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Effect of Adding Cow's Milk, Soy Milk, Rice Milk and Almond Milk on Antioxidant Capacity in English Breakfast Tea

Research Article

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Abstract

Tea is one of the popular beverages worldwide, with various health benefits,that have been studied. There is increasing popularity of drinking tea in Thailand and some people prefer drinking milk tea. However, many studies suggest that polyphenols in tea that contribute to the antioxidant capacity of tea may interact with milk protein and may alter the antioxidant capacity of tea. This study aims to evaluate the effect of adding the various types of milk including cow's milk, soy milk, rice milk, and almond milk on antioxidant capacity in English breakfast tea by comparing total polyphenol content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant capacities of 200 mL tea infusion with and without the addition of 50 mL of each milk. We hypothesize that adding milk will decrease the total polyphenol content and antioxidant capacity of English Breakfast tea. The result showsthat the total polyphenol content and DPPH antioxidant capacities of English breakfast tea infusion with the addition of each type of milk do not change significantly compared totea infusion without the addition of each milk. Soy milk, rice milk, and almond milk can be used as an alternative to cow's milk in adding to tea infusion without disturbing antioxidant capacity of tea.

Keywords: Antioxidant Capacity; Total Polyphenol Content; Black Tea; Milk; DPPH.

Abbreviations: ABTS, 2,2-azinobis-3-ethylbenzthiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; FRAP, ferric reducing antioxidant-power.

Introduction

Among many types of beverages around the world, tea is one of the most popular healthy beverages, following only water and also has various health benefits. Tea contains many polyphenols which act as antioxidants that may contribute to the reduction of many chronic diseases such as coronary heart disease, stroke, and cancers [1].

Polyphenols in tea contribute to the antioxidant properties of tea. Both green tea and black tea, which are two types of the most well-known tea, have a different composition of flavonols. In the process of making green tea, tea leaves are steamed immediately after harvesting to prevent oxidation of polyphenols, then dried. For black tea, tea leaves undergo enzymatic fermentation by disrupting cellular compartment and making oxidation process of phenolic compounds in tea leaves before drying. In this process, polyphenols in tea leaves which are mainly epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) are converted by polyphenols oxidase to new complex condensation products such as theaflavins and thearubigins [2].

Black tea composition can differ markedly due to preparation and fermentation processes. The approximate composition of black tea beverage measure in weight % of solid compositions are thearubigins (12-18%), phenolic acids and depsides (10-12%), amino acids (13-15%), methylxanthines (8-11%), carbohydrates (15%), flavonols (6-8%), catechins (3-10%), the aflavins (3-6%),protein (1%),mineral matter (10%), and volatiles (less than 0.1%) [3].

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Catechins and theaflavins in black tea possess antioxidant capacities. Catechins can inhibit free radical generation, act as electron donors. Moreover, catechins can also chelate metal ions and scavenge free radicals [4]. Like catechins, theaflavins can also inhibit reactive oxygen species generation by scavenging reactive oxygen species.

In Thailand, many people prefer drinking milk tea. However, adding milk to tea may affect the antioxidant capacity of tea as casein protein in cow's milk can form a complex with polyphenols which may affect antioxidant properties.

Experiment using fluorescence quenching found that casein protein in milk can interact with tannins in tea [5]. Adding alpha-casein to polyphenols can affect antioxidant properties of tea polyphenols. Previous studies show that adding milk protein which is casein to tea polyphenols leads to the reduction of antioxidant activity by 11-27% measured by ABTS (2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) free radical scavenging assay [6]. Other studies comparing whole milk, semi-skimmed milk, and skimmed milk found that adding skimmed milk to tea infusion decreasedthe total antioxidant capacity, measured by ferric reducing antioxidant power (FRAP) assay, significantly more than either whole milk or semi-skimmed milk [7].

Many studies suggested that adding cow's milk to tea infusion may decrease antioxidant capacity.There are still limitedpieces of evidence about the effect of adding plant-based milk to tea infusion. This study aimed to compare the effect of adding different types of milk (cow's milk, soy milk, rice milk, and almond milk) on the total polyphenol content and antioxidant capacity of black tea. We hypothesize that adding milk will decrease the total polyphenol contentand antioxidant capacity of English Breakfast tea infusion measured by colorimetric method using Folin–Ciocalteu reagent and 1,1-diphenyl-2-picrilhydrazyl (DPPH) radical scavenging assay, respectively. The total polyphenol content and antioxidant capacity of tea infusion and milk tea mixtures were examined.

Methods and Materials

Tea and four types of milk

Commercially available English breakfast tea, cow's milk,soy milk, rice milk, and almond milk were purchased from a local supermarket. The experiment in this study was performed at the Tea laboratory at Tea and Coffee Institute of Mae Fah Luang University. The laboratory is well equipped with the equipment instrument, materials, and chemical meet with ISO/IEC 17025.

Tea Preparation

Tea infusion was prepared according to the Standard Operating Protocol of Tea Laboratory, Tea Institute, Mae Fah Luang University. Two grams of dry English breakfast tea was infused with 200 mL of distilled water and heated at 100 degrees Celsius for 5 minutesand then filteredthrough filter paper. Control samples were added with distilled water 50mL.Milk tea, soy milk tea, rice milk tea, and almond milk tea samples were prepared by adding 50 mL of cow's milk, soy milk, rice milk, and almond milk, respectively, to the tea infusion.Four samples in each type of tea were performed in four independent experiments which carried out exactly in the same way.

Total Polyphenol Content Measurement

Total polyphenol was measured by colorimetric method using Folin–Ciocalteu reagent according to Molyneux [8] and ISO 14502-1:2005 [9]. Folin–Ciocalteu reagent composed of phosphomolybdic-phosphotungstic acid reagents which will reduce phenolic hydroxyl groups of total polyphenols and turn to a blue complex. This blue complex can be measured by spectrophotometry at 765 nm [10].

Briefly, gallic acid standard solution 1000 µg/mL was prepared by mixing 0.1 g of gallic acid with distilled water and adjusted volume to 100 mL and then diluted gallic acid standard solution to 10, 20, 40, 60, 80, and 100 µg/mL.The diluted gallic acid standard solutions in each concentration was mixed with 5 mL of Folin–Ciocalteu phenol reagent (10% v/v) and 4 mL of Na_2CO_3 7.5%(w/v). Tea samples of five types, four samples for each type, were diluted to 1:100. The diluted tea samples were also mixed with Folin–Ciocalteu phenol reagent and Na_2CO_3 in the same manner. All final solutionswere incubated at room temperature for 1 hour then measured photoabsorption by spectrophotometry at 765 nm. The standard graph was made using the absorbance measuredvsthe six concentrations ofgallic acid standard solution. Total polyphenol contents of all tea sampleswerethen calculated using the absorbance of the samples and the parameters retrieved from the standard graph.

Antioxidant Capacity by DPPH Radical Scavenging Activity Measurement

Antioxidant is measured by DPPH radical scavenging assay. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with one delocalization of the spare electron around the molecule. This delocalization gives a deep violet color with the absorption of light in ethanol solution at around 517 nm. After DPPH reacts with antioxidants, the deep violet color will decrease. [8-10]

Briefly, DPPH solution 60 µmol was prepared by mixing DPPH 0.00236 g with methanol and adjusted volume to 100 mL. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) standard solutions at concentration of 0, 200, 400, 600, 800, and 1000 µmol were prepared. The Trolox standard solutionin each concentration was mixed with 1.95 mL of DPPH solution. Tea samples of five types, four samples for each type, were diluted to 1:50. The diluted tea samples were mixed with DPPH solution in the same manner. All final solutions were incubated in a dark room for 30 minutes then measured absorbance with a spectrophotometer at 517 nm. The standard graphwas made using the photoabsorption values vs the six concentrations of Trolox. Antioxidant capacities of all tea samples were then calculated and reported in µmol Trolox/1 g dry basis.

Statistical Analysis

The experiment was performed in four independent samples in each group. The results were calculated and reportedas a group. Data were analyzed using analysis of variance to compare the differences between the mean value of each group. The statistically significant level was set at p-value < 0.05. The test was assumed that antioxidant capacity values in each group sample were distrib-

uted in a normal distribution manner and variation values in each group were not significantly different.

After the normality test for normal distribution of values and homogeneity of variance test for variances were performed, the one-way ANOVA test was done. If the ANOVA result showed that there was at least one pair of between-group differences, the post hoc analysis was performed using Fisher's LSD test to evaluate the differences between antioxidant capacity values in each group sample.

Results

Total Polyphenol Content

The total polyphenol contents of tea samples are shown in Table 1 and Figure 1. The total polyphenols in % weight/weight dry basis of tea samples can be arranged in order as English breakfast tea (mean ± SD, 11.51 ± 0.93), almond milk tea (11.43 ± 0.47), soy milk tea (10.86 ± 0.63), rice milk tea (10.45 ± 0.61),and cow's milk tea (10.43 ± 1.08).

The values of total polyphenol content were compared whether there was any difference between each pair of groups among English breakfast tea, cow's milk tea, soy milk tea, rice milk tea, and almond milk tea groups using one-way ANOVA. We found no statistical differences among the groups (p-value = 0.189).

Antioxidant Capacity by DPPH Radical Scavenging Activity

The antioxidant capacity in µmol Trolox/1 g dry basis of tea sample (Table 2 and Figure 2) can be arranged in order asEnglish breakfast tea (2253.05 ± 181.46), almond milk tea (2150.68 ± 264.57), soy milk tea (2088.40 ± 164.98), cow's milk tea (1991.76 ± 172.66), and rice milk tea (1957.37 ± 152.92). DPPH radical scavenging activity values of each pair of groups were evaluated statistically usingone-way ANOVA. No statistical differences among the groups were found (p-value = 0.234).

Discussion

Our results found that the total polyphenol content of English breakfast tea infusion after adding different types of milk can be arranged in order as English breakfast tea, almond milk tea, soy milk tea, rice milk tea, andcow's milk tea. However, the total polyphenol content is not statistically different among the groups. Hence, the hypothesis was rejected.

The antioxidant capacity of tea depends on polyphenolic compounds in tea infusion. In our study, the result found that the antioxidant capacity measured by DPPH assay in µmol Trolox/1 g dry basis of tea sample can be arranged in order as English breakfast tea, almond milk tea, soy milk tea, cow's milk tea, and rice milk tea. Nevertheless, there is no statistical differencein antioxidant capacity among different types of milk added to tea infusion.

There are conflicting evidenceson whether adding milk to tea infusions affect the polyphenol content and antioxidant capacity of tea. Previous studies showed three types of results, i.e., no effect, dual (increasing or decreasing) effect based on antioxidant capacity measurement, and decreasing antioxidant capacity.

Kyle et al. [11] found that adding milk to black tea infusion did not change the plasma total polyphenols in healthy volunteer within 80 minutes after consumption. van der Burg-Koorevaar et al. [12] evaluatedthe effect of milk on black tea catechin bio-accessibility

Table 1. Total polyphenol content shown as % weight/weight dry basis of tea samples using gallic acid as a standard in English breakfast tea, English breakfast tea + cow's milk,English breakfast tea + soy milk, English breakfast tea + rice milk, and English breakfast tea + almond milk.

Sample	Total polyphenol (% w/w dry basis)					
	#1	#2	#3	#4	mean	SD
EB tea + distilled water	11.06	12.82	10.68	11.47	11.51	0.93
EB tea + cow's milk	9.07	10.79	11.65	10.23	10.43	1.08
EB tea + soy milk	11.76	10.59	10.78	10.32	10.86	0.63
EB tea + rice milk	10.38	10.21	9.9	11.32	10.45	0.61
EB tea + almond milk	11.18	11.51	10.99	12.06	11.43	0.47

EB tea = English breakfast tea

Table 2. The antioxidant capacity by DPPH radical scavenging activity shown as µmol Trolox/1 g dry basis of tea sample using Trolox as a standard in tea samples.

Sample	DPPH-Radical scavenging activity (µmol Trolox/1 g dry basis)					
	#1	#2	#3	#4	mean	SD
EB tea + distilled water	2392.22	2375.44	1999.33	2245.22	2253.05	181.46
EB tea + cow's milk	2176.59	2011.68	2019.61	1759.16	1991.76	172.66
EB tea + soy milk	2177.27	2250.99	2051.3	1874.04	2088.4	164.98
EB tea + rice milk	2126.47	1755.58	1986.13	1961.3	1957.37	152.92
EB tea + almond milk	2297.27	2434.49	2023.07	1847.9	2150.68	264.57

EB tea = English breakfast tea.

Figure 1. Total polyphenol content shown as % weight/weight dry basis of tea sample using gallic acid as a standard. Bars represent mean ± standard deviation of each sample groups. EBT = English breakfast tea, CM = cow's milk, SM = soy milk, RM = rice milk, AM = almond milk.

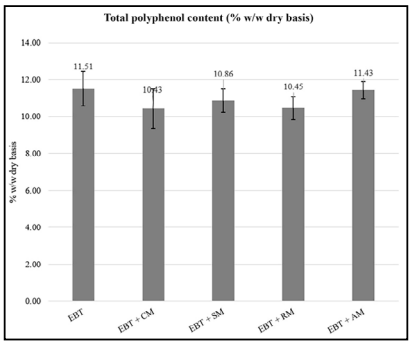
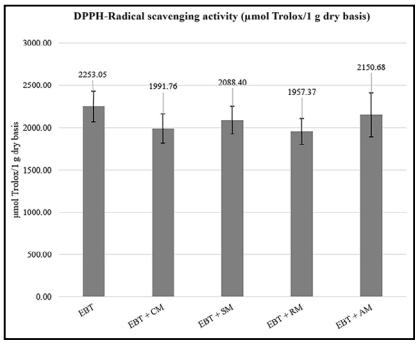


Figure 2. The antioxidant capacity by DPPH radical scavenging activity shown as µmol Trolox/1 g dry basis of tea sample using Trolox as a standard in each group. Bars represent mean ± standard deviation of each sample groups. EBT = English breakfast tea, CM = cow's milk, SM = soy milk, RM = rice milk, AM = almond milk.



and found that the bio-accessibilities of total catechins of tea and tea with milk were not statistically different.They suggested that polyphenol-protein complexes may degraded during digestion, andadding milk to tea infusion is unlikely to result in decreased plasma concentration of tea catechins.

Previous study by Bourassa et al. [6] demonstrated that adding alpha-casein to tea polyphenols affected antioxidant capacities of polyphenols depending on method of measurement. After adding alpha-casein, antioxidant capacities of tea polyphenols measured by ABTS free radical scavenging and voltammetry decreased. In contrast, addition of alpha-casein increased antioxidant capacity of polyphenols measured by lipid peroxidation inhibition method. Also, Dubeau et al. [13] suggested that adding milk to tea infusion can decrease or increase antioxidant capacities of green, Darjeeling, and English breakfast tea depending on the method used formeasuringantioxidant capacities. Similar to Bourassa et al. [6], the addition of milk decreased antioxidant capacities of all tea infusion measured by ABTS free radical scavenging and voltammetry method whereas the antioxidant capacity measured by lipid peroxidation inhibition method increased.Saovapakhiran et al. [14] found that adding milk to oolong tea decreased the antioxidant capacity of oolong tea measured by ABTS free radical scavenging method and cellular antioxidant activity assay. In contrast, antioxidant activity of oolong tea with addition of milk measured by ferrous ion-chelating assay increased.

Ryan and Petit [7] valuated the effect of adding whole, semi-skimmed, and skimmed bovine milk 10, 15 and 20 mL on antioxidant capacity of 200 mL black tea infusion. They found that adding skimmed milk decreased the total antioxidant capacity measured by ferric reducing antioxidant power (FRAP) assay more than eithersemi-skimmed milk or whole milk,and the higher volume of milk added tended to decrease antioxidant capacity more than the lower volume of milk. Simanjuntak et al. [15] evaluated the effect of adding skimmed milk to white tea and black tea on tea antioxidant capacity. Tea antioxidant capacity was measured by the DPPH method. The result found that, after adding skimmed milk with the volume of 1, 1.5, and 2 mL to tea infusions200 µL, the antioxidant capacities were decreased. Antioxidant activity of white tea mixed with skimmed milk was decreased by 84.39% whereas black tea mixed with skimmed milk was decreased by 73.54%. The author suggested that decreasing in antioxidant capacity after adding skimmed milk to tea infusion is because of the interaction between milk proteins and tea polyphenols, and that casein protein in milk can bind to tea polyphenols and cover the active groups on polyphenols result in decreasing free radical scavenging activity.Sharma et al. [16] observed that compare to plain black tea, black tea with milk brew prepared by tea 2 grams, milk 40 mL, and water 60 mL mixed and boiled for 2 minutes had lower antioxidant capacities measured by DPPH free scavenging activity assay.

Our findings that the decreases of the total polyphenol content and the antioxidant capacity, after adding the different types of milkto tea infusion, were not statistically significantbecause the interaction between milk protein and tea polyphenols could be varied by the proportion of the volume of tea infusion and the volume of milk, the types of tea and milk, the method of making tea infusion,or the method of measuring antioxidant capacity. In this study, we used the mixtures of 200 mL of English breakfast tea infusion, which were filtered and cooled down to room temperature, added with 50 mL of four different types of milk.

Hasni et al. [17] found that polyphenols from tea could bind weakly to both alpha- and beta-caseins which beta-caseins formed stronger complexes with tea polyphenols compared to alpha-caseins, and the polyphenols with more -OH groups can bind more caseins. Also, the type of tea can affect the polyphenolic compounds that contribute to antioxidants in tea infusions. Major polyphenols found in green tea are EC, ECG, EGC, and EGCG-whereas polyphenols in black tea are theaflavins and thearubigins [2]. As tea is a natural product from the plant (Camellia sinensis), environments such as light, temperature, water, and nutrition affect the quality of tea leaves [18]. The same type of tea including green tea, yellow tea, white tea, oolong tea, black tea, and dark tea from a different place of production possesses different phenolic profiles and antioxidant capacities [19]. In the process of producing black tea, polyphenols in tea leaves undergo enzymatic fermentation in a different method that results in varying polyphenols components [3]. Black tea from each manufacturer may contain varying levels of polyphenolic compounds which attribute to different interactions with milk protein.

Ryan and Sutherland [20] compared the effect of adding different types of soy milk and cow's milk on the antioxidant capacity of commercially available black tea. The result showed that adding soy milk also decreased the FRAP assay antioxidant capacity of black tea but toa lesser extent than cow's milk. The experiment done by Rawel et al. [21] examined that soy proteins (soy glycinin and soy trypsin inhibitor) also interact with some polyphenols and flavonoids. The different types of protein in each type of milk may alter the interaction between each milk and polyphenols in tea. However, there are still few pieces of evidence about the interaction between soy milk, rice milk, and almond milk with tea polyphenols. Further studies are needed to demonstrate the interaction between plant-based milk protein and tea polyphenols.

Despite that there are previous studies suggested that proteins can interact with phenolic compounds in the plant, our result demonstrated that adding different types of milk (cow's milk, soy milk, rice milk, and almond milk) in the volume of 50 mL to 200 mL English breakfast tea infusion did not significantly decrease the antioxidant capacitiesof tea infusion measured by DPPH radical scavenging assay.

Further study maybe performed using other methods to evaluate the antioxidant capacity of milk tea mixture or examine the interaction between plant-based milk protein and tea polyphenols.

Conclusion

Prior studies suggested that protein in milk can interact with tea polyphenols that leads to the reduction of antioxidant capacity in milk tea beverages. However, in this study, the result showed that after adding different types of milk (cow's milk, soy milk, rice milk, and almond milk) the antioxidant capacities of tea did not decrease significantly. Soy milk, rice milk, and almond milk can be used as an alternative to cow's milk in adding to tea infusion without disturbing the antioxidant capacity of tea.

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Appendix A

Materials

Figure A.1 Material: (a) Thai-Denmark cow's milk; (b) Pureharvest™ soy milk (unsweetened); (c) Pureharvest™ rice milk (unsweetened); (d) 137 degrees™ almond milk (original unsweetened); (e) English breakfast tea (Twinings™).

Table A.1Nutrition information.

	(a)	(b)	(c)	(d)
Serving Size, mL	250	250	250	180
Total Energy, kcal	160	123	127	60
Total Fat, g	9	8.5	2.75	5
Saturated Fat, g	5	1	0.25	1
Cholesterol, mg	30	Not detected	Not Detected	0
Protein, g	8	7.5	0.75	2
Total Carbohydrate, g	12	3.75	24.7	2
Dietary Fiber, g	0	Not detected	0	0
Sugar, g	11	1.5	13	0
Sodium, mg	85	40	145	15
Calcium, mg	240	300	6.75	0
Vitamin A, μg RE	64	N/A	N/A	360
Vitamin B1, mg	0.09	N/A	N/A	0.45
Vitamin B2, mg	0.425	N/A	N/A	0.85
Folic Acid, μg	N/A	N/A	N/A	120
Vitamin E, mgα-TE	N/A	N/A	N/A	2
Iron, mg	0	N/A	N/A	0

(a) Thai-Denmark cow's milk; (b) Pureharvest™ soy milk (unsweetened); (c) Pureharvest™ rice milk (unsweetened); (d) 137 degrees™ almond milk (original unsweetened)

RE = Retinol equivalent, α-TE = α-Tocopherol equivalent, N/A = not available