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Nutritional and phytochemical properties of Chaya leaves (*Cnidoscolus chayamansa* Mc Vaugh) planted in Northeastern Thailand

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Abstract

Chaya (*Cnidoscolus chayamansa* Mc.Vaugh) is commonly found in Thailand, especially in the northeastern (Isan) region where the consumption of this plant has been cooked for its nutritional benefits and taste. To assess the nutritional composition and antioxidant properties of raw and cooked chaya leaves. Chaya plants from Isan were heat-treated by boiling and drying and compared with raw leaves. Analysis of nutritional composition, phenolic compounds, total phenolic content (TPC) and antioxidant capacity were standardized techniques. Cooked leaves showed a significant ($p \leq 0.05$) decrease in protein, carbohydrate, potassium, sodium, and hydrocyanic acid compared to raw leaves. Chaya leaves are rich in protein (32.41-34.02 g/100g dry weight), but the preliminary protein quality by a digestible indispensable amino acid score is low with isoleucine being the limiting amino acid. Cooking was also significantly ($p \leq 0.05$) eliminated hydrocyanic acid (HCN) Raw (1121.72), Cooked (1.71) mg/kg dry weight]. The major phenolic compounds of both samples were sinapic acid and caffeic acid. Cooked leaves have TPC decreased significantly ($p \leq 0.05$). TPC and antioxidant capacity are significantly ($p \leq 0.05$) decreased by three assays. The results proved that chaya plants found in Thailand are rich sources of nutritional and phytochemical properties. Some plants must require a heat-treated process for safe consumption. Although, some nutritional and phytochemical properties diminish.

Keywords: *Cnidoscolus chayamansa*, Nutritional, Amino acid, Phenolic compound, TPC, Antioxidant capacity

1. Introduction

Cnidoscolus chayamansa (Mc Vaugh), is a leafy edible plant generally known as "Chaya," "Tree spinach," or "Mexican spinach." This plant is often grown in Central America, Mexico and tropical or subtropical areas such as the Yucatan Peninsula and Guatemala [1,2]. It has spread throughout the world, including in Thailand, especially found in the northeastern region. Indigenous peoples in some countries have used Chaya as food and health properties [3]. The plant leaves have been studied to determine their potential benefits, including anti-inflammatory, hepatoprotective, anti-diabetic, and cardioprotective properties, and their utility in medical treatment for protein energy malnutrition [3-5]. Some studies suggest that Chaya leaves have greater nutritional value than other leafy vegetables such as spinach [2]. Chaya is rich in important macro-and micronutrients, especially protein content and an amino acid profile [6-7]. However, the quantity and quality of plant protein should be evaluated which is based on both quantity and amino acid balance.

Previous studies using HPLC analysis found that Chaya leaves contain several phytochemicals such as phenolic and flavonoid compounds, including kaempferol and quercetin glycosides [3], moretenol, moretenyl, and kaempferol-3-7-dimethyl ether [1], gallic acid, chlorogenic acid, caffeic acid, rosemarinic acid, apigenin and resveratrol [8]. In addition, Chaya extract's potential antioxidant capacity was identified by standard methods

based on several oxidative products [4,9,10]. Anti-nutrients such as tannins, saponins and lignin were also detected. In addition, this plant was also found to contain hydrocyanic acid or cyanogenic glycosides (as linamarin). This compound is highly toxic to organisms. Therefore, a toxin in this plant must be eliminated by heat- treated processing [11,12].

The local people of Thailand consume Chaya vegetables for tasty and nutritious, which Chaya vegetables have been cooked to eliminate toxins before they are consumed. However, the data of Chaya leaves after easily heat-treated processing to remove undesirable substances is limited. Therefore, this study aimed to assess the chemical composition (including carbohydrate, fat, protein, potassium, sodium, phosphorus, and HCN), amino acid profile, preliminary protein quality, phenolic compounds, TPC, and antioxidant capacity of raw and cooked Chaya leaves.

2. Materials and methods

2.1 Plant material

C. chayamansa leaves are widely grown. The plants were identified by expert botanists and deposited at the Khon Kean University herbarium as voucher specimen KK25547. Chaya was randomly collected from selected areas and planted in gardens in Khon Kaen and Mahasarakham province (northeastern Thailand) in May and June 2019. The samples from both areas were pooled for chemical analysis. The edible leaves were harvested at leaflet and early mature stages (Table 1). The samples were immediately divided for analyses as raw and cooked samples. Dry samples were prepared by boiling at 100°C for 15 min and then cooked to obtain a constant weight in a tray dryer at 60°C [13]. The samples were assessed for variation of nutrient compositions, amino acid profile, protein quality, phenolic compounds, TPC and antioxidant capacity. Sample was thoroughly milled with a laboratory grinder and stored in a plastic bag under vacuum conditions, which raw and cooked samples were kept at around -18°C for further analysis.

Table 1 Description of chaya leaves in this study.

C. chayamansa	Description
Raw leaves	Dark green, shiny leaves with 3-5 lobes (like maple leaves); the first 3-5 pairs of leaves from the top of the plant were collected.
Cooked leaves	Leaves were treated via heat process; they were boiled, then dried.

2.2 Determination of proximate analysis and mineral content

Each sample was analyzed for proximate composition. The moisture content was determined following the Association of Official Analytical Chemists (AOAC) 925.10 method. Samples were dried in a hot air oven at 100±5°C to a constant weight. The ash content was determined by incineration at 550°C (AOAC, 942.05 method). Kjeldahl's method, was used to analyze protein content. Total fat was analyzed using AOAC 922.06 method. Total carbohydrate was calculated by subtracting the sum of moisture, fat, protein and ash percentages from 100%. Mineral content analysis was determined by the AOAC 2013.06 method. All determinations were carried out in triplicate and reported in grams/100gram dry weight samples (g/100g dw).

2.3 Determination of Hydrocyanic acid (HCN)

The HCN assay was performed according to Brimer L et al. with some modification [14]. Approximately 5 g. of grounded raw leaves and 1 g. of cooked leaves were mixed with 10 mL. of extraction media (0.1 M phosphoric acid (H3PO4) containing 25% v ethanol) for 2 min, then were centrifuged at 12,000 rpm for 10 min. An aliquot of 0.1 mL of each sample was mixed with 0.4 mL of 0.1 M phosphate buffer pH 7.0 (0.1 M H_3PO_4 and 0.1 M trisodium phosphate (Na_3PO_4) with adjusted to pH 6.0 and 7.0), then was added and mixed with 0.1 mL of linamarase (Enzyme 5 unit per ml. was dissolved by phosphate buffer pH 6.0). After the mixtures were incubated at 30°C for 15 min. 0.2 M sodium hydroxide (NaOH) (0.6 mL), 0.1 M phosphate buffer pH 6.0 (2.8 mL) was added and followed by 0.5% chloramine-T solution (0.2 mL) were mixed. After that, the mixtures were incubated in the water 0-4°C for 5 min. 0.8 mL of Pyridine/Pyrazolone solution was pipetted into the mixtures, then placed at room temperature for 90 min before absorbance was measured at 620 nm. HCN assays were calibrated by using potassium cyanide (KCN) standards. KCN solution was diluted to containing the concentration between 0.25-2.50 µg KCN. (equilibrium to 0.1-1.0 µg. HCN). Each concentration of KCN solution was added with the same reagent and method as above. The absorbance and concentration of KCN were standardized curves to determine the slope. Triplicate analyses were performed for each sample. The total cyanide was calculated as described by Brimer L et al. in mg HCN / kg dw [14].

2.4 Amino acid profiles analysis

The amino acid (AA) profile was analyzed according to Sawar, et al. with some modifications [15]. An EZ: faast (TM) free amino acid kit was used for AA determination by liquid chromatography and mass spectrometry (LC/MS; Agilent model 1100TM LC). The mobile phases were prepared as follows: (A) the water was added with 10 mm ammonium formate and (B) the methanol was added with 10 mm ammonium formate. The LC/MS was conducted under the following conditions: the flow rate was set as 0.25 mL/min at 35°C. Elution of the standard and samples was achieved with the following solvent gradients: 32% A to 17%A (13.00 min), 17% A to 32 % A (0.01 min) and 32% A isocratic (17.00 min).

2.5 Preliminary determination of the digestible indispensable amino acid (IAA) reference ratio and %DIASS

Digestible indispensable amino acid (DIAA) score of edible raw leaves was expressed as a percentage (%DIASS). The calculation was stipulated by the FAO (2013) as the following equation: DIASS % = 100 x lowest (mg of digestible IAA in 1 g of the sample protein) / (mg of the same digestible IAA in 1 g of the reference protein), which digestible IAA content for each IAA in 1 g of the sample protein were multiplied by the true ileal digestibility coefficient for the same IAA. In this study, the true ileal digestibility values for selected human foods were assumed with other plant values and carried out in accordance with FAO (2013) and Sarwar et al. [16,17] The amino acid pattern of the reference protein to be used for calculating and comparing are as follows: infants (birth to 6 months), young children (≥6 months to 3 years), and older children/adolescent/adults.

2.6 Sample preparation for phenolic compound determination by HPLC

Fresh leaves were harvested, cleaned in water, and stored in a refrigerator at 5°C for analysis within 12 h. Raw samples were ground for 3 min. Pulverized samples were extracted using a minor modification of Kuti and Konuru's methods [12]. Fresh samples (1 g dry basis in triplicate) were combined with 50 mL of methanol/HCl (100:1, V/V) and then mixed by shaker incubator at 150 rpm for 12 h under dark conditions (35°C). After centrifugation for 10 min at 3,000 rpm, the supernatant was filtered through filter paper (Whatman No.4) and evaporated under vacuum until dry (35-40°C). The residue was re-extracted 2 times in 25 mL of methanol/water (50:50, V/V) and filtered on a fine nylon membrane (0.45 μm) before injection (20 μL) into the HPLC system. The cooked leaf samples (1 g dw in triplicate) were prepared in the same as the raw leaves.

2.7 Analysis of phenolic compounds by HPLC

HPLC analysis was performed using a Shimadzu LC-20 AC series HPLC system with an SPD-M20A diode array detector (DAD) and the separation was carried out in a C-18 column (250 mm x 4.6 mm, 5 μm, GL Sciences Inc., Tokyo, Japan). The solvent components and gradient elution conditions were determined according to Kubola and Siriamornpun with some modification [18]. The mobile phase consisted of acetic acid in purified water (pH 2.75) (solvent A) and acetonitrile (solvent B) with a flow rate of 0.8 mL/min. The solvent gradient was performed as follows: 0-5 min, 0-9% B; 5-15 min, 9% B; 15-22 min, 9-11% B; 22-38 min, 11-18% B; 38-43 min, 18-23% B; 43-44 min, 23-90% B; 45-55 min, 90-80% B; isocratic at 80% B, 44-45 min; linear gradient from 80 to 5% B, from 55 to 60 min and re-equilibrium of 5 min with 5% B. The injection volume of sample was 20 μL; the detection wavelength was set 280 nm for hydroxybenzoic acids, 320 nm for hydroxycinnamic acids and 370 nm for flavonoids. The phenolic acids were identified by their retention times and compared with the area of each sample's corresponding peak within the calibration curve.

2.8 Sample extraction for total phenolic content (TPC) and antioxidant capacity analysis

All raw and dried leaves samples were thoroughly milled into 5 g and 1 g samples, respectively. The samples were combined with 50 mL of ethanol and extracted via sonication in a sonication bath (Crest Ultrasonics, Tru-sweep, NJ, USA) at 60°C for 30 min; the mixtures were then shaken for 2 h, then centrifuged at 3,000 rpm for 10 min. The mixtures were filtered through filter paper (Whatman No.1). The residuals were re-extracted twice in 30 mL solvent using the same extraction procedure as described above and the three fractions were combined. All samples were kept at -18°C until these were analyzed.

2.9 Determination of TPC

Total phenolic content was determined with Folin-Ciocalteu reagent according to Dewanto, et al.'s method with some modification. The reaction mixture contained 125 μL of extract (with appropriate dilution) and 250

μL Folin-Ciocalteu reagent; 3 mL distilled water was added and mixed, and after 6 min, 2.5 mL of 7% Na_2CO_3 solution was added. Finally, the reaction mixture was left for 90 min at room temperature before absorbance was measured at 760 nm (Shimadzu UV-1800 spectrophotometer, Japan). The result was expressed as mg of gallic acid equivalents (GAE)/100 g sample dw.

2.10 Determination of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging

DPPH• scavenging activity was determined according to the method reported by Loarca-Pina, et al. 's method with minor modification. A solution of 0.1 mm methanol solution of DPPH• was newly prepared with 1.0 AU at 517 nm. An aliquot of 100 μL of each sample (with appropriate dilution) was mixed with 4.0 mL of DPPH• solution, and incubated in darkness at room temperature for 30 min. The antioxidant capacity was expressed as mg Trolox equivalent/100 g sample dw.

2.11 Determination of 2,2' – Azinobis(3-ethylbenzothiazoline-6-sulphonic) ($ABTS^{\bullet+}$) scavenging

Investigation of the samples' ability to scavenge ABTS•+ free radicals was conducted according to the procedure described by Wootton-Beard, et al. 's method with little modification. For the standard assay, ABTS•+ was generated by reacting ABTS stock solution (7 mm) with K2O8S2 (4.95 mm) at a ratio of 1:1(v/v), with the mixture left at room temperature in the dark for 12 h before use. The ABTS•+working solution was diluted with phosphate buffer saline (PBS, pH 7.4) until the absorbance was 1.0 AU at 734 nm. Reaction mixtures contained 40 μL sample extracts (with appropriate dilution) and 4 mL working solution and were left to stand for 10 min before measurement. The result was expressed as mg Trolox equivalent/100 g sample dw and calculated from a Trolox calibration curve.

2.12 Determination of ferric reducing ability of power (FRAP)

Benzie and Strain's FRAP assay method was performed with slightly modifications. The working FRAP reagent was prepared by mixing 1 volume of TPTZ (10 mm) solution in HCl (40 mm) with 1 volume of $FeCI_3 \bullet 6H_2O$ (20 mm) and 10 volume of acetate buffer pH 3.6 (300 mm) (3.1 g sodium acetate and 16 mL acetic acid/liter). Subsequently, 150 μL sample extracts (with appropriate dilution) were added to 2.9 mL FRAP reagent and then were placed for 30 min in the dark. The mixture was performed at the absorbance at 593 nm and was expressed as mg $FeSO_4$ equivalent/100 g sample dw. A calibration curve standard was prepared with fresh $FeSO_4$.

2.13 Statistical analysis

All measurement values were conducted in triplicate and data were expressed as mean ± standard deviations (SD). Mann-Whitney test was applied to interpreted statistically significant at $p \leq 0.05$. and was performed using statistical software (SPSS 19.0, SPSS Inc., USA).

3. Results and discussion

C.chayamansa (Chaya) is a leafy green vegetable used as food (especially in South America and Africa) that draws wide interest due to its in phytochemical content. Recently, Chaya has been grown in Asian countries such as Thailand, which has a subtropical climate. Both raw and cooked leaves, the latter of which are safe for consumption, were examined for nutrient composition and other food components (Table 2). Only raw leaf moisture was expressed by fresh weight (fw). Other values were carried out using dry weight (dw).

Table 2 The chemical compositions in raw and cooked leaves of *C. chayamansa*.

Chemical Compositions (g/100 g)	Raw	Cooked
Moisture	82.86 ± 0.25*	8.17 ± 0.02*
Dry matter	17.14 ± 0.23	91.83 ± 0.03
Ash	10.45 ± 0.16*	7.04 ± 0.01*
Protein	34.02 ± 0.84*	32.41 ± 0.50*
Fat	5.63 ± 0.49*	8.46 ± 0.21*
Carbohydrate	49.91± 1.48*	43.92 ± 0.70*
Caloric value**	386.39	381.46
Potassium	3.46 ± 0.07*	1.56 ± 0.04*
Sodium	0.32 ± 0.03*	0.12 ± 0.01*
Phosphorus	0.53 ± 0.07*	0.33 ± 0.02*
Other (mg/kg)		
HCN	1121.72± 22.46*	1.71± 0.04*

* Statistically significant at $p \leq 0.05$ in the same row to denote significant difference between raw and cooked leaves and the values [Nutrient composition (mg/100g dw)], [Other(mg/kg)] was expressed as mean ± standard deviation of triplicate measurement. ** The unit of Caloric value = Kcal/100 g dw.; NA = not applicable

The plant samples were heated by boiling and then dried to assess their nutrient content as actual consumption. There are many differences between the nutrient composition of raw and cooked Chaya leaves. The moisture content of the cooked leaves was reduced to 8.17% dw, which extends shelf life and prevents microbial spoilage. Raw leaves were found to contain significantly higher protein, carbohydrate and ash content than cooked leaves ($p \leq 0.05$). Raw and cooked leaves contained 34.02% and 32.41% protein, 49.91% and 43.92% carbohydrates, and 5.63% and 8.46% of fat, respectively, based on dry weight. The results of this study are similar to the finding of Traoré K, et al. [13] that demonstrated how food processing affects the nutrient content. In their study of amaranth (Amaranthus cruentus L.), another leafy green vegetable, they found fresh, boiled and dried (at 60°C) leaves had a protein content of 25.68, 25.94 and 23.62%, and a carbohydrate content of 25.68, 46.69 and 12.96% respectively, by dry weight.

The results of this study also found that the macronutrient content of Chaya by fresh weight (fw), including protein, carbohydrates, and fat (5.83%, 8.56% and 0.96%, respectively), differed by planting area. Previous studies found that macronutrients in Chaya plants in African countries ranged from 3.6 to 5.2% protein, 4.00 to 8.52% carbohydrate and 0.47 to 1.90% fat [6,9,19,20]. These differences might be due to the plant age, environment, and harvest method. In comparison with other fresh leafy vegetables, Chaya had considerably higher protein content than other fresh leafy vegetables such as spinach (2.99% ww) and kale (5.12%ww), as reported by Agarwal, et al. [21], or amaranth (4.02%ww), as reported by Traore, et al. [13]. Previous studies have been reported that Chaya is also attractive as other sources of protein are considered "incomplete." The Chaya plant may be useful in areas facing a shortage of protein sources [2,4,5].

According to the results of this study, the cooked leaves contained 1.56 potassium (K), 0.12 sodium (Na) and 0.33 phosphorus (P) g/100 g dw, which was two times lower than the mineral content of fresh leaves (3.46, 0.32 and 0.53 g/100 g dw., respectively) (Table 2). It can be assumed that any culinary or technological preparation, especially the use of heat such as boiling, blanching, and drying, may result in the plant's loss of mineral elements. In this study, the plants were heat-treated by boiling and drying to eliminate undesirable substances, then dried into a dry powder that was easy to store and use for other purposes. The mineral content of raw Chaya leaf in this study was higher than that found in other areas, as reported by Jimenez-Aguilar and Grusak (1.84 K, 3.70 P g/100 g dw, and 0.14 mg/100 g dw) [8]. The mineral content of K, Na and P in Chaya leaves was lower than that of dried spinach leaves, as reported by Gedi, MA et al.(12.95, 0.64 and 0.87 g/100 g dw.); this may be due to plant type and heat treatment processes. In general, green and dark green leafy vegetables had higher K content than other vegetables such as spinach (5.84) and kale (2.77 g/100 g dw) [22]. The mineral content may vary according to the soil minerals and proportions of individual mineral absorption by each plant. In addition, these minerals are electrolytes that the body needs in appropriate amounts.

Other composition such as hydrocyanic acid (HCN), were presented in Table 2. The raw Chaya leaves contained 1121.72 mg/kg dw. As for cooked leaves, HCN was also significantly ($p \leq 0.05$) eliminated by more than 99% (1.72 mg/kg dw.). This finding showed that cooking processes could significantly reduce the toxicity of this substance. The HCN content of Chaya leaves has been reported as 2.50 mg/kg dw. [12]. The difference in the amount of this substance depends on many factors such as plant type, maturity, planting area, and climate. This study agreed with the previous studies, which demonstrated that some leafy vegetables such as Asystasia gangetica, Ceratotheca triloba, and Physalis viscosa had significantly different decreases in HCN after 15 min of boiling [23]. However, cyanide released from cyanogenic glycosides can generate hydrocyanic acids (such as cyanide and prussic acid) via beta-glucosides in food; in case HCN enters the body in small amounts, HCN is detoxified by the Rhodanase enzyme to form Thiocyanate (SCN), which becomes less toxic before it is excreted

in the urine. In addition, a Center for Disease Control and Prevention (CDC) fact sheet reported that natural cyanide is also found in some plants such as cassava, lima beans, almonds and apricot seeds. For some plant, elimination of toxin is an essential purpose for safe consumption [1,12].

Total amino acid of raw and cooked leaves was represented as Figure 1 and found between 27.09 and 20.66 g/100 g dw., respectively. Among total amino acids, the essential amino acids in raw and cooked leaves accounted for 36.54 and 40.45%. The results of this study show that the amino acid contents of Chaya vegetables were quite different from those found in the previous study. Victor M (2016) indicated that the total amino acids as 81.67 g/100 g protein in dried Chaya leaves in Africa, while the proportion of essential amino acids was 42.77% [7]. The variations in quantity of each amino acid may be due to planting area, atmosphere, and post-harvest handling methods. The existence of 9 essential amino acids in both raw and cooked Chaya leaves were found in Chaya vegetable. It is notable that humans cannot synthesize essential amino acids but must consume them through the diet [23]. Amino acid content alterations were affected by thermal decomposition. Moreover, heated leaves had increased amounts of essential amino acids (including histidine, isoleucine, leucine and valine) and non-essential amino acids (including alanine, glycine, proline, tyrosine, and glutamine). Chemical composition changes were observed in vegetables resulting from culinary methods such as boiling, blanching, and drying. In this study, the plant samples were treated by boiling in water and drying with a tray dryer at a moderate temperature (60°C) to get rid of undesirable substances before actual intake. The study described above noted that amino acid variations might be related to compactness and density of vegetable tissue and solubility in water. In addition, the increase in amino acids could be associated with high decomposition of protein fractions or degradation products of lipids in Stecker's reaction and the Maillard sugar process [15,24].

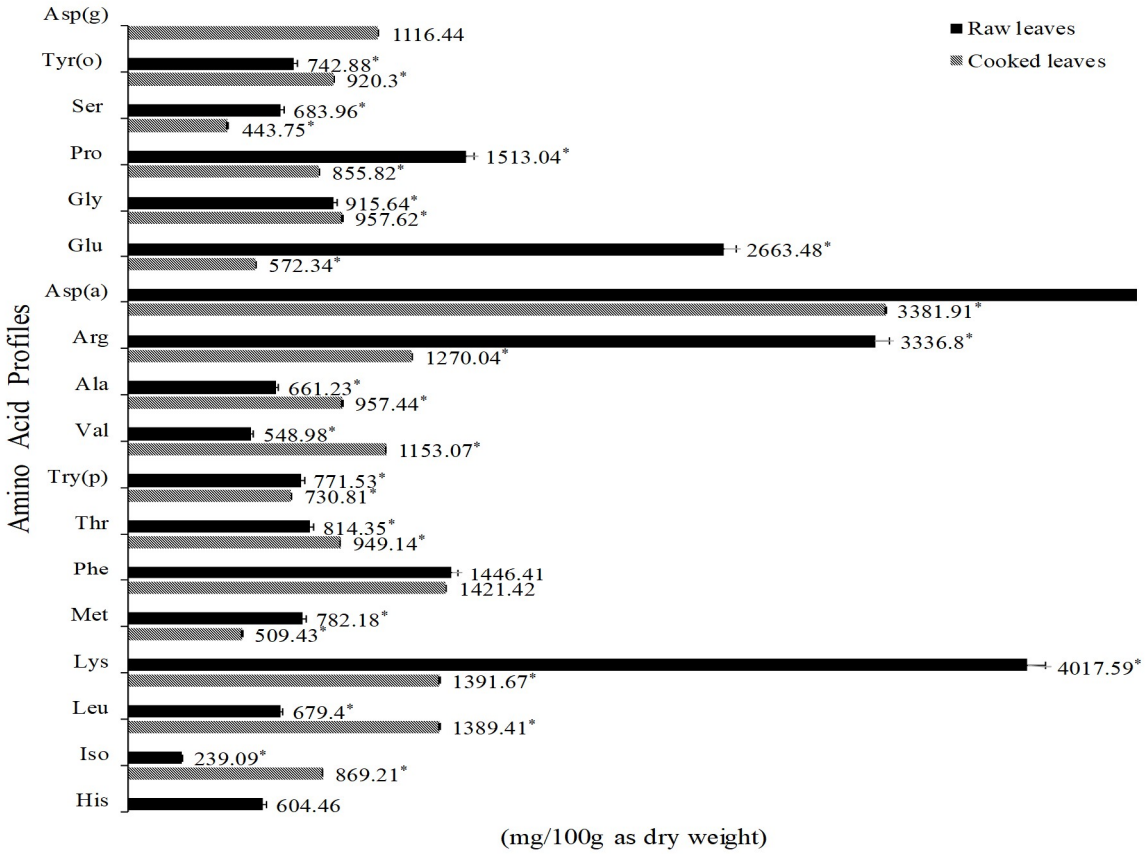

Figure 1 The amino acid profiles in the raw and cooked leaves of *C.chayamansa*. *Statistically significant at $p \leq 0.05$ comparing raw and cooked and the values. Data were reported as mg/100 g dw.

This study performed a preliminary evaluation of DIAAS to describe the protein quality of plant leaves. These values were calculated in accordance with the Food and Agriculture Organization (FAO) recommendation and the results shown in Table 3. DIAAS were calculated based on the true ileal digestibility of each amino acid with reference to other plants and were used the amino acid pattern of the reference protein as follows infants, infants to older children, and adolescents to Adults [16,25,26], the Chaya leaves had a DIAAS range of 12-22%, with isoleucine as a limiting amino acid. In addition, the previous studies reported a DIAAS

for other plants, including wild vegetable leaves such as Pandiaka heudelotii (47.60-68.40% with lysine as the limiting amino acid) [24], seitan and wheat protein (20.00-31.00% with lysine as limiting amino acid) [25] and pea protein (45.00-84.00%, with the limiting amino acids of sulfur, phenylalanine, and tyrosine) [27]. These findings show that Chaya has a high protein content, however, the quality of protein is lower than that of other plants or meat protein when the imbalance of essential amino acids is considered.

Table 3 Preliminary result of the digestible indispensable amino acid (IAA) score of raw *C.chayamansa* leaves.

Amino acid	g/100g sample	mg/g protein	DIAAS reference protein ratio (mg/g protein) */**		
			Infant	Child	Older child, Adolescent, Adult
Histidine	0.10	17.77	0.80	0.84	1.06
Isoleucine	0.04	7.03	0.12	0.20	0.22
Leucine	0.12	19.97	0.20	0.29	0.31
Lysine	0.69	118.01	1.62	1.97	2.34
Threonine	0.14	23.84	0.50	0.71	0.88
Tryptophan	0.13	22.71	1.28	2.56	3.30
Valine	0.09	16.12	0.28	0.36	0.38
Methionine+Cysteine	0.13	23.00	0.64	0.78	0.92
Phenylalanine+Tyrosine	0.38	64.20	0.66	1.19	1.50
DIAAS (%)			12 (ILE)	20 (ILE)	22 (ILE)

*Ratios were calculated using the recommended amino acid scoring patterns for three age groups: infant (birth to 6 months), child (6 months to 3 years) and older child (with adolescent and adult) (FAO, 2013).
**DIAAS were multiplied by the true ileal digestibility coefficient based on some plants (FAO, 2013).

A fraction of both Chaya leaves samples was separated and quantified for their phenolic acid and flavonoid content by HPLC-DAD. All data on these compounds were listed in Table 4. This study found that sinapic acid (7.85 ± 0.21 mg/g dw), caffeic acid (5.62 ± 0.28 mg/g dw), and p-hydroxybenzoic acid (1.04 ± 0.10 mg/g dw) were the major soluble phenolic acids in the fresh leaves extract, accounting for 47%, 33% and 6%, respectively, while caffeic acid, syringic acid, and sinapic acid were the major phenolic acids in cooked leaves extract, accounting for 51%, 28% and 14%, respectively. In both raw and cooked Chaya leaves, the minor phenolic acids were gallic acid and ferulic acid (0.05 ± 0.004 and 0.05 ± 0.03 mg/g dw, respectively). In addition, myricetin and apigenin (3.82 ± 0.13 and 3.78 ± 0.19 mg/g dw, respectively) were the most abundant flavonoids in raw leaves, accounting for 42%, while cooked leaves contained three flavonoids: myricetin, apigenin, and rutin (1.92, 1.51 and 1.23 mg/g dw, respectively). The main phenolic compounds of this study were sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid), and hydroxybenzoic acid, which belong to a family of phenolics with remarkable antioxidant potential such as the ability to scavenge stable radicals or to scavenge the highly reactive hydroxyl radicals and other free radicals (hydroperoxyl radicals, nitric oxide radicals, peroxynitrite) by suppressing lipid peroxidation.

Table 4 Phenolic acid and flavonoid content of *C. chayamansa* methanol extract.

Chemical Formula		Raw	Cooked
Phenolic compounds (mg/100 g dry weight)*			
Gallic acid	$C_7H_6O_5$	4.92 ± 0.16*	2.34 ± 0.08*
Protocatechuic acid	$C_7H_6O_4$	67.98 ± 1.94*	20.25 ± 1.10*
p-hydroxybenzoic acid	$C_7H_6O_3$	104.28 ± 9.90*	18.01 ± 1.89*
Chlorogenic acid	$C_{16}H_{18}O_9$	82.14 ± 4.61*	7.31 ± 1.64*
Caffeic acid	$C_9H_8O_4$	561.71 ± 27.66*	406.98 ± 16.55*
Syringic acid	$C_9H_{10}O_5$	70.71 ± 5.10*	225 ± 8.77*
p-coumaric acid	$C_9H_8O_3$	3.86 ± 2.04*	6.75 ± 1.73*
Ferulic acid	$C_{10}H_{10}O_4$	5.25 ± 2.58*	0.86 ± 0.02*
Sinapic acid	$C_{11}H_{12}O_5$	784.50 ± 20.76*	112.50 ± 6.88*
Flavonoid compound (mg/100 g dry weight)*			
Rutin	$C_{27}H_{30}O_{16}$	52.00 ± 0.06*	123.03 ± 6.99*
Myricetin	$C_{15}H_{10}O_8$	381.54 ± 12.84*	192.26 ± 7.48*
Quercetin	$C_{15}H_{10}O_7$	95.36 ± 3.11*	9.55± 0.34*
Apigenin	$C_{15}H_{10}O_5$	377.57 ± 18.84*	151.50 ± 19.11*

*Statistically significant at $p \leq 0.05$ in the same row to denote significant difference between raw and cooked leaves. The values (mg/100g dw) were expressed as mean ± standard deviation of triplicate experiments.

This study found that cooked leaves have a significant decrease in the individual phenolic and flavonoid compounds from the raw leaves ($p \leq 0.05$) (Table 4). Previous studies have reported that flavonoid compounds in raw and cooked Chaya leaves extract exist in the form of kaempferol and quercetin; cooking leads to reduction of the total flavonoid content in samples about 13-17% [3]. Conversely, several studies have reported that differences in extraction processes tend to increase the phenolic content with elevated temperatures, such as the study of Godinez-Santillan et al. (2019) has reported that the methanol or ethanol extract from thermal treatment (boiling the leaf in water only 5 min) was obtained with greater contents in the thermal leave extraction [11]. The loss of compound in this study may be caused by heat-treated leaves by boiling for up to 15 min prior to drying to ensure removal of unwanted substances. As the result, this seemed to contribute to large phenolic and flavonoid compound losses.

However, the previous HPLC analysis showed that the main phenolic and flavonoid compounds were slightly different from this study in terms of using many solvent extraction types. For example, the phenolic group consisted of rosmarinic acid, chlorogenic acid, and 4-hydroxybenzoic acid (26.89, 8.67, and 8.13 mg/g fw, respectively) and the flavonoid group consisted of epigallocatechin gallate, hesperidin, and vanillin (27.42, 16.25, and 11.38 mg/g fw, respectively) extracted in aqueous [5] or coumaric acid and quercitrin (4.08 and 6.94 mg/g dw, respectively) were extracted in ethyl acetate fraction [28] or coumaric acid, chlorogenic acid and resveratrol (1843, 1272, and 1263 mg/100 g, respectively) were extracted in lyophilized extract [29].

Table 5 Total phenolic content and antioxidant capacity of raw and cooked leaves extract of *C. chayamansa*.

Samples	Raw	Cooked
Total phenolic content		
TPC (mg gallic eq/g_{dw})	$22.91 \pm 0.23^{*}$	$10.81 \pm 0.80^{*}$
Antioxidant capacity		
FRAP (mg $FeSO_4$ eq/g_{dw})	$112.97 \pm 2.33^{*}$	$42.20 \pm 3.18^{*}$
ABTS (mg Trolox eq/g_{dw})	$29.35 \pm 0.39^{*}$	$12.95 \pm 0.37^{*}$
DPPH (mg Trolox eq/g_{dw})	$10.72 \pm 0.24^{*}$	$3.52 \pm 0.14^{*}$

*Statistically significant at $p \leq 0.05$ in the same row to denote significant difference between raw and cooked leaves. The values were expressed as mean ± standard deviation of triplicate experiments.

The results of TPC and antioxidant capacity from the raw and cooked leaves extracts are shown in Table 5. The cooked leaves extract had significantly lower TPC (10.81 mg gallic eq./g dw) than raw leaf extract (22.91 mg gallic eq./g dw) ($p \leq 0.05$). The results from FRAP, ABTS, and DPPH assays showed that the antioxidant capacity of the cooked leaves sample was 50% lower than that of raw leaves. This result agreed with the findings of Kuti and Konoru reported that the phenolic content and antioxidant capacity of Chaya vegetables were reduced when heated with a microwave [3] and the study of Babalola and Alabi found that TPC decreased by 50% after heat-treats by blanching and boiling [9]. However, this study showed different results from some research, which indicated that TPC increases when plants were cooked, as Godinez-Santillan R.I. et al. reports. They also found that an ethanol extract of boiled Chaya significantly increased TPC and antioxidant capacity when measured by DPPH, ABTS and TEAC methods [11]. Therefore, the effects of heat-treats process and other factors such as temperature and time, that may increase or decrease TPC as well. Additionally, heat-treats process can affect the plant cell walls, causing their rupture and the release of chemicals or the loss of heat-sensitive antioxidants [9,10,30]. The results indicated that the TPC of fresh Chaya extract in ethanol (2.88 mg gallic eq/g fw., data not shown) was lower than that reported by Ramos-Gomez, et al., (3.35 mg gallic eq/g fw., of the water extract) [5] and Jimenez-Aguilar and Grusak,[8] , which indicated TPC of C. aconitifolius and other green leafy vegetables, including Solanum scabrum and Crotalaria longgirostrata (3.30, 2.76 and 2.36 mg gallic eq./g fw, respectively). The differences in phytonutrient content in plant specimens may depend on plant type, extraction method, extract solvent, planting area, crop age and post-harvest handling [19,30].

4. Conclusion

Chaya is a leafy vegetable from northeastern Thailand demonstrated that are rich in nutrients, especially protein, carbohydrate, and amino acids. However, the preliminary protein quality was shown to be less than animal protein. Cooking may affect the reduction of nutrients and metabolites such as phenolic compounds and antioxidant properties. However, cooking has made the consumption of this plant safer by eliminating toxins such as HCN. Consequently, this plant may be useful in further studies of food and folk medicine.

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6. Conflicts of interest

The authors declare no conflicts of interest.

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