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Antioxidant Activities of Selected Milk Teas and Fruit Teas Using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

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ABSTRACT

Milk teas and fruit teas are popular as a refreshment, but their percent antioxidant properties need to be determined. In this study, the antioxidant activities of selected commercialized milk teas (Matcha, Okinawa, and Winter Melon) and fruit teas (Green Apple, Kiwi, and Lychee) were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in terms of % scavenging activity. In that assay, the plates with milk teas and fruit teas were incubated in the dark and covered with aluminum foil for 30 minutes at room temperature before being measured for absorbance at 570 nm with a microplate reader. They were kept out of the light until they were evaluated for analysis. Within 30 minutes of incubation, the discoloration from purple to yellow was observed. Three (3) trials with three (3) replicates were carried out with negative control (water with sugar and creamer for milk tea and water with sugar for fruit tea) and positive control (Vitamin C). Using one-way ANOVA, the Matcha (mean = 62.9, sd = 2.6) Okinawa (mean = 46.4, sd = 5.9), and Winter Melon (mean = 61.6, sd = 2.8) milkteas have anti-oxidant activities when compared to the negative control (mean = 0.0, sd = 0.0) ($p < .05$) Both Matcha and Winter Melon have the best antioxidant activities, but not comparable to the positive control, Vitamin C (mean = 98.7; sd = 0.62) ($p < .05$). While the Green apple fruit tea (mean = 95.5; SD = 2.4) Kiwi fruit tea (mean = 97.3, SD = 1.5), and Lychee fruit tea (mean = 94.4; sd = 1.3) ($p > .05$) have comparable antioxidant activities when compared to the negative control (mean sd = 0.0). Kiwi fruit tea has the best antioxidant activity and comparable to Vitamin C (mean = 98.7; sd = 0.62). This study scientifically established that both Matcha and Winter Melon commercial milk teas have antioxidant activities of 61.6 to 62.9%. While the commercial Kiwi, Green apple, and Lychee fruit tea ranged from 94.4 to 97.3%. These antioxidant activities can be useful in preventing diseases brought by free radicals, which can be verified by conducting more studies.

INTRODUCTION

The scientific community is very interested in antioxidants and their effects in a variety of sectors, including food engineering, medicine, and pharmacy (Munteanu & Apetrei, 2021). According to Xiao *et al.* (2020), reactive oxygen species (ROS) have the potential to harm biological macromolecules, resulting in oxidative stress-related diseases, lipid, protein, and DNA damage, as well as cell aging. An imbalance between the production and neutralization of oxidants leads to oxidative stress. Oxidative stress causes a variety of illnesses and disorders, including cancer, chronic renal disease, neurological diseases, cardiovascular diseases (CVDs), and chronic obstructive lung disease (Sharma *et al.*, 2022). Oxidative stress could be avoided using antioxidants.

The antioxidant properties of several types of tea have been recognized. As a beverage, milk teas grew in popularity (Ong *et al.*, 2021). By experimenting with different creamers, flavors, and sweeteners, it opened the door for tea lovers to enter the industry (Bastasa *et al.*, 2022). On the other hand, fruit teas gained popularity as cool alternatives. A range of fruits, herbal infusions, and occasionally even boba pearls for texture are frequently included. These beverages transcended tradition and became a phenomenon in society and culture. Millennials and Generation Z Filipinos become much more interested in milk tea (National Nutrition Council, 2020).

Matcha tea, one of the milk teas, was examined for its nutritional makeup and demonstrated antioxidant qualities (Koláčková *et al.*, 2019). High concentrations of catechins, including EGCG, which have anti-inflammatory and antioxidant properties, were found in matcha. There are numerous possible health advantages of matcha green tea. Compared to other green tea kinds, it had higher quantities of phenolic acids, quercetin, rutin, theanine, and chlorophyll (Kochman *et al.*, 2020). Additionally, general studies on matcha tea indicated that it had positive effects on anti-tumorigenesis, cardio-metabolic health, and cognitive function (Sokary *et al.*, 2023).

On the other hand, the primary constituents of Okinawa milk tea are often tapioca pearls, black tea, milk, and kokuto, a unique Okinawan brown sugar (Saiful, 2022). In the Zaiyar *et al.* study. Using the DPPH assay, black tea showed high antioxidant contents in 2020. Using DPPH, the ABTS test (2,2'-azino-bis 3-ethylbenzothiazoline-6-

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sulfonic), and FRAP (ferric reducing antioxidant power), Stobiecka *et al.* (2022) found that cow milk, the most widely used type of milk worldwide, had the lowest antioxidant potential when compared to other animal milk. In the Kinjo *et al.* (2019) study, kokuto, a non-centrifuged cane sugar, showed antioxidant and anti-stress properties.

Tapioca pearls are made from cassava (Manihot esculenta). Zekarias *et al.* (2019) stated that it was thought to be a treatment for a number of ailments, such as diabetes, celiac disease, bone and neurological health, cardiovascular disease, allergens and prostate issues, GIT issues, and blood pressure issues.

Winter melon (Benincasa hispida), was evaluated as a nutrient-dense vegetable in Alsaadi and Abass's (2020) study because it was a good source of natural sugars, amino acids, organic acids, minerals, and vitamins. According to the pharmacological investigations, the plant had a wide range of pharmacological properties, such as analgesic, antioxidant, anti-inflammatory, and central nervous system effects. antibacterial, antidiabetic, nephroprotective, diuretic, and hypolipidemic properties. Triterpenes, phenolics, sterols, glycosides, and other functionally significant bioactives and medicines are abundant in it. Using water-immersing restraint stress (WRS) and Indometacin [INDO]-induced gastric mucosal damage in rats, as well as DPPH to investigate the antioxidation effect and free radical scavenging of watermelon seed methanol extract, winter melon seed extract demonstrated an improved correlation between dependent DPPH concentration and pyloric association action (Purohit *et al.*, 2019).

Fruit tea is another type of tea offered in milk tea establishments. Dried fruit, flowers, or leaves are combined to make fruit teas. Antioxidant qualities are also present in the fruits used to make these teas. Green apples (Malus domestica) have been shown in laboratory experiments to have a potent antioxidant impact that lowers cholesterol, lipid oxidation, and the growth of cancer cells. Quercetin, catechin, fluorine, and chlorogenic acid are among the many phytochemicals found in apples that have strong antioxidant properties (Aksoy & Ötleş, 2022).

Conversely, kiwi (Actinidia deliciosa) fruits are incredibly rich in vitamin C and also contain a variety of other nutrients, such as nutritionally significant amounts of dietary fiber, potassium, vitamin E, and folate, as well as several bioactive substances, such as a variety of antioxidants, phytonutrients, and enzymes, that contribute to their metabolic and functional advantages. A rising body of evidence from human intervention research was drawing special attention to the role that kiwi fruit plays in digestive health (Richardson *et al.*, 2018).

Additionally, phytochemical components with anticancer, antibacterial, antioxidative, and antiglycating effects have been discovered in lychee (Litchi chinensis Sonn.). In addition to improving insulin resistance, lychee seed, lychee seed extracts, and related compounds have been shown to have promising antihyperglycemic properties, including lipid regulation, neuroprotection, anti-inflammatory, anti-neurotoxic, and renoprotective effects (Zhang *et al.*, 2021). To completely grasp the potential of these many teas and tastes, more research is needed, as the total health impact depends on individual circumstances and consumption habits.

The antioxidant qualities of several plant extracts have been the subject of numerous investigations. Nevertheless, no research had been done on the antioxidant content of commercial milk teas or fruit teas, which were becoming more and more popular. Thus, this investigation of the antioxidants found in particular fruit and milk teas has been carried out.

This study aimed to determine the antioxidant activities of the selected milk teas (matcha, okinawa, and winter melon) and fruit teas (green apple, kiwi, and lychee) using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay in terms of % scavenging activity. The study also determined the best milk tea and fruit tea that exhibited the highest antioxidant, and they were compared to the positive control used (Ascorbic Acid).

This study established the antioxidant activities of one commercial brand of milk teas (matcha, okinawa, and winter melon) and fruit teas (green apple, kiwi, and lychee). This may benefit drinkers of these milk teas and fruit tea

MATERIALS AND METHODS

Only a post-test experimental design was used in this study. Using the DPPH assay, various milk and fruit teas were screened for antioxidants. The Ethics Committee of a private university in Iloilo City reviewed this study, which was carried out there. This research was verified by a licensed chemist. In order to ensure dependability, this study used three replicates with three trials. Every instrument had the proper calibration.

Acquisition of Milk Tea and Fruit Tea

The selected milk and fruit teas were acquired from a local seller in Iloilo City

Dpph Assay

Three distinct flavored milk teas, three distinct flavored fruit teas, as well as positive and negative controls, were evaluated for antioxidant activity in this study. 180 µL of DPPH solution and 20 µL of the commercially available fruit teas (green apple, kiwi, and lychee) and milk teas (matcha, okinawa, and winter melon) were added to each well of a 96-well plate. The stock solution was created by dissolving 0.00591 g of DPPH (0.3 mM) in 50 mL of methanol. Another stock solution was made with 50 mL of methanol and 0.5 g of ascorbic acid (10 mg/mL).

In the wells of the 96-well plate, 180 µL of DPPH solution was mixed with 20 µL of distilled water with creamer and sugar (a negative control for milk teas). To prevent false positive results in the antioxidant activity of the samples, a non-dairy creamer was employed in the production of negative control for milk teas from the local vendor. Additionally, 180 µL of DPPH solution and

20 µL of distilled water with sugar (the negative control for fruit teas) were poured into the 96-well plate's wells. The positive controls were 180 µL of DPPH solution and 20 µL of ascorbic acid (10 mg/mL) that were dispersed in the 96-well plate's wells.

A microplate reader was used to measure the absorbance at 570 nm after the plate had been incubated for 30 minutes at room temperature in the dark while covered with aluminum foil (Suarez *et al.*, 2021). The materials are kept out of the light until they are assessed for analysis since DPPH is photosensitive (Lim *et al.*, 2023). The purple-to-yellow staining was noticed within 30 minutes of incubation. Three replicates and three trials were used to conduct the test. The following formula was used to calculate the chosen milk teas' and fruit teas' DPPH radical scavenging activity.

The plate was incubated in the dark, covered with aluminum foil, for 30 minutes at room temperature before being measured for absorbance at 570 nm with a microplate reader (Suarez *et al.*, 2021). DPPH is photosensitive; the materials are kept out of light until they are evaluated for analysis (Lim *et al.*, 2023). Within 30 minutes of incubation, the discoloration of the purple-to-yellow color was observed. The test was carried out in three replicates with three trials. The DPPH radical scavenging activity of the selected milk teas and fruit teas was determined using the formula:

% DPPH Radical Scavenging Activity = {(absorbance of negative control - absorbance of sample)/ (absorbance of negative control)}X100

Waste Disposal

Segregation and proper garbage disposal procedures were adhered to. For local rubbish collection, general waste was disposed away in the appropriate bins. Chemical wastes were divided into different containers and categorized based on their content. Ascorbic acid and DPPH were categorized as organic halogenated trash and were electronically and physically recorded with the appropriate waste codes before being submitted to the Pollution Control Officer.

Statistical Analysis

In this study, the mean and standard deviation were computed, and comparisons were performed using One-Way Analysis of Variance (ANOVA) followed by Tukey's Honest Significant ($p \leq 0.05$).

RESULTS AND DISCUSSION

In contrast to the negative control (mean sd = 0.0), Table 1 demonstrates that the milk teas made from matcha (mean = 62.9, sd = 2.6), okinawa (mean = 46.4, sd = 5.9), and winter melon (mean = 61.6, sd = 2.8) exhibit antioxidant properties. = 0. Winter melon and matcha both exhibit the highest levels of antioxidant activity, however they are not on par with ascorbic acid, the positive control.

In contrast to the negative control (mean sd = 0.0), the antioxidant activities of the green apple fruit tea (mean = 95.5; SD = 2.4), kiwi fruit tea (mean = 97.3, SD = 1.5), and lychee fruit tea (mean = 94.4; sd = 1.3) are comparable. The greatest antioxidant activity, on par with vitamin C, is seen in kiwi fruit tea (mean = 98.7; sd = 0.62).

Table 1: Antioxidants of selected milk teas and fruit teas using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay through % scavenging activity

Milk Teas and Fruit Teas	**% Scavenging Activity**	
	Mean	**SD**
Matcha with tea	62.9^{c}	2.6
Okinawa with tea	46.4^{b}	5.9
Winter melon with tea	61.6^{c}	2.8
Negative Control for Milk Teas (Distilled water with creamer and sugar)	0.0^{a}	0.0
Green apple with tea	95.5^{d}	2.4
Kiwi fruit with tea	97.3d,^{e}	1.5
Lychee with tea	94.4^{c}	1.3
Pure Tea	91.5^{b}	0.5
Negative control for Fruit Teas (water with sugar)	0.0^{a}	0.0
Positive Control (Ascorbic acid)	98.7^{e}	0.62

Note. Significant if p-value <0.05

Discussions

There was antioxidant activity in every flavor of milk tea, matcha, okinawa, winter melon, and fruit tea, as well as in green apple, kiwi, and lychee. This is because flavonoids, a subgroup of polyphenols, are present. A wide range of fruits and plant-based flavorings include them. Much research has focused on their ability to scavenge free radicals and reduce oxidative stress. Flavonoids have the ability to neutralize free radicals such as DPPH radicals and give hydrogen atoms or electrons (Kumar & Pandey, 2013).

Because fruits, even when powdered, are rich in flavonoids, fruit teas have higher antioxidant activity than milk teas. Our study's results demonstrate this, since

while both displayed the highest antioxidant activity within their respective groups, kiwi fruit with tea had a higher antioxidant activity than matcha with tea. The concentration of antioxidant chemicals during drying procedures may be the reason for the release of flavonoids from the cell's fruits (Nunes *et al*., 2016). Catechins are abundant in tea, which was used as one of the flavorings. Tea can also help reduce inflammation and cancer (Musial *et al*., 2020).The findings indicated that matcha tea had the highest percentage of scavenging activity among the milk teas. This is consistent with a comparable study that was previously carried out in which matcha tea's nutritional composition was examined and antioxidant qualities were found (Koláčková *et al*., 2019). High concentrations of catechins, including EGCG, are found in matcha (Kochman *et al*., 2020).

Out of all the fruit teas examined, kiwi fruit had the highest antioxidant activity. According to a related study about the fruit's potential medical benefits, the kiwi fruit itself has significant levels of phenolic acid, with protocatechuic acid having the highest concentration (Zhang *et al*., 2021). One kind of phytochemical that is frequently referred to as a secondary metabolite of plants is phenolic acid, a non-nutritive substance that may improve human cell function (Yoo *et al*., 2018). Numerous bioactivities, including anti-tumor, anti-inflammatory, antioxidant, hypoglycemic, and hypolipidemic effects, are also attributed to them. The total antioxidant qualities of the fruit tea are enhanced by the inclusion of tea, which contains phytochemical qualities of its own (Bag *et al*., 2022).

Ascorbic acid, which is the positive control of this study, appears in the body in the form of ascorbate, which is recognized for its powerful antioxidant and anti-inflammatory characteristics. It is clear that the radical anion monodehydroascorbate largely reacts with radicals, while ascorbic acid functions mostly as a donor of single hydrogen atoms (Njus *et al*., 2020). All of these characteristics work together to provide ascorbic acid with exceptional antioxidant benefits that are higher compared to milk teas and fruit teas, which makes it ideal to use as a positive control.

So far, this study is the first attempt to determine the antioxidant of the commercial of selected milk tea and fruit teas.

Thus, future researchers could try a different approach to address the limitations of our study, which includes selected samples of milk teas (matcha, okinawa, and winter melon) and fruit teas (green apple, kiwi, and lychee) along with pure tea. In addition to our analysis of the data, this study was limited to using two negative controls specific to milk teas and fruit teas. Distilled water with creamer and sugar was utilized as a negative control for milk teas. Distilled water and sugar were utilized as negative controls for fruit teas.

This adds to our growing research body, further enriching our understanding of antioxidant activities of commercial milk teas (matcha, okinawa, and winter melon) and fruit teas (green apple, kiwi, and lychee).

CONCLUSION

The study demonstrated the antioxidant activity of the selected milk teas (matcha, okinawa, and winter melon) and fruit teas (green apple, kiwi, and lychee) in terms of % scavenging activity. The best milk tea and fruit tea that exhibited antioxidant activity were matcha and winter melon and kiwi, for fruit tea, respectively. They can be useful in preventing diseases like cancer, inflammatory and cardiovascular disorders, and other conditions brought on by free radicals, subject to further studies. It is recommended to explore other health benefits of milk teas and fruit teas aside from their antioxidant properties, and may also opt to use vitamin E as a positive control.

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