# Biological activity of dried infused sapodilla (*Manilkara zapota*) leaf tea formulated with different milk and sugar contents

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## Abstract

Milk tea is a popular global beverage, but it also has high sugar and fat contents that can lead to chronic diseases. Sapodilla (*Manilkara zapota*) leaves contain various phenolic compounds with significant antioxidant and health benefits, but the biological impacts of interactions between sapodilla and other food ingredients in food systems have not been investigated. This study assessed ingredient interactions with the biological properties of functional sapodilla milk tea. Dried sapodilla leaves were infused with hot water at 1 g/120 mL and 1 g/68 mL and formulated with almond milk, soy milk, fat-free cow milk, and lactose-free cow milk. Each formulation was determined for color, total phenolic content, and total antioxidant activity using ABTS and FRAP assays. Two sweeteners, white sugar and smart-branded sugar, were added and tested for antioxidant capacity and sensory acceptability. The formulations were measured for estimated glycemic index (GI), antioxidant capacity, and sensory acceptability compared to commercial products. Results revealed a prototype with a lower estimated GI than commercial products ($p<0.05$) and high overall liking score of 7.6 (n=30). Functional ingredients from natural sources such as sapodilla leaves can be successfully incorporated into foods, providing novel functional product categories and new commercial opportunities.

**Keywords:** *Manilkara zapota*; phenolic compounds; glycemic index; antioxidant capacity; ingredients in food systems; functional beverage.

**Practical Application:** Sapodilla leaf extract contains phytochemicals with significant antioxidant capabilities and benefits for lowering blood sugar.

## 1. Introduction

Milk tea is a famous tea-based drink throughout Asia, with the global market value estimated to reach US$3.49 billion by 2027. Excessive consumption of sugar-sweetened beverages, which include milk tea, increases the risk of developing chronic diseases such as diabetes and obesity (Malik et al., 2010). Milk is the main source of saturated fat, with a high impact on the risk of metabolic syndrome (Bhavadharini et al., 2020). The recent growth of healthy milk tea alternatives is mainly attributed to increasing public health consciousness (Fortune Business Insights, 2020; Yang et al., 2023). Low-sugar milk tea and/or milk tea made from different species of tea have also attracted interest in diabetes prevention (GutiErrez-Grijalva et al., 2016; Saibandith et al., 2017; Yang et al., 2014).

*Manilkara zapota*, also called Sapodilla or Sapota, belongs to the Sapotaceae family and is native to Mexico and Central America (Bano & Ahmed, 2017). Sapodilla leaf extract has antidiabetic, anti-inflammatory, antipyretic, and analgesic biological activities previously investigated by Fayek et al. (2012), Islam et al. (2020), and Maslikah et al. (2021). Phytochemicals are crucial components in sapodilla, especially myricetin-3-O-α-L-rhaminopyranoside, apigenin-7-O-α-L-rhamnopyranoside, and gallic acid. Species, age, production technique, and food interaction all affect plant phenolic compounds and biological activities (Cosme et al., 2020; Natnoi & Pirak, 2019; Owolabi et al., 2018; Pothinuch & Tongchitpakdee, 2019; Suravanichnirachorn et al., 2018). Factors impacting phenolic compound contents in *Manilkara zapota* leaf botanical drinks have also been studied, including age of leaves, drying temperature, and extraction method (Putson et al., 2022). This research developed beverage products from sapodilla leaves and assessed the impact of food ingredient interactions in food systems on biological activities.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Standard reagents including gallic acid, catechin, Trolox, tannic acid, and a Glucose (GO) Assay Kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pepsin, pancreatin, and amyloglucosidase enzymes and other chemicals were bought from MT Instrument Co., Ltd. (Bangkok, Thailand), A&A

Reagent Ltd. (Songkhla, Thailand), and Smart Sugar (Green Food Asia Co., Thailand).

### 2.2. Ethical approval

Kasetsart University Research Ethics Committee approved this project, which involved using human research subjects (COE No. 65/113). The investigations began in August 2022 and ended in September 2022. Kasetsart University registered the trial. All subjects provided written informed consent, and their data were kept confidential. The procedure remained unchanged throughout the trial.

### 2.3 Cooking methods

#### 2.3.1. Dried leaves

Mature sapodilla leaves in the color range $L^* \approx 45$ $a^* \approx -11$ $b^* \approx 28$ using a Chromameter (Konica Minolta, NJ, USA) were harvested from a plantation in Pak Chong, Nakhon Ratchasima, in November 2020. The sapodilla leaves were washed with clean water, then dried in a hot air oven at 55 °C for 2.5 h and blended thoroughly into powder (60 mesh).

#### 2.3.2. Effect of water-soluble extract type

Water-soluble extract and sapodilla leaf tea were combined using a factorial experiment at 5:20 (mL). The two experimental factors were four commercial water-soluble extract varieties as soy (Lactasoy), almond (Almond Breeze), fat-free and lactose-free (Meiji) milk, and two levels of sapodilla leaf tea (SPT) concentration as 1 g of sapodilla leaf powder per 68 mL of water (SPT high) and 1 g of sapodilla leaf powder per 120 mL of water (SPT low). The leaf powders were heated at 60 °C for 3 min, and the experiment comprised 14 samples called the control group.

#### 2.3.3. Effect of sugar type

In Thailand, sucrose from sugar cane almost became a commercial sugar in beverage recipes. In the factorial experiment, sugar and novel sweetener were used to modify the taste of the drink. Smart sugar (Thai FDA No.: 10-1-05950-1-0003) contains brown sugar (99.5%) and stevia extract (0.5%) and is two times sweeter with half the calorie value of sucrose. Sugar concentrations when making sapodilla tea were the same for SPT high and SPT low.

### 2.4. Sample extraction

Water-soluble samples were extracted with acetone following the modified procedure of Niero et al. (2018). The milk tea was diluted with water (1:20), and then 0.1 mL of the diluted milk tea was mixed with 1 ml of acetone (80%). Samples were incubated for 10 min at room temperature before centrifuging for 10 min at 7,500 × g to precipitate milk proteins.

### 2.5. Color analysis

Milk tea colors were analyzed (MAV) using a color spectrophotometer Ultrascan PRO (Hunter Lab, VA, USA) equipped with a measuring transmittance mode of medium measurement channel. All measurements were conducted in triplicate. Lightness ($L^*$), red-green component ($a^*$), and blue-yellow component ($b^*$) of the CIE Lab scale were the color parameters examined.

### 2.6. Total phenolic content determination

Total phenolic content (TPC) was determined following the method of Ademiluyi et al. (2018). Briefly, 200 μl of the extracts were diluted appropriately, oxidized with 62.5 μL of 10% (v/v) Folin-Ciocalteu reagent, and neutralized with 50 μL of 7.5% (w/v) sodium carbonate solution. The reaction mixtures were then incubated for 90 min at 25 °C absorbance at 765 nm was measured using a microplate reader (Bioteksynergy HTX multimode reader). The equivalent of gallic acid (0.01–0.05 mg/mL) was used to calculate TPC.

### 2.7. Antioxidant activity

The antioxidant tests employed a microplate reader as the detector and Trolox (0.36–1.8 mg/mL) as the reference (Ajiboye et al., 2018).

The reaction of 7 mM of ABTS aqueous solution with 2.45 mM of $K_2S_2O_8$ in the absence of light for 16 h produced ABTS. Ethanol was added to raise the absorbance at 734 nm to 0.70±0.02. After 15 min, absorbance was measured at 734 nm by adding 50 μL of an appropriate extract dilution to 100 μL of ABTS solution.

The FRAP assay was conducted by combining 5 mL of 1% (w/v) potassium ferricyanide and 5 mL of 200 mM sodium phosphate buffer (pH 6.6). After 20 min of incubation at 50 °C, 5 mL of 10% (w/v) TCA was added to the mixture. A 15 mL aliquot of the solution was combined with 2 ml of 0.1% (w/v) ferric chloride and an equivalent volume of distilled water. Finally, 150 μL of FRAP solution and 50 μl of the sample were combined, and the absorbance was measured at 700 nm.

### 2.8. Estimated glycemic index

The method used for glycemic index (GI) estimation was modified from Aribas et al. (2020). An aliquot of 100 mL of the sample was placed in a 50 mL tube containing 10 glass beads (6 mm in diameter), 2 mL of HCl (0.05 M) and pepsin (5 mg/mL), and incubated at 37 °C in a water-bath shaker for 30 min. Each tube received 4 mL of sodium acetate buffer (pH 5.2, 0.5 M), 1 ml of a solution containing 14.45 U of amyloglucosidase, and 0.104 g of pancreatin buffer. The tubes were incubated in a water-bath shaker at 37 °C. At intervals between 0 and 90 min, 100 μL of the resultant aqueous solution was placed in an Eppendorf tube and combined with 1 mL of pure ethanol for extraction and centrifugation at 2,500 rpm for 10 min. Glucose residue was tested in the samples using the Glucose (GO) Assay Kit and a microplate reader measured the absorbance at 540 nm.

### 2.9. Sensory analysis

A food and beverage primary sensory test requires 30–40 testers (Stone, 2018), while laboratory-scale testing recommends

25–50 testers per product (Stone and Sidel, 2004). We had a short period to test, so we used 30 testers who had drunk milk tea in the just-about-right sensory test. Sensory testing was conducted by 30 individuals to reduce the possibility of adverse reactions when consuming sapodilla leaves for the first time in Thailand, using a 9-point hedonic scale and "Just About Right." The volunteers were university staff members aged between 18 and 60 years who were not allergic to soy products. The volunteers were asked to provide extra feedback about the product by writing in the suggestions section. All volunteers were instructed to thoroughly clean their mouths with water before tasting the samples. Each tasting test took 30 min to complete.

### 2.10. Statistical analysis

Three replicate trial findings were combined, and the results were presented as mean and standard deviation (SD). Mean values were examined by one-way analysis of variance and post hoc analysis using SPSS, with statistical significance set at $p \leq 0.05$ (Version 12, SPSS Inc., Chicago, IL, USA).

## 3. Results and discussion

### 3.1. Effect of water-soluble extract on total phenolic and antioxidant activity

Functional sapodilla (*Manilkara zapota*) leaf milk tea (FSLMT) was created after testing various milk tea recipes using a 20:5 (mL) tea-to-milk ratio. The experiment comprised two tea concentrations and four types of water-soluble extract. In the control group with only milk, the highest lightness (L* value) was found in lactose-free cow milk, with the lowest in soy milk. Soy milk gave the highest a* red-green value and b* blue-yellow value (p<0.05). The SPT high (1 g/68 mL) had a darker color (p<0.05). Red and yellow values were also higher than SPT low (1 g/120 mL) (Table 1). Milk combined with SPT was darker than the control. Milk combined with SPT became darker due to the anthocyanin/anthocyanidin pigments found in plants. The most common types are glycosides. Ganguly et al.

(2013) discovered glycosides in sapodilla leaves. Depending on the pH of the environment, glycosides can generate hues ranging from bright red to deep purple.

As shown in Figure 1, soy milk had the highest antioxidant capacity and TPC compared to the other types of water-soluble extract. A significant difference was found when soy milk was combined with SPT. Isoflavones are the primary active component in soy milk that impact anti-inflammation activity because of their antioxidant mechanisms. Isoflavones are also effective antidiabetic drugs by targeting various cell signaling pathways implicated in the pathogenesis of diabetes through pleiotropic actions (Duru et al., 2018). The presence of the C-2-C-3 double bond and the C-4 ketonic group are two crucial structural components for flavonoid bioactivity and particularly for their antidiabetic properties. The antioxidant and antidiabetic effects of flavonoids in vitro were diminished by methylation and acetylation of hydroxyl groups (Sarian et al., 2017). Rizzo (2020) found that the combination of soy milk and coffee extract improved digestibility, which was considered by Sęczyk et al. (2017) to result from the interaction between soy milk and coffee extract. Tryptophan and other amino acids such as $-NH_2$ and -SH in soy milk encourage the bonding of phenol-protein complexes (Ozdal et al., 2013; Rawel et al., 2002). Schefer et al. (2021) found that interactions between some proteins and phenolic acids impacted the physicochemical and nutritional properties of the proteins. Our results showed that adding SPT to cow milk had no significant effect, whereas the antioxidant capacity and TPC of almond milk increased. As a result, soy milk was chosen to create four milk tea recipes.

### 3.2 Effect of sugar on total phenolic content and antioxidant activity

Soy milk was chosen for the next step in recipe development, and the impacts of sugar and Smart Sugar were investigated. Smart Sugar contains brown sugar with stevia extract. Sugar content was added to the recipes at 5% because ready-to-drink

**Table 1**. Color analysis of milk tea samples.

| | Sample | L* | a* | b* |
|---|---|---|---|---|
| Control (water-soluble extract only) | Soy milk | 80.00±0.34a | 2.15±0.05d | 14.78±0.31b |
| | Almond milk | 81.93±0.02b | 0.34±0.29c | 8.79±0.04a |
| | Lactose-free milk | 93.55±0.08d | -1.76±0.03b | 8.98±0.04a |
| | Fat-free milk | 88.56±1.08c | -4.46±0.23a | 8.87±0.35a |
| SPT low (1 g/120 mL) | Sapodilla tea (1 g/120 mL) | 68.06±0.06e | 17.99±0.09e | 55.94±0.12e |
| | Soy milk | 61.20±0.14c | 3.49±0.01c | 14.38±0.02b |
| | Almond milk | 49.26±0.04a | 4.80±0.05d | 16.03±0.05d |
| | Lactose-free milk | 67.05±0.02d | 1.95±0.02b | 15.62±0.02c |
| | Fat-free milk | 55.92±0.03b | -0.29±0.01a | 12.24±0.06a |
| SPT high (1 g/68 mL) | Sapodilla tea (1 g/68 mL) | 62.93±0.17d | 26.39±0.06e | 71.23±0.15e |
| | Soy milk | 61.32±0.22c | 5.22±0.13c | 16.57±0.32b |
| | Almond milk | 45.44±0.01a | 7.52±0.06d | 18.59±0.05d |
| | Lactose-free milk | 65.54±0.01e | 3.43±0.01b | 17.98±0.02c |
| | Fat-free milk | 56.04±0.43b | 1.36±0.07a | 14.51±0.57a |

Data are reported as mean±SD (n=3). Values in columns with different letters are significantly different (p≤0.05). L* represents lightness from 0 (dark) to 100 (bright), a* shows green (-a*) to red (+a*), and b* shows blue (-b*) to yellow (+b*).
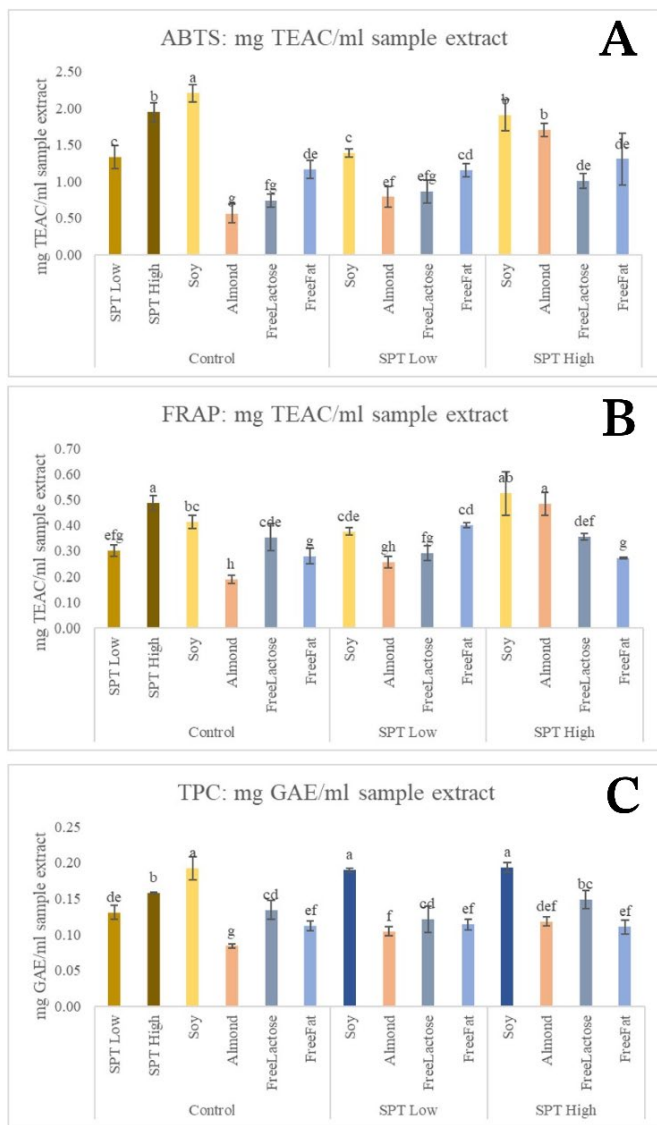
**Figure 1**. Antioxidant activity and total phenolic content of milk tea samples. (A) Antioxidant activity by ABTS assay, (B) antioxidant activity by FRAP assay, and (C) total phenolic content of samples. Columns with different letters indicate significant differences (p≤0.05).

**Table 2**. Sapodilla leaf tea soy milk recipes.

| Formula | 1* | 2* | 3** | 4** |
|---|---|---|---|---|
| | (%) | (%) | (%) | (%) |
| Sapodilla leaf tea | 75 | 78 | 75 | 78 |
| Soy milk | 20 | 20 | 20 | 20 |
| Sugar | 5 | 2*** | 5 | 2*** |

*Milk tea with concentrated tea 1 g/120 mL; **Milk tea with concentrated tea 1/ 68 mL; ***Use Smart Sugar instead of granulated sugar.
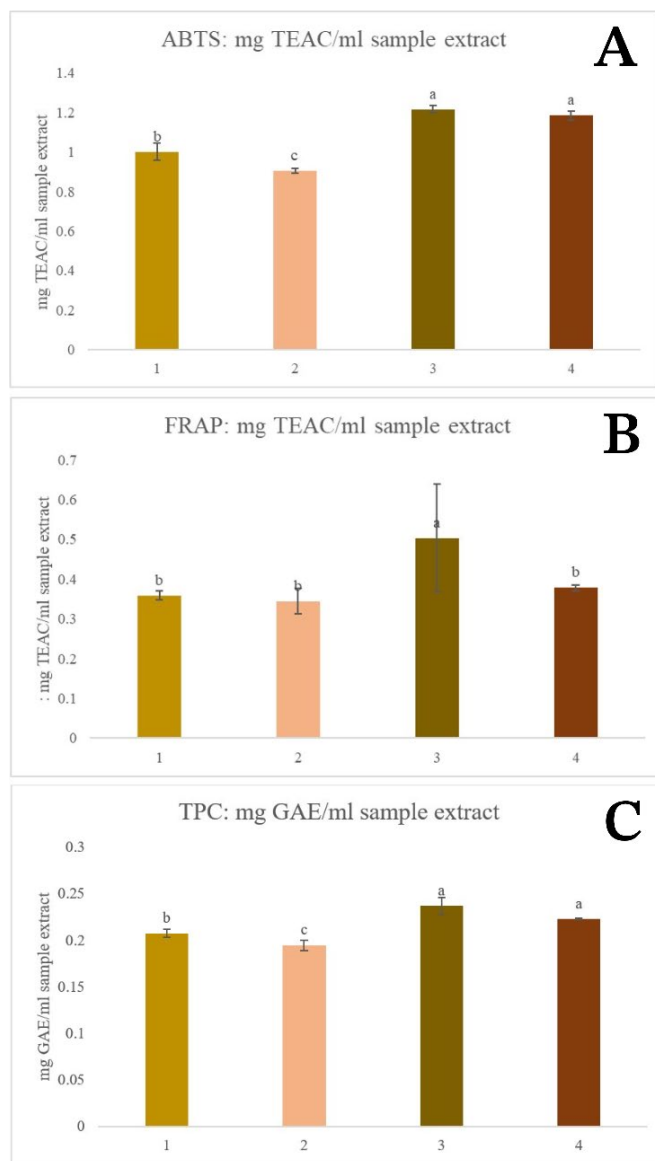


**Figure 2**. Biological activity of four recipes of sapodilla leaf milk tea. (A) Antioxidant activity by ABTS assay, (B) antioxidant activity by FRAP assay, and (C) total phenolic content of samples. Columns with different letters indicate significant differences (p≤0.05).

milk tea products in the market have sugar content range of 5–9%. High and low SPT were also investigated for their impact on product taste. Four recipes were tested, as presented in Table 2.

Blends of SPT high and low and soy milk tea were tested for bioactivity, as presented in Figure 2. Soy milk combined with SPT high had greater antioxidant capacity and TPC than soy milk combined with SPT low (p<0.05). Sugar added to water-soluble extract for ABTS assay at SPT high concentration (p<0.05) showed higher antioxidant activity than Smart Sugar. The influence of milk and sugar on the antioxidant potential was also reported by Sharma et al. (2008). They found that black tea showed the highest radical scavenging activity, followed by black tea combined with sugar and milk tea containing sugar. Milk tea without sugar showed the lowest activity. The antioxidant activity of tea was enhanced and stabilized by

adding milk or sugar. The condensation interaction between the hydroxyl groups caused a decline in antioxidant ability. Glycosides were created from the phenolic components in tea extract and the hydroxyl groups in sucrose molecules (Shalaby et al., 2016).

Samples of sapodilla leaf tea blended with soy milk were evaluated by volunteers for sensory analysis. Only 30 subjects were included in this study because Short et al. (2021) suggested n~20 as sufficient for a trial hedonic test. Sapodilla leaf tea has never been consumed in Thailand. Therefore, the probability of negative reactions should be reduced as much as possible. Further trials are necessary with larger sample sizes to better gauge the public's acceptability of this new beverage.

Results in Figure 3 show that the four recipes had similar preferences for sweetness, with soy milk tea recipes containing smart sugar obtaining a favorable evaluation. Volunteers who participated in the sensory evaluation noted that the milk tea had a sweet taste on the tongue. The SPT high with sugar recipe gave the highest preference for each component and was chosen for further experiments.

Based on previous experimental results, we chose sample number 4 to develop the prototype. The result of the Just About Right score given by the volunteers in the sensory test (Table 3) indicated how we should reduce or increase concentrations of tea flavor and sweetness. Results between -20 and 20 did not need revision, while results over 20 and lower than -20 required addition and reduction of ingredients, respectively (Gómez-Corona et al., 2016).

### 3.3. Milk tea formulations

The product prototype was developed using a net effect technique to test for antioxidant activity, total phenolic index, and GI. Results were compared with commercial milk tea to assess product potential using the sample in Table 4.
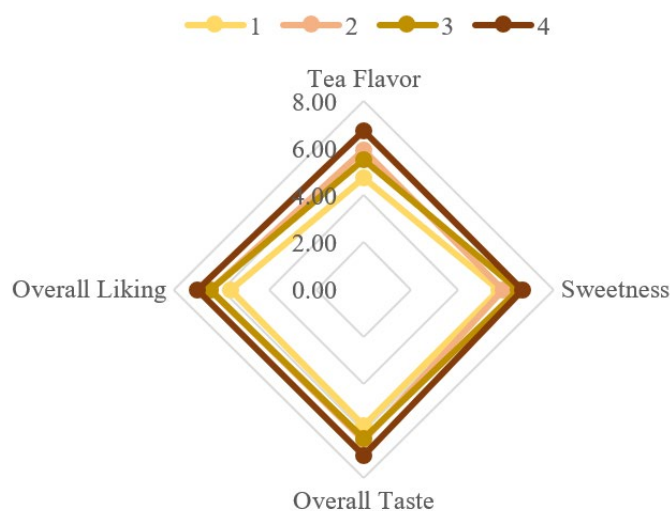


**Figure 3**. Sensory preference scores of the samples (n=30).

Results in Figure 4 demonstrate that normal milk tea has antioxidant capacity related to TPC. Black tea contains many catechins and flavonoids including epigallocatechins, gallates, theaflavins, thearubigins, L-theanine amino acids, and several other groups of polyphenols that have high biological efficacy (Rasheed, 2019). Estimated GI values in Table 5 revealed that food ingestion raised blood glucose levels when the samples were digested with enzymes that mimicked human digestion (Medicine Plus, 2020). FSLMT produced a statistically significant decrease in estimated GI (p<0.05), suggesting that sapodilla leaves can slow down the breakdown of glucose and manage diabetes by inhibiting α-glucosidase activity and enhancing glucose uptake. The combination of free energy values of phenolic compounds in sapodilla produced a synergistic effect. This result concurred with recent studies by Islam et al. (2020), Maslikah et al. (2021), and Putson et al. (2022).

Figure 5 displays liking scores of 30 volunteers for FSLMT products. The overall liking score for the product was moderate (7.6).

Products made from sapodilla leaves may be employed as active components in the production of functional beverages. No volunteers in this study experienced any negative side effects from consuming sapodilla leaf tea at a concentration of 1.15 g per 100 mL of beverage during the sensory tests. Recently, research interest in using plants as functional food and beverage has increased (Cilla et al., 2019; Jiang et al., 2020; Maleš et al., 2022; Rivera et al., 2010).

## 4. Conclusion

Phenolic chemical contents in beverages made from sapodilla leaves change with the addition of ingredients. The water-soluble extract impacted the functionality of drinks made from sapodilla leaves. The phenolic component content's capacity to combat free radicals was best shown in soy milk, while

**Table 4**. Final sample of FSLMT and other milk teas in the market.

| Sample | FSLMT | Commercial soy milk tea | Commercial milk tea |
|---|---|---|---|
| | (%) | (%) | (%) |
| Sapodilla tea powder | 1.15 | – | – |
| Water | 77.85 | – | 88.2 |
| Black tea powder | – | 0.4 | 0.8 |
| Soy milk | 18 | 93 | – |
| Milk powder | – | – | 5.5 |
| Sugar | 3 | 5 | 5.5 |
| Food additive | – | + | + |
| Total | 100 | 100 | 100 |

**Table 3**. Net effect of sample number 4 (n=30).

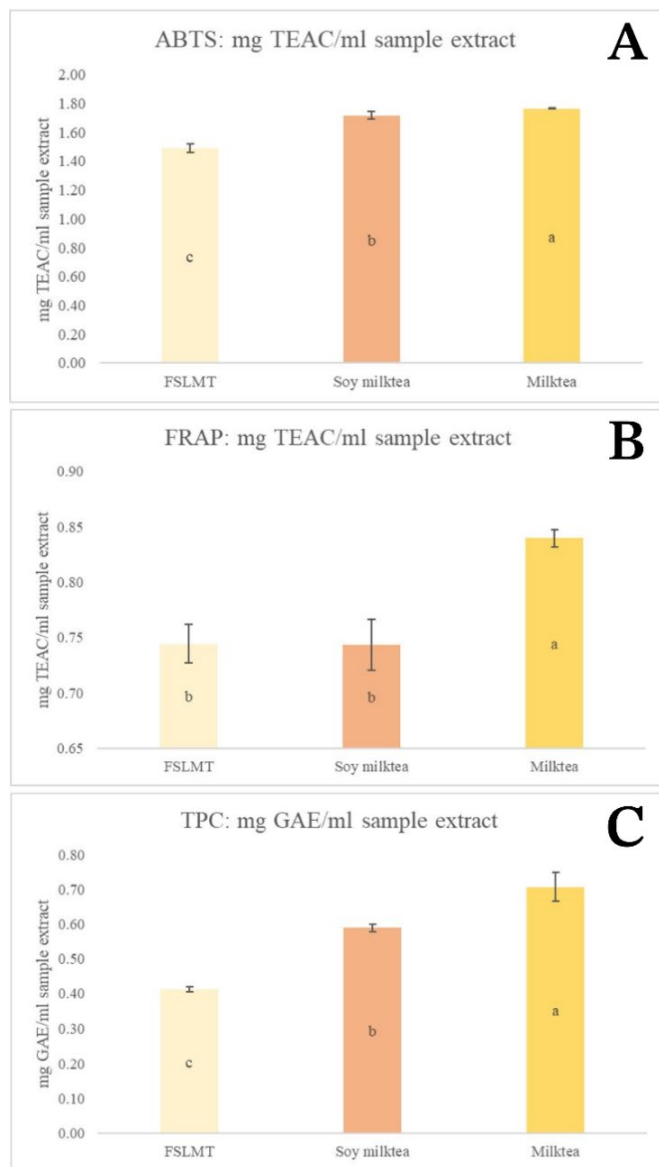| Attribute | Need for improvement (%) | | | | | |
|---|---|---|---|---|---|---|
| | Great increase | Slight increase | No improvement | Slightly reduced | Greatly reduced | Net effect |
| Tea flavor | 13.3 | 23.2 | 56.7 | 6.7 | 0 | 29.8 |
| Sweetness | 0 | 0 | 63.3 | 26.7 | 10 | -36.7 |

**Figure 4**. Bioactivity of the final formulation of FSLMT and other milk teas in the market. (A) Antioxidant activity by ABTS assay, (B) Antioxidant activity by FRAP assay, and (C) Total phenolic content. Columns with different letters indicate significant differences (p≤0.05).

**Table 5**. Estimated glycemic index values of the final sapodilla leaf tea soy milk recipe and commercial milk teas.

| Sample | Sapodilla leaf soy milk tea | Commercial soy milk tea | Commercial milk tea |
|---|---|---|---|
| Estimated GI | 20.18±0.10b | 20.75±0.18a | 21.03±0.34a |

Data are mean±SD (n=3). The letters represent statistically significant differences in each row at confidence level of 95%.

sugar combined with SPT high had high antioxidant activity and TPC. The estimated GI of FSLMT was lower than that of commercial milk tea, and this was recognized by the volunteers. This was the first sensory analysis of a sapodilla leaf product that showed beneficial health properties such as high antioxidant content to control blood sugar levels. The bioavailability test
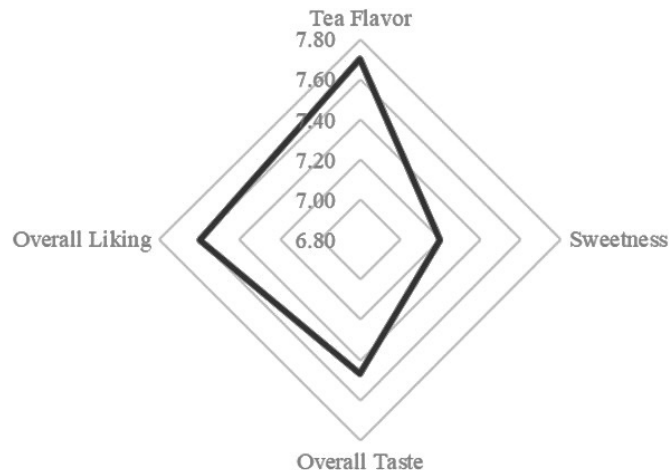


**Figure 5**. Sensory analysis of the final recipe of FSLMT (n=30).

was only carried out in vitro. Additional in-depth studies are necessary to evaluate GI values at both animal and human levels. Sapodilla leaves should be used in more culinary preparations to give consumers extra choices and increase the potential uses of this innovative, health-promoting product.

## Acknowledgment

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