Evaluation of Physicochemical, Sensory, Antioxidant and Nutritional Properties of Latte Drinks from Chaya *(Cnidoscolus aconitifolius)* Leaves

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Abstract

This study aimed to develop a latte drink from Chaya *(Cnidoscolus aconitifolius)* leaves and evaluate its physicochemical, antioxidant, nutritional and sensory properties. The cyanide content and physicochemical properties of dried leaf powder were determined. The results showed that the cyanide level of Chaya leaf powder was less than 0.20 mg/L. The color of dried leaf powder had a brightness (L*) of 49.04 ± 0.45 with green (-5.93±0.09) and yellow (24.50±0.29). The water activity and percent moisture of the powder were 0.26±0.00 and 5.51±0.21, respectively. A suite of latte drinks using different amounts of powder ranging from 0.50-3.00 g of Chaya powder were developed. Adding 0.50-1.00 g of Chaya powder to the latte drink did not significantly affect the color value. The viscosity of latte drinks ranged from 16.48 to 32.80 cp. Moreover, the phenolic and flavonoid contents, as well as the antioxidant activity of the latte drinks were quantified. The Chaya latte drinks contained phenolics and flavonoids in the range of 146.71 to 280.39 mgGAE/100 mL and 26.04 to 53.39 mgQE/100 mL, respectively. The antioxidant activity based on percent inhibition of latte drinks ranged from 30.81 to 53.48. A sensory test conducted using 50 participants showed that the formula with 0.50 g of powder had the highest overall acceptance score (7.04±1.47), but this score did not significantly differ from the formula with 1.00 g of powder (6.34±1.38). Based on the results, 1.00 g of powder (37.2 kcal) was a suitable amount of Chaya powder for a latte drink product.

Keywords: Latte drink, *Cnidoscolus aconitifolius*, Physicochemical properties, Antioxidant properties, Sensory properties, Nutritional properties

Introduction

Consumers of the 21st century are becoming more concerned about their health, which results in an increased awareness of the importance of consuming more fruits and vegetables[1]. The benefits of plant-based foods have been well-documented and shown obvious benefits to health including reduced risk of cardiovascular disease, type II diabetes, non-gallstone related acute pancreatitis, various cancers and cognitive decline[2]. One of the plants that is gaining popularity in the plant-based food community is *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst., of the family Euphobiaceae, commonly called Mexican kale or Chaya. Studies have shown that Chaya is effective in treating numerous aliments including insomnia, diabetes, gout, and scorpion stings[3], and has been found to have antibacterial, hepatoprotective, anti-inflammatory, hematinic and analgesic properties[4,5]. It has also been used to strengthen nails, treat alcoholism and improve vision[6]. Moreover, Chaya leaves are rich in nutrients such as protein, carbohydrate, fiber, vitamins, calcium, iron, sodium, potassium, magnesium, zinc and copper[7,8], as well as antioxidant compounds such as cumarin, flavonoids, phenolics, tannin, antraquinine, triterpenoid, flabotanin, kaempherol, cyanogenic glycosides[9-11]. These antioxidant compounds are associated with the prevention of degenerative diseases such as cardiovascular disease, cancer, obesity, diabetes, and inflammation[12]. Despite these benefits, Chaya consumption comes with a caveat in that it has to be cooked by heat for at least 5 minutes to destroy hydrocyanic acid (HCN), a compound that may cause toxicity if consumed in a large amount or consecutively[3,7,11].

Due to the many health benefits of Chaya, and the increasing consumption of latte among the Thai population, it is interesting to substitute Matcha green tea latte with the caffeine-free Chaya latte for serving to people who are allergic or hypersensitive to caffeine. Thus, this study aimed to formulate a Chaya drink in the form of a milk latte, which is a drink made with steamed milk as its main ingredient[13]. Furthermore, different formulas of the latte were evaluated for their physicochemical, antioxidant, nutritional and sensory properties.

Materials and Methods

Material preparation

Young Chaya leaves were harvested from Valaya Alongkorn Rajabhat University under the Royal Patronage, Klong Luang district, Pathumthani province, Thailand. The sample was identified by a forestry technical expert. The sample was cleaned with water and dried at 60°C for 24 h in a hot air oven (Binder, Germany). The dried sample was ground and passed through a sieve (50 mesh number). Then, the percent yield of dried powder was calculated.

Evaluation of cyanide in dried and fresh samples were performed by mixing 10 mL of 0.10 g/mL dried sample in a distilled water solution with a drop of 95% concentrated sulfuric acid (Qrec, New Zealand) and shaking immediately. Hydrocyanic acid gas was formed and could be detected at the boundary layer between water and air. After shaking, 10 mm of Cyantesmo Test paper (Macherey-nagel, Germany) was dipped into the acidified solution for 15 minutes. The color reaction on the pale green test paper changed to pale blue and dark blue according to the concentration of hydrocyanic acid, which correlates with cyanide level. The method has a minimum limit of cyanide detection of 0.20 mg/L.

Evaluation of the physicochemical properties of the dried sample powder was done by measuring the moisture content, water activity and color (CIE system) using a moisture analyzer MA37 (Sartorius, Germany), water activity meter LabSwift-aw (Novasina, Switzerland) and Chroma Meter CR-400 (Konica Minotta, Japan), respectively. The moisture content measurement was performed using the fully automatic mode at 105°C. Approximately 8.00 g of sample was weighed on the moisture analyzer and recorded as percent moisture. Water activity was determined using the auto start mode. The sample was added to a sample dish then put into the water activity meter. After the analysis, the actual value in aw was read. The color meter was calibrated using a white plate CR-A43 (y = 85.70, x = 0.3177 and y = 0.3340) and assessed using the DP mode. The analyzed color parameters were L* (lightness: from 0 (black) to 100 (white), a* from - (green) to + (red) and b* from – (blue) to + (yellow). All physicochemical measurements were performed in triplicate and expressed as mean ± SD.

Preparation of latte drinks from Chaya leaf powder

The Chaya leaf powders (0.50, 1.00, 2.00, 3.00 g) were added to 40 mL of boiling water and blended with an electric hand held frother (Zulay kitchen, U.S.A.). After cooling, all drinks were combined with 60 mL whole milk.

Evaluation of physicochemical properties of Chaya latte drinks

Physicochemical properties of all four Chaya latte drink formulas were investigated. The evaluation of viscosity and color were performed using a DVE Viscometer (AMETEK Brookfield, U.S.A.) and Chroma Meter CR-400 colorimeter (Konica Minotta, Japan), respectively. The viscosity of 100 mL sample was measured at 100 rpm using a no. 2 needle. Color was analyzed as described above. Measurement of viscosity and color were carried out in triplicate and expressed as centipoise and L*, a*, b* (mean ± SD), respectively.

Evaluation of the antioxidant property of Chaya latte drinks

Folin-Ciocalteu method

The Folin-Ciocalteu method was used to determine total phenolic content. Yellow F-C reagent containing molybdotungstate (Mo) oxidizes the electron-transfer reaction between reductants (phenolate) and molybdotungstate, changing the color to blue (equation below)[14]. The Folin-Ciocalteu method was performed as described by Herald et al.[15]. Gallic acid standard (20-200 μg/mL) was prepared. Either gallic acid standard (25 μL) (Sisco research Laboratories Pvt. Ltd., India) or Chaya latte sample solution (25 μL) was mixed with 75 μL of distilled water and 25 μL of 0.2 N Folin-Ciocalteu reagent (Sisco research Laboratories Pvt. Ltd., India). After the solution was mixed and left for 6 minutes, 100 μL of 75 g/L Na_2CO_3 (Ajax Finechem Pty Ltd, Australia) was added. The solutions were incubated in the dark at room temperature for 90 minutes Then, the absorbance was measured at 765 nm using a microplate reader (Synergy HTX BioTek instruments, U.S.A.). The sample solutions were analyzed in triplicate. Total phenolic content of samples was measured against the gallic acid standard calibration curve (y = 0.0014x+0.0381, R^2 = 0.9943). Total phenolics was expressed as milligram gallic acid equivalent (GAE)/100 mL of latte drink (mean ± SD).

$$\text{Mo(VI) (yellow)} + \text{Phenolic } (e^-) \longrightarrow \text{Mo(V) (blue)}$$

Aluminum chloride method

The aluminum chloride ($AlCl_3$) method was used to determine total flavonoid content. The principle of the aluminium chloride colorimetric method involves the formation of acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl groups of flavones and flavonols. In addition, it also forms acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids[16]. The aluminum chloride method was modified from Pal et al.[16] Gallic acid standard (0.6-21 μg/mL) was prepared. In each reaction, 130 μL of either quercetin standard (Sisco research Laboratories Pvt. Ltd., India) or Chaya latte sample solution was added into the mixture 130 μL of 10% aluminum chloride (Elago Enterprises Pty Ltd, Australia) and 130 μL of 1 M sodium acetate (Carlo Erba, Italy). After mixing, the reaction mixture was allowed to stand for 30 minutes at room temperature. The 200 μL of mixture solutions were pipetted into 96 well plates and the absorbance was read at 415 nm using a microplate reader. Each sample solution was analyzed in triplicate. The flavonoid content of samples was measured against the quercetin standard calibration curve (y = 0.0015x+0.036, R^2 = 0.9932). The result was expressed as milligram quercetin equivalents (QE) in 100 mL of latte drinks (mean ± SD).

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging method

The 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging method was used to determine total antioxidant activity. This method is based on the measurement of the reducing ability of antioxidant compounds toward DPPH radical which is one of a few stable and commercially available organic nitrogen radicals bearing a deep purple color. Antioxidant ability can be evaluated by measuring the change of DPPH color from violet to yellow upon reduction by either the process of hydrogen or electron donation[17]. The method for total antioxidant activity was modified from Khettaf et al.[17]. Different concentrations of standard (1-10 μg/mL) were prepared. One-hundred microliter of either standard (ascorbic acid, Sigma-Aldrich, U.S.A.) or Chaya latte sample solution was mixed with 100 μL of 208 μM DPPH (Sigma-Aldrich, U.S.A.) in methanol. After incubating in darkness for 30 minutes, the absorbance was measured at 517 nm using a microplate reader. All reactions of samples were carried out in triplicate. Percent inhibition was calculated using the following formula:

$$\%\text{ inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where: $A_{control}$ is the absorbance of the control solution

A_{sample} is the absorbance of sample solution or standard

Evaluation of nutritional properties

Energy and nutrients (carbohydrate, protein, fat, and sugar) of the latte formulations were calculated using the reference of the U.S. Department of Agriculture database (USDA, 2020). The obtained data were reported as 100 mL of each composite latte sample.

Evaluation of sensory properties on Chaya latte drinks

The sensory scores of four formulas were evaluated by 50 participants who were not allergic to dairy and who drank latte drinks. Participants were students at Valaya Alongkorn Rajabhat University under the Royal Patronage, and all consented to join this study. The sample drinks were labeled with a three letter-digit randomized code. Twenty milliliters of sample drinks (5-7°C) were served to participants. Appearance, color, texture, taste, flavor, and overall preference of samples were assessed using the 9-point hedonic scale (1: extremely dislike to 9: extremely like). The study protocol was approved by the Valaya Alongkorn Rajabhat University under the Royal Patronage regional research ethics committee (Ref. no. 0008/62). The result was expressed as mean ± SD.

Data analysis

Statistical data were analyzed using ANOVA at $p \leq 0.05$ significance level. The data were shown as average and standard deviation.

Results

Cyanide and physicochemical properties of dried plant powder

Chaya leaf sample was processed by drying and grinding, giving a final dry mass yield of 26.85 w/w. The Cyantesmo test papers of dried and fresh samples are shown in Figure 1. The Cyantesmo test paper of dried sample appeared green, which indicated that detectable cyanide in dried leaf powder was less than 0.20 mg/L.

The results from the physicochemical characteristic analysis showed that the brightness of Chaya leaf powder (L*) was 49.04 ± 0.45 with green (a* = -5.93±0.09) and yellow (b* = 24.50±0.29) (Table 1). The water activity (a_w) and the percent moisture content were 0.26 ± 0.00 and 5.51 ± 0.21 (Table 1), respectively.

Physicochemical properties of latte drinks from Chaya leaf powder

The result showed that the formula with the highest Chaya leaf powder content (3.00 g) was the most viscous and the viscosity decreased with decreasing Chaya leaf powder content (Table 2). The brightness (L*) of Chaya latte drinks decreased, when adding more Chaya leaf powder (in the range of 35.40±0.97 - 25.94±0.41) with color green (-a*) and yellow (+ b*). However, the a* and b* colors in formulations with 0.50 and 1.00 g of Chaya leaf powder were not significantly different ($p > 0.05$), suggesting that the addition of Chaya

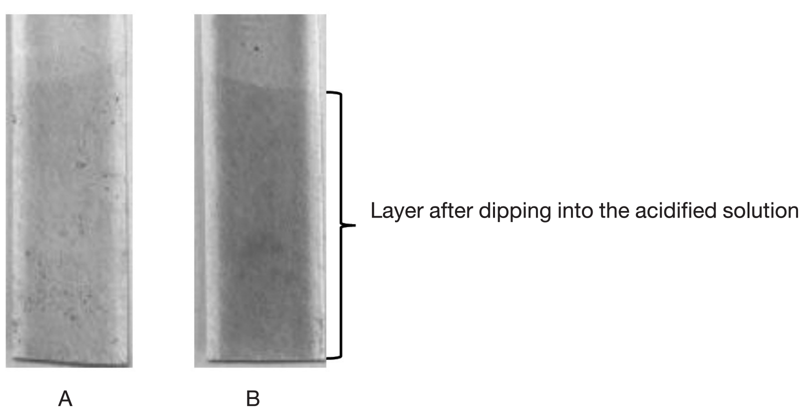

Figure 1 Cyantesmo Test papers after dipping into the solution of (A) dried Chaya leaf powder and (B) fresh Chaya leaf

Table 1 Physicochemical properties of Chaya leaf powder

Sample	Water activity (a_w)	Moisture (%)	Color L*	Color a *	Color b *
Chaya leaf powder	0.26±0.00	5.51±0.21	49.04±0.45	-5.93±0.09	24.50±0.29

Table 2 Color and viscosity of latte drink formulations containing Chaya leaf powder

Latte drinks Formulation with Chaya leaf powder (g)	Color L*	Color a*	Color b*	Viscosity (cp)
0.50	35.40±0.97[a]	-6.52±0.26[a]	10.40±0.43[a]	16.48±0.43[a]
1.00	31.45±0.95[b]	-5.95±0.27[ab]	9.59±0.44[ab]	17.42±0.20[b]
2.00	29.28±0.65[c]	-5.60±0.22[b]	9.09±0.36[b]	29.75±0.15[c]
3.00	25.94±0.41[d]	-4.40±0.19[c]	7.32±0.28[c]	32.80±0.30[d]

[a-d] Different letters in the same column indicate values are significantly different ($p \leq 0.05$)

powder in the range of 0.50-1.00 g did not affect the changes of green and yellow colors.

Antioxidant activity of latte drinks from Chaya leaf powder

The antioxidant activity, phenolic and flavonoid contents of Chaya latte drinks are presented in Table 3. The Chaya latte drinks contained phenolic and flavonoid contents in the range of 146.71-280.39 mgGAE/100 mL of latte drinks and 26.01-53.39 gQE/100 mL of latte drinks, respectively. The results showed a positive correlation between the amount of Chaya leaf powder and the amounts of phenolic and flavonoid compounds. The free radical inhibition of latte drinks was in the range of 30.81±2.07-53.48±1.11%.

Nutritional properties of latte drinks from Chaya leaf powder

The energy of each formula was mostly attributed to whole milk. Therefore, each formula did not significantly differ in energy and composition. The result showed that the carbohydrate, protein, fat and sugar contents of all formulas of the latte drink were 2.75, 2.00, 2.00 and 2.75 g, respectively. The energy of the latte drink was 37.2 kcal.

Sensory properties of latte drinks from Chaya leaf powder

The development of latte drinks from Chaya leaf powder consisted of four formulations (Table 4). The formulation with 0.50 g of Chaya leaf powder exhibited the highest flavor score (6.94 ± 1.73) and was significantly different from other formulas. As for texture characteristic scores, the formulation with 1.00 g of Chaya leaf powder had the highest preference scores (7.64±11.52), but was not significantly different (p>0.05) from the 0.50 g formula (6.44±1.26). However, the taste, appearance and color characteristics of latte drink formulations with 0.50, 1.00 and 2.00 g of powder were not significantly different in their preferences scores (p > 0.05). Moreover, the overall acceptance score of the formulation with 0.50 and 1.00 g of Chaya leaf powder was not significantly different (p> 0.05). The result indicated that the addition of Chaya leaf powder in the range of 0.50-1.00 g did not affect consumer perceptions of taste, appearance, color and overall acceptance.

Table 3 Antioxidant activity, phenolic and flavonoid contents of Chaya latte drinks

Latte drinks Formulation with Chaya leaf powder (g)	Phenolic content (mgGAE/100 mL of latte drinks)	Flavonoid content (mgQE/100 mL of latte drinks)	Antioxidant activity (% inhibition)
0.5	146.71 ± 3.56^{a}	26.01 ± 0.62^{a}	30.81 ± 2.07^{a}
1	164.34 ± 3.73^{b}	31.82 ± 1.45^{b}	34.04 ± 1.43^{b}
2	239.75 ± 9.17^{c}	48.56 ± 0.63^{c}	41.20 ± 2.06^{c}
3	280.39 ± 10.58^{d}	53.39 ± 1.06^{d}	53.48 ± 1.11^{d}

$^{a-d}$ Different letters in the same column indicate values are significantly different ($p\leq0.05$)

Table 4 Sensory properties of latte drinks from Chaya leaf powder

Characteristics	Latte drink formulation with Chaya leaf powder			
	0.50 g	1.00 g	2.00 g	3.00 g
Appearance	6.42 ± 1.25^{a}	6.42 ± 1.21^{a}	5.76 ± 1.51^{a}	4.78 ± 1.76^{b}
Color	6.82 ± 1.45^{a}	6.74 ± 1.21^{a}	5.88 ± 1.55^{a}	4.86 ± 1.88^{b}
Texture	6.44 ± 1.26^{a}	7.64 ± 1.52^{a}	5.30 ± 1.45^{b}	4.36 ± 1.70^{c}
Taste	5.80 ± 1.54^{a}	5.68 ± 1.46^{ab}	5.28 ± 1.40^{ab}	4.84 ± 1.66^{b}
Flavor	6.94 ± 1.73^{a}	5.80 ± 1.77^{b}	4.78 ± 1.74^{bc}	3.88 ± 1.90^{c}
Overall acceptance	7.04 ± 1.47^{a}	6.34 ± 1.38^{a}	5.18 ± 1.57^{b}	4.16 ± 1.62^{c}

$^{a-c}$ Different letters in the same row indicate values are significantly different ($p\leq0.05$)

Discussion

The results found that drying Chaya leaves at 60°C for 24 hours in a hot air oven was able to eliminate volatile HCN to a residual amount of less than 0.20 mg/L. The World Health Organization (WHO, 2004) reported that the toxic level of cyanide to humans was 20-40 mg/mL or 20,000 mg/L[18]. Therefore, the result concluded that the Chaya powder used in the latte formulas were safe for consumption. The green and yellow colors of Chaya powder were likely attributed to the presence of chlorophyll, rutine, quercetin, and keampferol[19]. The water activity (a_w) was within the range that most microorganisms cannot multiply[20]. The percent moisture content was within an acceptable range according to the Thai Community Product Standard (TCPS 120/2558) of the Thai Industrial Standards Institute (TISI), who requires that the moisture content of tea powder (similar to a Chaya powder) does not exceed 8%.

A suite of latte drinks using different amounts of powder ranging from 0.50–3.00 g of Chaya powder was developed. The physicochemical properties of Chaya latte drinks were of a similar green color to that of Matcha green tea latte. The viscosity of Chaya latte drinks increased with increasing Chaya leaf powder, possibly due to the dietary fiber from Chaya powder[7]. Although the molecular weight of dietary fibers may alter the viscosity of a solution, the alteration may not proportionally affect physiological responses[21]. The phenolic and flavonoid contents, and the antioxidant activity, of the Chaya latte drinks were quantified. The results showed that a higher ratio of Chaya leaf powder increased antioxidant, phenolic and flavonoid contents. This result could be attributed to the presence of gallic acid, chlorogenic acid, caffeic acid, ferulic acid, luteolin, apigenin, coumaric acid, kaempferol, quercetin and rutin[22,23]. These compounds are associated with the prevention of degenerative diseases such as cardiovascular disease, cancer, obesity, diabetes, and inflammation[13]. However, product development should be considered in conjunction with consumer acceptance. Sensory properties analysis revealed that adding more Chaya leaf powder in the latte drinks resulted in a lower taste score. This may be due to flavonoids in the powder of Chaya leaf, which increased the bitterness and resulted in lower consumer preference scores[24]. However, adding Chaya leaf powder in the range of 0.50-1.00 g did not affect consumer acceptance in terms of appearance, color, texture, taste, and overall acceptance. Therefore, 1.00 g of Chaya leaf powder was the most appropriate because of an antioxidant level that was higher than the drink containing 0.50 g of Chaya leaf powder. Moreover, participants commented that the flavor of the Chaya latte drink was similar to that of green tea latte, indicating that the drink could be a substitute for consumers who are sensitive to caffeine, and who are concerned about their health. Furthermore, the analysis of its nutritional properties showed that the latte drink was suitable for consumption between meals due to its low energy content (less than 200 kcal), as recommended by the Thai dietary reference intakes (Thai DRI), Ministry of Public Health.

Conclusion

This study showed that Chaya leaf powder could be formulated into a latte drink. The powder was a yellowish-green color and heat treatment was able to reduce cyanide in the leaves to an undetectable level. The water activity of the Chaya leaf powder was within the range that microorganisms could not propagate, and the moisture level was within the acceptable standard. Formulated Chaya latte drinks had a color which was similar to that of Matcha green tea latte. Studies showed that latte drink with 1.00 g of Chaya leaf powder was the most appropriate formula for health-aware consumers due to the consumer acceptance score, physicochemical property, antioxidant property, and nutritional values.

Author Contributions

PJ designed the study and performed experiments on antioxidant activities, latte formulation, and physico-chemical property. SM collected samples and performed the sensory and nutritional studies. PJ and SM wrote the manuscript. Both authors read and approved the manuscript prior to submission for publication.

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Conflicts of Interest

The authors have no conflicts of interest.

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