenous sources (e.g., mitochondrial respiration and inflammatory processes) and exogenous sources (e.g., environmental pollutants and ultraviolet radiation). Chronic oxidative stress, resulting from an imbalance between free radical production and antioxidant defenses, has been implicated in the etiology of various pathological conditions, including cardiovascular diseases, neurodegenerative disorders, diabetes mellitus, and neoplastic diseases (Mukherjee et al., 2024).

Antioxidants serve as a critical defense mechanism against oxidative stress by neutralizing free radicals and mitigating cellular damage. These compounds can be categorized into endogenous antioxidants, synthesized by the body, and exogenous antioxidants, obtained through dietary sources. The consumption of antioxidant-rich foods has been associated with a plethora of health benefits, primarily through the attenuation of oxidative stress and the concomitant reduction in chronic disease risk. For instance, antioxidants play a pivotal role in cancer prevention by neutralizing free radicals capable of inducing DNA mutations and genomic instability (Sharifi-Rad et al., 2020).

Despite the well-documented health benefits of matcha, its consumption often involves the addition of milk to enhance palatability and texture. This practice has raised concerns regarding the potential impact of milk on the bioavailability and anti-oxidant capacity of matcha's bioactive compounds. Previous research has indicated that milk proteins, particularly caseins, can form complexes with tea catechins, potentially reducing their bioavailability and antioxidant efficacy (Chen et al., 2024). This interaction presents a critical research gap: while matcha is celebrated for its health-promoting properties due to its high antioxidant content, the impact of milk addition on these benefits remains inadequately explored.

The primary objective of this study is to elucidate the effects of various types of milk on the antioxidant activity of matcha. By examining how different types of milk impact matcha's antioxidant capacity, total phenolic content, flavonoid content, and various antioxidant assays, we aim to provide comprehensive insights into optimizing the health benefits associated with matcha consumption. This research is of particular significance as it has the potential to inform evidence-based dietary recommendations for maximizing the health-promoting properties of this increasingly popular beverage.

Our study hypothesizes that the addition of milk will significantly attenuate the antioxidant capacity of matcha, with the magnitude of reduction correlating with the protein concentration of different milk types. We posit that cow's milk, due to its higher casein content (Lamothe et al., 2014), will exhibit the most pronounced effect on reducing matcha's antioxidant activity, followed by soy milk, while plant-based alternatives such as almond and oat milk may demonstrate less interference.

To prove our hypothesis, we will employ a multifaceted approach incorporating various spectrophotometric assays to assess antioxidant capacity, including the Folin-Ciocalteu method for total phenolic content (Kupina et al., 2019), the aluminum chloride colorimetric assay for flavonoid content (Shraim et al., 2021), DPPH and hydrogen peroxide assays for radical scavenging activity (B. Al-Ghafari et al., 2017), and assessments of metal chelating activity and ferric reducing power (Doghaither et al., 2017). These methodologies will provide a comprehensive evaluation of the antioxidant profile of matcha and how it is modulated by different milk types.

The findings of this research have the potential to significantly impact both consumer behavior and public health recommendations. By elucidating the optimal methods for consuming matcha to maximize its health benefits, this study may contribute to more in-formed dietary choices and potentially enhance the preventive and therapeutic applications of matcha in the con-text of oxidative stress-related disorders.

Materials and Methods

Materials

Matcha green tea powder (ceremony matcha) was purchased from (Merlin Bird, Gungan, China) Cow milk (Almarai company, Riyadh, Saudi Arabia) and different plant-based including coconut milk and oat milk (Alpro company,

Belgium), oat milk (Saudia company, Riyadh, Saudi Arabia) and almond milk (Australia's own, Newcastle). All animal and plant-based milk were purchased from local supermarket at Jeddah, Saudi Arabia. The nutritional information per 120 mL of milk is provided in tab. 1.

Table 1. Nutritional composition of different types of milk per 120 mL of milk.

Type of milk	Protein	Fat	Carbohydrate
Low fat Cow milk	4.2 g	1.2 g	6 g
Coconut milk	0.12 g	2.98 g	0.84 g
Almond milk	0.96 g	1.08 g	3.31 g
Oat milk	0.96 g	3.6 g	8.04 g
Saudia-soy milk	3.6 g	2.16 g	3.6 g

Milk-tea model preparation

The types of milk and their proportions were selected based on responses from a questionnaire administered to 300 participants, reflecting common consumption patterns. Matcha tea samples were prepared according to the preferences reported by the respondents. Each sample was prepared by adding 2 g of matcha powder to 40 mL of hot water (80°C) and whisking for two minutes. For milk added samples, 120 mL of each milk type was added, then the volume has been completed to 250 mL. For all analysis, 2 mL aliquot of the solution was diluted by adding 8 mL of distilled water. This step is important to ensure all spectrophotometric absorbance measurements fall within the calibration curve range.

Determination of total phenolic contents

Total phenolic content was determined using Folin-Ciocalteu method (Kupina et al., 2019; Lamuela-Raventós, 2018). Briefly, 1 mL of sample was mixed with 100 µL of Folin-Ciocalteu reagent and 1580 µL of distilled water. After five minutes of incubation at room temperature, 300 µL of 5% (w/v) sodium carbonate solution was added. The mixture was then incubated at 40°C for 30 minutes in a water bath. Absorbance was measured at 765 nm using a spectrophotometer (UV-1900 Shimadzu), with a blank sample as reference. Calibration curve was prepared from serial dilution (50 µg/mL, 100 µg/mL, 200 µg/mL, and 500 µg/mL) of gallic acid (Sigma-Aldrich St. Louis, MO) dissolved in methanol (liquid chromatography grade, ≥ 99.8%, Merck).

Determination of total flavonoid contents

The aluminum chloride colorimetric method was used to determine the total flavonoid content (Shraim et al., 2021). Sample (500 µL) was mixed with 3 mL of deionized water and 150 µL of 5% sodium nitrite solution (Fluka Chemika). The mixture was then incubated at room temperature for five minutes. Then, 300 µL of 10% aluminum chloride solution (Sigma-Aldrich St. Louis, MO) was added, followed by the addition of 0.5 mL of 1 M sodium hydroxide (VWR Chemicals). The absorbance was measured at 510 nm using a spectrophotometer (UV-1900 Shimadzu). To quantify the results, a standard curve was prepared using a series of β-Catechin (Sigma-Aldrich St. Louis, MO) solutions ranging in concentration from 0 to 500 ppm dissolved in methanol (Sigma-Aldrich St. Louis, MO) (Matić et al., 2017).

Determination of radical scavenging activity

The radical scavenging activity of matcha samples was assessed using two assays: Hydrogen Peroxide (H_2O_2) scavenging assay and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay.

For the H2O2 scavenging assay, the method described by Ruch et al (Ruch et al., 1989). was used. About 1 mL aliquot of the sample was mixed with 2 mL of 30% H_2O_2 (fisher chemical, Fair Lawn). The mixture was incubated at room temperature for 10 minutes, then absorbance was measured at 230 nm using a UV-Vis spectrophotometer (UV-1900 Shimadzu). The percentage of radical scavenging was calculated using the following equation:

$$\text{H2O2 Scavenging activity (\%)} = \frac{\text{A(Blank)} - \text{A(Sample)}}{\text{A(Blank)}} \times 100$$

The DPPH radical scavenging activity was determined using Brand-William's method (Brand-Williams et al., 1995). DPPH (Sigma-Aldrich, Poole, UK) solution (0.1 mM) was prepared by dissolving it in ethanol. Samples (1 mL) was mixed with 3 mL of DPPH (0.1 mM). The reaction was then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. The percentage of radical scavenging was calculated using the following equation:

$$\text{DPPH Scavenging activity (\%)} = \frac{\text{A(Blank)} - \text{A(Sample)}}{\text{A(Blank)}} \times 100$$

Determination of metal chelating activity

The metal chelating activity of the samples was determined using the ferrozine method (Gulcin & Alwasel, 2022) Sample (1 ml) was mixed with 100 µL of freshly prepared FeCl2 (2 mm) (Sigma-Aldrich, Poole, UK) and 3 ml of distilled water and incubated at room temperature for 30 seconds. Then, 200 µL of freshly prepared (5 mm) ferrozine (Sigma-Aldrich, Poole, UK) was added and the mixture was incubated at room temperature for 10 minutes. The absorbance was measured spectrophotometrically (UV-1900 Shimadzu) at 562 nm. The percentage of ferrous ion chelating activity was calculated using the equation:

$$\text{Chelating Activity (\%)} = \frac{\text{A(Blank)} - \text{A(Sample)}}{\text{A(Blank)}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay

Ferric Reducing Antioxidant Power (FRAP) was performed according to Berker et al., 2010 method (Zhong & Shahidi, 2015). Sample (1 mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) (Honeywell, Germany) and 1.25 mL of 1% potassium ferricyanide (Sigma-Aldrich, Poole, UK). The reaction was incubated at 50°C for 30 minutes. After incubation, 1.25 mL of 10% trichloroacetic acid (Sigma-Aldrich, Poole, UK) was added to stop the reaction. The mixture was centrifuged at 6000 rpm for 10 minutes. The supernatant (1 mL) was mixed with an equal volume of distilled water and 1 mL of 1% ferric chloride (Sigma-Aldrich, Poole, UK). The absorbance was measured immediately at 700 nm using a UV-1900 Shimadzu spectrophotometer. The reducing power activity was calculated using the equation:

$$\text{Reducing Power Activity (\%)} = \frac{\text{A(Blank)} - \text{A(Sample)}}{\text{A(Blank)}} \times 100$$

Statistical analysis

GraphPad Prism software was used to analyze the results. Three independent experiments were performed for each assay and three technical replicates. Statistical differences between different groups were assessed using a one-way ANOVA test with Bonferroni's test correction. The data were expressed as mean ± SD and results were considered statistically significant when $P<0.0001$.

Results

In this study, a questionnaire was administered to 300 participants to gather insights about consumption patterns. The results revealed that 70.7% (n=213) of respondents reported regular consumption of matcha and the main reasons for consuming matcha were its taste and health benefits. Of the matcha consumers, 213 individuals indicated using additives to enhance flavor, with 145 individuals incorporated milk to their preparations. About 80.3% of participants preferred adding 120 mL of various milk types to their matcha, including cow's milk, almond milk, coconut milk, oat milk, and soy milk. Based on these findings, this study aimed to investigate the effects of these commonly used milk types and their typical concentrations on the antioxidant activities of matcha green tea.

Effect of different types of milk on the total phenolic content and total flavonoids of matcha tea

The impact of various types of milk including cow milk and different types of plant-based milk on the Total Phenolic Content (TPC) of matcha tea was quantified using the Folin-Ciocalteu assay. There was an overall significant reduction in the TPC of matcha tea when milk was added, regardless of the type of milk (Fig. 1a). Specifically, the addition of cow milk resulted in 56 % reduction in TPC, while plant-based milks decreased TPC by 26% to 35%. Among the plant-based options, coconut milk caused the least reduction compared to soy milk.

In addition to phenolic content, the Total Flavonoids Content (TFC) of matcha samples was also evaluated using the aluminum chloride colorimetric assay. Similar to the TPC, the TFC of matcha tea was significantly reduced across different types of milk including cow's milk, almond milk, soy milk, coconut milk, and oat milk (Fig. 1b). However, cow's milk led to a more pronounced reduction of TFC decreasing it by 82%. In contrast, plant-based milks only caused a decrease in TFC by 29% to 57%. Among the plant milks, coconut milk caused the least reduction while soymilk has the highest reduction in TFC. We noticed here that flavonoids are the most phenolic class that is affected by cow milk and to less extent by almond milk, soy milk and oat milk.

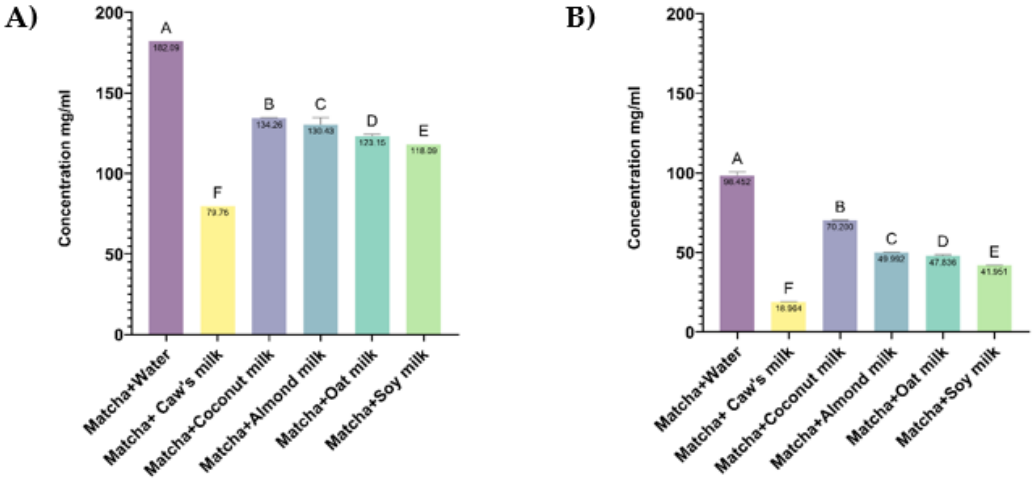

Figure 1. Total phenolic and flavonoids content in matcha mixed with different milk. A) Total phenolic content of matcha alone and matcha combined with cow milk, coconut milk almond milk, oat milk and soy milk. B) Total flavonoids content of matcha alone and matcha combined with animal and plant-based milk. Data are presented as mean of three biological replicates and three technical replicates ± SD. Different letters indicate statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA followed by post-hoc analysis.

Effect of different types of milk on antioxidant activity of matcha

The radical and H_2O_2 scavenging activity of matcha samples, both with and without various types of milk, was evaluated using DPPH and H_2O_2 assays (Fig. 2a, 2b). Adding cow milk or soy milk reduced the scavenging activity of matcha by half. However, almond milk and oat milk has less effect. Interestingly, adding coconut milk to matcha teat did not alter the scavenging activity of matcha (Fig. 2a).

The antioxidant activity of matcha with and without milk against H_2O_2 specifically was determined. Data showed that adding any type of milk to matcha significantly reduced its scavenging ability toward H_2O_2. Matcha tea mixed with cow's milk exhibited more than a twofold decrease in H_2O_2 antioxidant activity. In contrast, the addition of coconut milk had the least impact on the H_2O_2 antioxidant capacity of matcha (Fig. 2b).

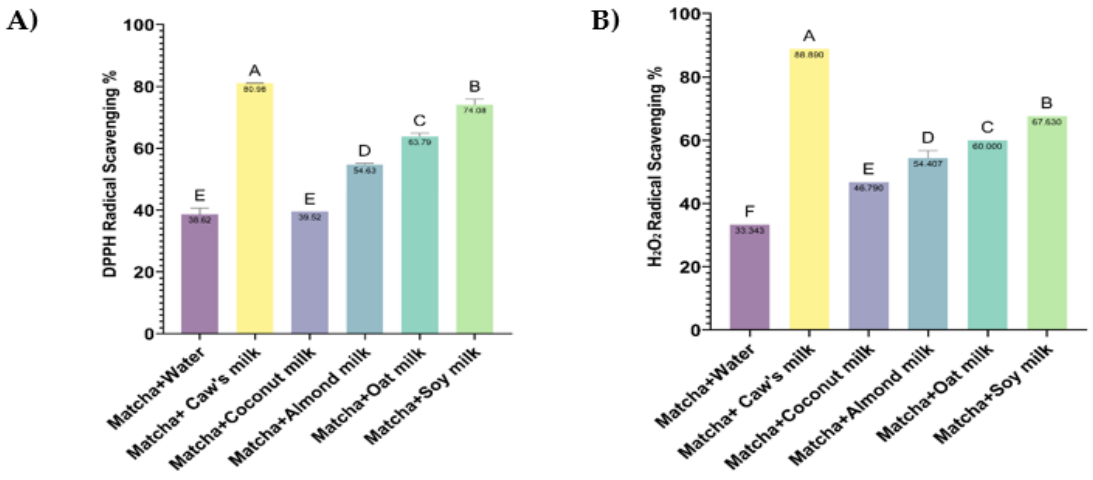

Figure 2. Antioxidant scavenging activity of matcha tea with and without milk. A) Antioxidant scavenging measured as DPPH radical scavenging activity of matcha alone and matcha combined with different milk types. B) Antioxidant scavenging measured H_2O_2 radical scavenging activity of matcha alone and matcha combined with different milk types. Data are presented as mean of three biological replicates and three technical replicates ± SD. Different letters indicate statistically significant differences between groups ($p<0.05$) as determined by one-way ANOVA followed by post-hoc analysis.

Effect of different types of milk on metal chelating activity on matcha

Reactive metal species is one of the radicals that may catalyze oxidation reaction. Therefore, effective sequestering of this radical species is an important characteristic of antioxidants. To assess if adding different types of milk affects metal ion binding ability of matcha tea, a metal chelating test was performed. Data showed that incorporation of milk, irrespective of type of milk, led to a significant reduction in the metal-chelating activity of matcha (Fig. 3a). The highest reduction is caused by cow's milk followed by oat and soy milk. while coconut milk and almond milk results in less reduction in metal chelating activity of matcha. These findings suggest that the interaction between milk constituents and matcha components alters the matcha's ability to chelate metal ions, potentially impacting its antioxidant efficacy.

Effect of different types of milk on Ferric reducing antioxidant power of matcha

The overall reducing power of an antioxidant could be assessed by quantifying its capacity to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This test provides insight into the electron-donating ability of the antioxidants reflecting their potential to counteract damaging oxidative reactions. Here, to evaluate the effects of different types of milk on the electron donating property of matcha tea, FRAP test was performed. The results indicated that cow's milk significantly decreased the reducing power of matcha tea by sixfold, while coconut milk reduced it by twofold (Fig. 3b). Other plant-based milks resulted in a maximum reduction of fourfold.

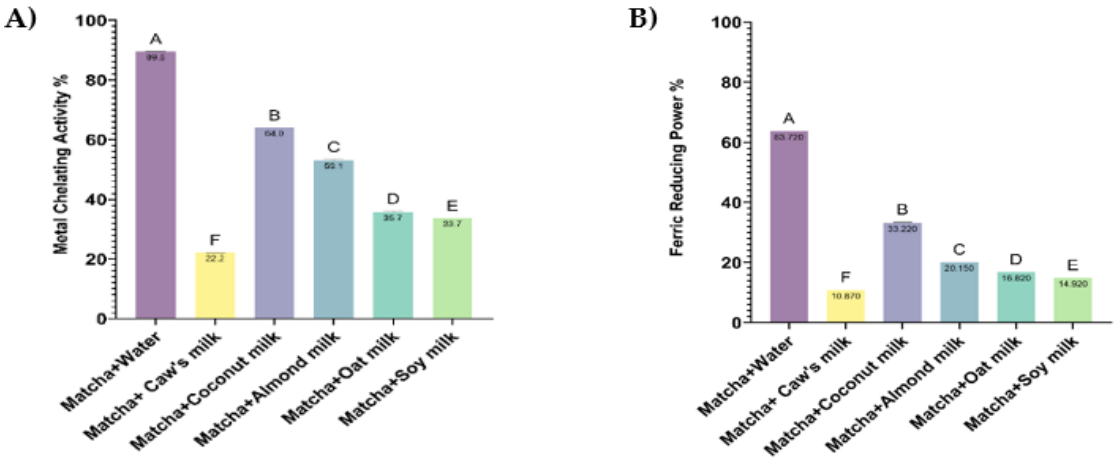

Figure 3. Radical chelating activity of matcha tea with and without milk. A) Metal ion chelating activity of matcha alone and matcha combined with different milk types (cow's milk, almond milk, soy milk, oat milk, and coconut milk). B) FRAP activity of matcha alone and matcha combined with different milk types (cow's milk, almond milk, soy milk, oat milk, and coconut milk). Data are presented as mean of three biological replicates and three technical replicates ± SD. Different letters indicate statistically significant differences between groups ($p < 0.05$) as determined by one-way ANOVA followed by post-hoc analysis.

Discussion

Matcha, a finely ground powder derived from specially grown and processed green tea leaves, has gained immense popularity worldwide due to its unique flavor and numerous potential health benefits. Rich in antioxidants, particularly catechins, matcha is celebrated for enhancing metabolism, improving mental clarity, and providing a rich source of vitamins and minerals. However, the health benefits of matcha can be influenced by various factors, including the addition of other substances. Numerous studies have indicated that incorporating different additives into tea can alter its bioactive compounds, potentially affecting its antioxidant properties and overall biological activity. One common addition is milk, which raises questions about how this combination influences matcha's nutritional profile.

The present study investigated the impact of various milk types on the antioxidant properties of matcha green tea, focusing on Total Phenolic Content (TPC), Total Flavonoid Content (TFC), radical scavenging activity, metal chelating activity, and reducing power. Our findings reveal significant alterations in matcha's antioxidant profile when combined with different milk sources, with implications for its potential health benefits. The observed decrease in TPC and TFC upon milk addition aligns with previous studies on tea-milk interactions. A study reported that milk proteins could bind to polyphenols, potentially reducing their bioavailability and antioxidant capacity (Sharma et al., 2008). Our results extend these findings to matcha, demonstrating that this effect is consistent across various milk types, with cow's milk showing the most pronounced impact. The reduction in antioxi-dant compounds can be attributed to the formation of protein-polyphenol complexes, primarily through hydrophobic interactions and hydrogen bonding (Lorenz et al., 2007).

The significant decrease in DPPH radical scavenging activity and H_2O_2 scavenging activity with milk addition corroborates the findings of a study investigated the impact of skimmed milk addition on the antioxidant capacity of white and black tea, as measured by the DPPH method. The research revealed that incorporating skimmed milk into tea infusions resulted in a significant reduction of antioxidant activity. Specifically, the antioxidant capacity of white tea decreased by 84.39%, while black tea experienced a 73.54% reduction when mixed with skimmed milk, the authors proposed that this diminished antioxidant activity is attributed to the interaction between milk proteins, particularly casein, and tea polyphenols (Simanjuntak & Nurhayati, 2017). They suggest that casein binds to tea polyphenols, effectively masking their active groups and consequently reducing their free radical scavenging ability. This study highlights the potential impact of milk addition on the health benefits associated with tea consumption and underscores the complex interactions between dietary components that can affect their bioactive properties. The observed variations among different milk types suggest that protein composition and fat content play crucial roles in modulating these interactions. This is consistent with studies on other polyphenol-rich beverages like coffee, where milk addition has been shown to affect antioxidant capacity (Ryan & Petit, 2010). Our results on metal chelating activity and reducing power further support the notion that milk addition significantly alters matcha's antioxidant properties. The reduction in metal

chelating ability can be attributed to the formation of complexes between milk proteins, particularly casein, and tea polyphenols (Hasni et al., 2011).

These findings have important implications for consumers and health professionals. While matcha is celebrated for its high antioxidant content and potential health benefits (Truong & Jeong, 2021). the addition of milk may significantly alter its antioxidant properties. This suggests that individuals seeking to maximize the health benefits of matcha consumption might consider consuming it without milk or with milk alternatives that have less impact on its antioxidant activity. However, it is crucial to interpret these results with caution. In vitro studies may not always directly translate to *in* vivo effects, as the bioavailability and metabolism of tea polyphenols in the human body are complex processes influenced by various factors, including individual differences in gut microbiota and genetic polymorphisms (Manach et al., 2004). Furthermore, the nutritional value of milk itself should be considered when assessing the overall health impact of matcha-milk combinations.

Future research directions should focus on *in vivo* studies to better understand the bioavailability and potential health impacts of these interactions in the human body. Additionally, investigations into alternative preparation techniques or milk substitutes that minimally impact phenolic and flavonoid content could provide valuable information for consumers and the food industry. Exploring the effects of different milk-to-matcha ratios and preparation methods on antioxidant activity could also yield insights into optimizing matcha consumption for health benefits.

Conclusions

This study comprehensively analyzed the effects of various milk types on matcha green tea's antioxidant properties. The findings reveal that milk addition, regardless of source, significantly reduces matcha's antioxidant profile, including total phenolic content, total flavonoid content, DPPH radical scavenging activity, metal chelating ability, and ferric-reducing antioxidant power. Cow's milk showed the most pronounced effect, while plant-based alternatives had varying impacts. The observed decreases in antioxidant activity are likely due to the formation of protein-polyphenol complexes between milk proteins and matcha's catechins. While these in vitro results demonstrate reduced antioxidant activity, they should be interpreted cautiously due to the complex in vivo digestive processes and individual variations in gut microbiota. The study highlights the importance of considering preparation methods and milk selection when consuming matcha for health benefits and calls for future in vivo research to better understand the physiological relevance of these interactions.

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