Development of Phenolic-and-Flavonoid-containing Sugar-Free Jelly from Chaya (*Cnidoscolus aconitifolius*) Leaves and Evaluation of Physical and Nutritional Properties

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Abstract

The objective of this study was to develop sugar-free jelly from Chaya (*Cnidoscolus aconitifolius*) leaves and to evaluate its phenolic and flavonoid contents, physicochemical characteristics, and nutritional properties of the selected formula. Dried Chaya leaf powder's cyanide content and physicochemical properties were determined and evaluated. The results showed that the green-yellow Chaya leaf powder contained a cyanide level less than 0.20 mg/L. The water activity and percent moisture of Chaya leaf powder were below the standard criteria established for dry leaf powder. Different formulas of jellies were developed using various amounts of Chaya leaf powder ranging from 0.50 to 2.00 g. The control formulation was prepared using one gram of green tea powder instead of the Chaya leaf powder. The jellies were evaluated for their physical properties, including color and texture. The color of Chaya leaf jellies had a brightness (L^*) ranging from 31.62 to 33.58 with green (-3.61 to -5.14) and yellow (5.37 to 7.16). In terms of texture, the hardness of Chaya jellies ranged from 560.00 to 966.33 g. Total phenolic content was determined using the Folin-Ciocalteu and the Aluminum Chloride method was used to determine the flavonoid contents. The flavonoid content of the developed jellies ranged from 1,432.33 to 1,525.00 mg QE/20 g of sample, which was not significantly different from the control formula (1,520.33 mg QE/20 g of sample). The total phenolic content of the control formula (1.43 mg GAE/20 g of sample), Formula 3 (1.35 mg GAE/20 g of sample), and Formula 4 (1.45 mg GAE/20 g of sample) did not differ significantly ($p \leq 0.05$). Therefore, Formula 3 (1.5 g of Chaya leaf powder) was selected to study the nutritional properties. The Formula 3 (2.91 kcal per 100 g of sample) showed a 93% reduction in energy compared to the control formula (43.465 kcal per 100 g of sample). Therefore, Formula 3 represented a low-energy food source that could potentially be further developed into a commercial product.

Keywords: Jelly, Chaya (*Cnidoscolus aconitifolius*) leaves, Physicochemical properties, Phenolic content, Flavonoid content, nutritional properties

Introduction

Awareness of the role of diet in health is growing, driving a significant surge in demand for food products that are rich in antioxidants. This growing demand is largely driven by rising concerns over diseases associated with oxidative stress, which is caused by free radicals in the body. Free radicals are unstable molecules that can damage cells, proteins, and DNA, contributing to the development of various diseases, including cancer, cardiovascular disease, and neurodegenerative disorders (Rahman et al., 2022; Mutha et al., 2020). Antioxidants are compounds that neutralize free radicals, thereby protecting the body from their harmful effects.

Among the most studied antioxidants are flavonoids and phenolic compounds, both of which are abundant in fruits and vegetables (Djenidi et al., 2020; Gu et al., 2019). These compounds have gained significant attention in the fields of nutrition and food science due to their potential health benefits. Studies have demonstrated that regular consumption of foods rich in phenolic and flavonoid compounds can reduce the risk of chronic diseases, including cancer, diabetes, inflammation, and cardiovascular diseases, as well as mitigate the effects of neurodegenerative disorders like Alzheimer's and Parkinson's disease (Aryal et al., 2019; Al-Snafi, 2020). Research continues to support the notion that plant-based foods, which are naturally high in antioxidants, play a crucial role in improving overall health and preventing disease. These findings have contributed to an increase in consumer demand for plant-based products, as people seek to incorporate more nutrient-dense and antioxidant-rich foods into their diets. With this shift in consumer preferences, the food industry has witnessed the rise of functional foods and supplements, many of which focus on promoting the antioxidant properties of fruits, vegetables, and other plant-based ingredients (Altun & Aydemir, 2022). Given these findings, the interest in antioxidants, especially flavonoids and phenolics, will continue to grow as part of a larger movement towards healthier eating habits and preventive healthcare.

Green leafy vegetables are a rich source of nutrients, phytochemicals, and health-promoting components. *Cnidoscolus aconitifolius*, commonly known as Chaya or Tree Spinach, belongs to the Euphorbiaceae family (Kuri-Garcia, 2019). In Thailand, Chaya leaves are consumed as a food source, often in stir-fried dishes. Chaya is reported to have numerous medicinal properties, including antidiabetic, anti-inflammatory, analgesic, antihypertriglyceridemic, anticholesterolemic, neuroprotective, hepatoprotective, antimicrobial, hematopoietic, and antifertility effects, as well as its ability to improve erectile dysfunction and exhibit hypoglycemic effects (Bautista-Robles et al., 2020; Moura et al., 2019). Chaya leaves are rich in nutritional and phytochemical compounds, such as vitamins, minerals, proteins, carbohydrates, fatty acids, phenolics, flavonoids, tannins, terpenoids, alkaloids, saponins, and chlorophyll (Panghal et al., 2021; Gustiar et al., 2023). Additionally, Chaya contains antioxidants like coumarins, flavonoids, phenolics, tannins, anthraquinones, triterpenoids, flabotonins and kaempferol (Nnadiukwu & Nnadiukwu, 2024; Kuri-Garca et al., 2017). However, despite its beneficial properties, caution is necessary when consuming Chaya leaves in their raw form. The leaves naturally contain hydrocyanic acid (HCN), a potentially toxic compound that can cause adverse effects if consumed in large amounts or over extended periods. The toxicity of HCN can lead to symptoms such as headaches, nausea, and dizziness, and in severe cases, it can result in cyanide poisoning. To safely consume Chaya, it is recommended that the leaves be cooked for at least five minutes to break down the cyanogenic compounds and eliminate the harmful effects of HCN (Jaroennon & Manakla, 2021). Given its broad spectrum of nutrients and bioactive compounds, Chaya is increasingly considered a promising candidate for use in food applications aimed at improving public health.

The confectionery industry offers a vast array of products, many of which are commonly consumed as part of daily life. Among these products, jellies stand out due to their popularity, offering a variety of flavors and textures that appeal to a wide range of consumers. Traditionally, jellies have been made with high amounts of sugar, providing a sweet, satisfying treat, and sugars comprise 70-85% of the total content (Plotnikova et al., 2022). This high sugar content is a major concern, particularly as the global population becomes more health-conscious and increasingly aware of the risks posed by excessive sugar intake. To address these concerns, there has been a shift toward developing low-sugar or sugar-free jelly formulations that incorporate sugar substitutes such as erythritol, xylitol, sucralose, oligofructose, and *Stevia rebaudiana* extracts (Orbulescu et al., 2023). These sugar alternatives provide sweetness without the high calories and glycemic load associated with traditional sugar, making them appeal to individuals looking to reduce their sugar intake while still enjoying sweet treats. As sugar-free confectionery products primarily focus on sugar reduction, the development of nutrient-enriched jelly formulations offers a unique advantage by integrating both sugar reduction and enhanced micronutrient intake from natural sources. This dual benefit addresses both the desire of health-conscious consumers to reduce their sugar intake and the broader problem of nutritional deficiencies in certain populations. The rising demand for healthier confectionery options is not just limited to the desire for reduced sugar content. Consumers are increasingly seeking jelly products enriched with bioactive compounds that provide potential health benefits (Gunes et al., 2022). These bioactive compounds, such as antioxidants, phenolics, and flavonoids, are known for their protective effects against chronic diseases like heart disease, diabetes, and certain cancers (Gunes et al., 2022). The incorporation of these health-promoting ingredients into jelly products not only enhances their nutritional value but also responds to the rising demand for functional foods that support overall well-being. Additionally, there is also a growing awareness of the nutritional gaps in many diets. For example, in Thailand, a national survey found that the population consumes fewer vegetables than the World Health Organization (WHO) recommendations (Supachai et al., 2020). This indicates a significant opportunity for food developers to create functional foods, like nutrient-enriched jellies, that can help bridge the gap between the food people consume and the nutrients they need for optimal health. In addressing this issue, innovative strategies to enhance vegetable intake are crucial. Transforming leafy vegetables into convenient, easy-to-consume formats, such as jelly, represents a promising approach.

This study aimed to develop sugar-free jelly from Chaya (*Cnidoscolus aconitifolius*) leaves and to evaluate the phenolic and flavonoid contents, physicochemical characteristics, and nutritional properties of the selected formula. Through this research, we sought to contribute to the development of novel functional food products that promote healthier dietary habits. Our findings highlight the potential of Chaya-infused sugar-free jelly as a nutritious and functional food option, offering a valuable contribution to the field of food science and nutrition.

Materials and Methods

Preparation of Materials

Chaya leaves were thoroughly washed and then air-dried. Subsequently, they were dried in a hot air oven at 60 °C for 24 hr. Commercial green tea leaves were used as the control. The dried Chaya and green tea leaves were finely ground using an automatic grinder and sieved through a 50-mesh sieve to ensure uniform particle size.

Preliminary formulation

Preliminary palatability assessments were conducted to evaluate the efficacy of various ingredient combinations in optimizing the ratios of agar and sugar. The tested formulations included varying concentrations of sugar (20, 50, and 80 mg/500 mL of water), gelatin (1, 3, and 5 mg/500 mL of water), and carrageenan (1, 3, and 5 mL/500 mL of water), with results undisclosed. These combinations were analyzed to ensure acceptable sensory attributes. After establishing the optimal agar-to-sugar ratio, the concentration of green tea powder was adjusted (1, 5, and 15 g). Given that 1 gram of green tea powder had the highest preference score, and Chaya leaf powder tastes like green tea, 1 gram of Chaya leaf powder was initially used. This amount was then adjusted to 0.5, 1, 1.5, and 2 grams.

Evaluation of material properties

Cyanide detection in Chaya leaf powder was performed using Cyantesmo test paper (Macherey-nagel, Germany). Two grams of Chaya leaf powder were boiled in 500 mL of water (2 g/500 mL). One drop of 95% concentrated sulfuric acid (Qrec, New Zealand) was added to the solution and shaken immediately. Hydrocyanic acid gas was formed and could be detected at the boundary layer between the water and air. After shaking, 10 cm of Cyantesmo test paper was immersed in the acidified solution for 15 minutes. The color reaction on the pale green test paper changed to pale blue and dark blue depending on the concentration of hydrocyanic acid, which corresponds to cyanide levels. The method has a detection limit of 0.20 mg/L (0.20 ppm) for cyanide.

The physicochemical properties of the Chaya leaf powder and the green tea powder were assessed for moisture content, water activity, and color using appropriate instruments. The moisture content was determined with a moisture analyzer (MA37; Sartorius, Germany) at 105 °C in fully automatic mode, with approximately 5 grams of each sample powder weighed for analysis. Water activity was measured using a water activity meter (LabSwift-aw; Novasina, Switzerland) in auto-start mode, with sample powders placed in a sample dish of the analyzer. Color properties were evaluated using a colorimeter (Chroma Meter CR-400; Konica Minolta, Japan) calibrated with a white plate (CR-A43; y = 85.70, x = 0.3177, y = 0.3340) in DP mode. The analyzed color parameters included L^* (lightness, 0 = black to 100 = white), a^* (redness/greenness, with negative values indicating greenness and positive values indicating redness), and b^* (yellowness/blueness, with negative values indicating blueness and positive values indicating yellowness). All measurements were performed in triplicate, and the results were reported as % moisture, water activity (aw), and L^*, a^*, b^* values, respectively.

Preparation of Jelly formulations

The jelly formulas were prepared using Chaya leaf powder and green tea leaf powder in ratios as shown in Table 1. In brief, 100 mL of water at 60 °C was gradually mixed with either Chaya leaf powder (0.5 - 2 g) or green tea leaf powder (1 g). Then, 5 g of carrageenan and 3 g of gelatin dissolved in 10 mL of water were added. The 0.08 g of sucralose (The sweetness is 600 times that of sugar) was included in all formulations except for the control, which contained 50 g of sugar. After thorough mixing, 390 mL of water heated to 97-100 °C was added and stirred continuously for 5 min. Finally, 20 mL of the mixture was poured into molds and left at room temperature for 15 min before being refrigerated at 4 °C for 1 hr.

Table 1 Formulas of Jelly with Chaya Leaf Powder and Green Tea Leaf Powder (500 g per Formula)

	formulations				
Materials	**Control**	**1**	**2**	**3**	**4**
Chaya leaf powder (g)	-	0.5	1	1.5	2
green tea leaf powder (g)	1	-	-	-	-
Gelatin (g)	3	3	3	3	3
Carrageenan (g)	5	5	5	5	5
Sucralose (g)	-	0.08	0.08	0.08	0.08
Sugar (g)	50	-	-	-	-
Water (mL)	500	500	500	500	500

Physical property analysis of jellies

Color analysis

The color of the jelly formulations was analyzed using a Chroma Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan). The instrument was calibrated with a white plate (CR-A43) and operated in DP mode. Measurements were performed in triplicate, and the analyzed color parameters (L^*, a^*, b^*) were recorded as described above.

Texture analysis

The texture analysis of the jelly formulations was conducted using a CT3 Texture Analyzer (Ametek Brookfield, Middleborough, MA, USA) equipped with a TA25/1000 Cylinder probe (5 cm diameter). The analysis was conducted using the Texture Profile Analysis (TPA) mode with the following settings: distance of target type, 15 mm of target, 20 g of trigger loadg, 1.0 mm/s of test speed, 1 mm/s of return speed, and 2 of cycle count. Parameters including hardness, cohesiveness, springiness, gumminess, and chewiness were measured. Each measurement was performed in triplicate.

Determination of phenolic and flavonoid contents of jellies

Sample preparation

Twenty grams of each jelly formula were blended with 20 mL of water to form a homogeneous mixture. The mixtures were then filtered through filter paper (Susuki Coffee, USA). The solutions were used as samples to measure the phenolic contents using the Folin-Ciocalteu method and the flavonoid contents using the Aluminum Chloride method.

Folin-Ciocalteu method

The Folin-Ciocalteu method was performed as described by Jaroennon et al. (2021) to determine the jellies' phenolic content. A gallic acid standard (20-200 µg/mL) was prepared. A 25 µL aliquot of the gallic acid standard (Sisco Research Laboratories Pvt. Ltd., India) or 25 µL of sample solutions were mixed with 75 µL of distilled water and 25 µL of 0.2 N Folin-Ciocalteu reagent (Sisco Research Laboratories Pvt. Ltd., India). After mixing, the solution was left for 6 min, and 100 µL of 75 g/L Na_2CO_3 (Ajax Finechem Pty Ltd, Australia) was then added. The solutions were then incubated in the dark at room temperature for 90 min. 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 was calculated using the gallic acid standard calibration curve ($y = 0.0018x + 0.0514$, $R^2 = 0.9958$). Total phenolics were expressed as mg GAE per 20 g of sample.

Aluminum Chloride Method

The Aluminum Chloride Method was modified from Jaroennon et al. (2021) to determine the jellies' flavonoid content. A 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 to the mixture, which contained 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 min at room temperature. Then, 200 µL of the mixture was pipetted into 96-well plates, and absorbance was read at 415 nm using a microplate reader. Each sample solution was analyzed in triplicate. The flavonoid content of the samples was measured against the quercetin standard calibration curve ($y = 0.0015x + 0.036$, $R^2 = 0.9932$). The result was expressed as mg QE per 20 g of sample.

Nutritional Calculation

The control sample and selected sample were calculated for nutritional values using the nutritional analysis program (INMUCAL-Nutrients version 4.0, Institute of Nutrition, Mahidol University). The nutritional values include energy, carbohydrate, sugar, fiber, protein, fat, phosphorus, calcium, iron, sodium, potassium, magnesium, vitamin C, and carotenoids. The obtained data were reported per 20 g of jelly.

Data analysis

Statistical analysis using one-way ANOVA was conducted to assess changes in phenolic and flavonoid contents, as well as the physical properties of the jellies. Paired t-tests were used to analyze changes in the physical properties of the leaf powder.

Results and Discussion

Properties of Chaya Leaf Powder

The cyanide test on 2 grams of Chaya leaf powder, which was the maximum amount used in the jelly, showed that the Cyantesmo Test paper remained green (Fig. 1). The green color of the paper indicates that the cyanide content was less than 0.20 ppm. According to The World Health Organization (WHO, 1995), the permissible cyanide limit in food products is 10 ppm. (Poonsri et al., 2019). The cyanide content in the sample was therefore lower than the level recommended by the WHO and the Chaya leaf powder sample was safe for consumption.

Moisture content and water activity play a crucial role in microbial growth in food materials, potentially rendering the food unsafe for consumption and leading to health risks, including illness or death (Zambrano et al., 2019). The results for moisture content and water activity of the Chaya leaf powder and green tea leaf powder are presented in Table 2. The moisture content analysis revealed that the Chaya leaf powder (3.08±0.21) had significantly lower moisture content than the green tea leaf powder (8.13±1.00). Research suggests that a moisture content of approximately 10% or below is recommended for dry leaf powders (Raja et al., 2019; Zambrano et al., 2019). Based on these findings, the moisture content of both the Chaya leaf powder and the green tea leaf powder fell within the recommended range. The water activity value of the Chaya leaf powder was 0.23±0.01 and for the green tea leaf powder was 0.48±0.01. Studies indicate that maintaining water activity below 0.60–0.65 effectively prevents microbial contamination and spoilage (Zambrano et al., 2019). Therefore, the low moisture content and water activity of the sample powders indicated that they were safe for use in food products by limiting the moisture available for microbial activity.

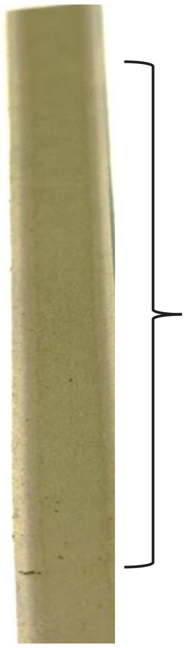

Figure 1 Cyantesmo test papers after dipping into the solution of dried Chaya leaf powder

The L^*, a^*, and b^* values presented in Table 2 showed a significant difference ($p < 0.05$) between the Chaya powder and the green tea leaf powders. The Chaya leaf powder exhibited a more negative a^* value than the green tea leaf powder, indicating a greater degree of greenness. Additionally, the yellowness (b^*) of Chaya leaf powder was higher, contributing to its higher brightness (L^*) value (50.00±0.19) compared to green tea leaf powder (40±6.56). Based on previous research, the green and yellow pigments are attributed to the presence of chlorophyll, rutin, quercetin, and kaempferol (Jaroennon & Manakla, 2021). These findings suggest that Chaya leaves, which displayed more intense green and yellow coloration than green tea leaves, likely contained higher levels of these compounds. Therefore, Chaya leaf powder is suitable for use as a substitute for green tea powder. Moreover, Chaya leaves are caffeine-free, making them suitable for serving to people who are allergic and people of all ages.

Table 2 Moisture, water activity, and color properties of Chaya leaf powder and green tea leaf powder

Properties	Sample		p-value
	green tea leaf powder	Chaya leaf powder	
Moisture (%)	8.13±1.00	3.08±0.21	0.010
Water activity (a_w)	0.48±0.01	0.23±0.01	0.000
color			
L^*	40.65±0.56	50.00±0.19	0.001
a^*	-2.70±0.06	-7.01±0.09	0.000
b^*	8.53±0.17	19.28±0.31	0.001

Mean ± SD within a row followed by significantly different at P $\leq$0.05

Properties of jellies

The characteristics of the five jelly formulas are shown in Fig. 2. They were green-yellow and had an odor similar to green tea.

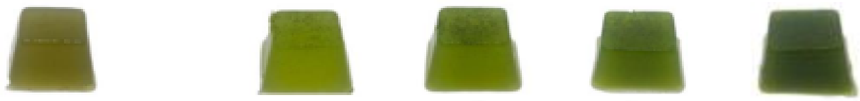

Figure 2 Color characteristics of the five jelly formulations. The control contained 1 g of green tea powder, and the experimental formulations (1-4) contained varying amounts of Chaya leaf powder: 0.5, 1, 1.5, and 2 g.

The color properties of jelly products made from Chaya and green tea leaf powders are summarized in Table 3. The L^* value represents the brightness of the jelly formulas, with the lightness of Chaya leaf jelly ranging from 31.62 ± 1.58 to 33.58 ± 1.17. Increasing the ratio of Chaya leaf powder did not result in a statistically significant difference in lightness ($p > 0.05$). All jelly formulas exhibited negative a^* and positive b^* values, indicating a green-yellow color. The results demonstrated that Chaya leaf jelly had higher green and yellow color intensities compared to the control formula made with tea leaves. Chaya leaves are known to contain flavonoids, which contribute to the yellow pigmentation and are pharmacologically relevant. These bioactive compounds have been associated with various health benefits, including antioxidant activity and disease prevention (Perez-Gonzalez et al., 2021). Formula 3 exhibited the highest b^* value (7.16 ± 0.51). However, it was not significantly different from Formula 4. This suggests that jelly containing 1.5 - 2 g of Chaya leaf powder may offer greater health benefits compared to other formulas. The high b^* value of Formula 3 may indicate the presence of yellow pigments, such as carotene. Carotene is known to possess antioxidant properties, which include reducing the risk of cancer, preventing vitamin A deficiency, supporting the immune system, and protecting against arthritis, cardiovascular diseases (CVD), and hypertension (Chatterjee et al., 2018). Furthermore, previous studies have shown that food products derived from green vegetables contain chlorophyll (Sun et al., 2020). This indicates that Chaya leaf jelly may have a higher chlorophyll content than green tea leaf jelly. Chaya leaves are also recognized as a rich source of chlorophyll (Gustiar et al., 2023), which provides health benefits such as antioxidant, antimutagenic, antigenotoxic, anti-cancer, and anti-obesogenic activities (Martins et al., 2023; Hayes & Ferruzzi, 2020). Although Formula 3 had the highest greenness intensity (-5.14 ± 0.14), it was not significantly different from Formula 1 and 2. Therefore, adding 0.5-1.5 g of Chaya leaf powder into the jelly may provide comparable levels of chlorophyll and a higher level of antioxidants.

Table 3 Color properties of Jelly

Jelly formulas	color		
	L^*	a^*	b^*
control	$28.36±0.48^{a}$	$-2.00±0.20^{a}$	$3.03±0.14^{a}$
1	$32.58±1.30^{b}$	$-4.28±0.86^{b}$	$5.37±0.97^{b}$
2	$32.27±1.13^{bc}$	$-4.36±0.81^{bc}$	$5.39±1.39^{bc}$
3	$31.62±1.58^{bcd}$	$-5.14±0.14^{bcd}$	$7.16±0.51^{d}$
4	$33.58±1.17^{bcde}$	$-3.61±0.74^{bce}$	$5.52±1.17^{bcde}$

Mean ± SD within a column with different superscript letters are significantly different at $P \leq 0.05$ from each other, ns: non-significant

The texture characteristics of all five jelly formulas are presented in Table 4. The hardness of the formulas ranged from 560.00 to 966.33 g, which corresponded to a level (from 550 to 5,000 g) that can be chewed (Kim & Iida, 2022). The gumminess of the developed jellies ranged from 85.00 to 130.00 g, which is higher than that of the control jelly. This indicates that the developed jellies (without sugar) required more energy to disintegrate into a state ready for swallowing than the control jelly (with added sugar) (Yusof et al., 2019; Masri et al., 2023). Chewiness is one of the important texture characteristics of jelly products. The results showed that the chewiness of the control jelly (1.60) was low and did not significantly differ from that of the other jellies (1.53 to 2.77). The developed jellies demonstrated a similarly low chewing energy requirement for solid food to reach a state ready for swallowing, comparable to the control jelly (Calvarro et al., 2016). The data demonstrates that an increase in the amount of Chaya leaf powder correlates with an increase in chewiness, suggesting an enhancement in the jelly's stickiness. This trend is especially pronounced in the formulations containing 1.5 grams and 2 grams of Chaya leaf powder, where a significant increase in toughness was observed. This can likely be attributed to the specific components within Chaya leaf powder, such as plant fibers (Panghal et al., 2021), which can absorb water and subsequently contribute to the thickening and solidifying of the gel matrix (Lian et al., 2023). These interactions may result in a more rigid and cohesive texture, which enhances the chewiness of the jelly. The highest springiness was observed in Formula 4 (2.60±0.83); however, it was not significantly different ($p > 0.05$) from that of the control, Formula 2, and Formula 3. All formulations exhibited similarly low cohesiveness values (0.03 to 0.04), with no significant differences ($p > 0.05$). Studies suggest that jellies with high springiness and low cohesiveness are easier to chew and swallow (Masri et al., 2023). These results indicate that Formulas 2, 3, and 4 have a similar softness when compared to the control formula. Moreover, research has reported that the texture of soft confectionery products is a critical factor for consumer acceptance and success of jelly products (Gunes et al., 2022). However, a sensory study in the future is essential for predicting market demands and enhancing the product's appeal.

Table 4 Texture properties of jellies

Jelly formulas	Hardness (g)	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mJ)
control	$647.00±96.7^{a}$	$0.04±0.01^{ns}$	$2.02±0.36^{a}$	$80.50±7.78^{a}$	$1.60±0.42^{a}$
1	$709.00±33.65^{ab}$	$0.03±0.01^{ns}$	$1.77±0.24^{ab}$	$87.33±15.57^{ab}$	$1.53±0.38^{ab}$
2	$560.00±63.02^{ac}$	$0.04±0.01^{ns}$	$2.04±0.19^{abc}$	$85.00±6.08^{abc}$	$1.67±0.21^{abc}$
3	$966.33±73.43^{d}$	$0.03±0.00^{ns}$	$2.16±0.17^{abcd}$	$130.00±2.65^{d}$	$2.77±0.23^{acd}$
4	$573.33±28.1^{ace}$	$0.03±0.01^{ns}$	$2.60±0.83^{acde}$	$98.33±13.32^{abce}$	$2.53±1.19^{abcd}$

Mean ± SD within a column with different superscript letters are significantly different at $P \leq 0.05$ from each other, ns: non-significant

Total phenolic and Flavonoid Contents of the Jellies

The total phenolic and flavonoid contents of the jellies are given in Table 5. The developed jellies contained total phenolic contents in the range of 1,432.33 – 1,525.00 mgGAE/20 g of sample and total flavonoid contents of 0.22 - 1.45 mgQE/20 g of sample. These results showed a positive correlation between the amount of Chaya leaf powder and the amounts of phenolic and flavonoid compounds. Many lines of studies have indicated that phenolic and flavonoid compounds play a significant role in antioxidant activity (Mutha & Surana, 2021). The flavonoid content of the developed jellies ranged from 1,432.33 to 1,525.00 mg GAE/20 g, which did not significantly differ from the control. Statistical analysis further revealed that the phenolic content among the control formula (1.43), Formula 3 (1.35), and Formula 4 (1.45) showed no significant difference. These results indicate that consuming jelly from any of these three formulas provides a similar phenolic content. In commercial applications, it is advisable to use smaller quantities of ingredients that offer the same benefits as larger quantities. Therefore, Formula 3 was selected to study its nutritional properties.

Table 5 Phenolic and flavonoid contents of jellies

Jelly formulas	Total Flavonoid content (mg QE/20 g Sample)	Total Phenolic content (mg GAE/20 g Sample)
control	1,520.33±30.09 [ns]	1.43±0.22[a]
1	1,432.33±19.14[ns]	0.54±0.02[b]
2	1,458.33±83.86[ns]	0.86±0.04[c]
3	1,516.00±53.93[ns]	1.35±0.04[ad]
4	1,525.00±65.34[ns]	1.45±0.07[ade]

Mean ± SD within a column with different superscript letters are significantly different at $P \leq 0.05$ from each other, ns: non-significant

Nutritional properties

The nutritional properties of Formula 3 and the control formula were analyzed and are presented in Table 6. The Formula 3 contained higher levels of calcium, potassium, phosphorus, and vitamin C. Additionally, Formula 3 was found to contain carotenoids. The total energy content of Formula 3 was 0.582 kcal per 20 g (2.91 kcal per 100 g), whereas the control formula contained 8.693 kcal per 20 g (43.465 kcal per 100 g). This demonstrates that substituting sugar with sucralose resulted in a 93% reduction in energy in Formula 3 compared to the control formula. According to the guidelines for nutrition and health, solid foods with an energy content of less than 40 kcal per 100 grams may be labeled as low-energy foods (Nutrition and Health Claims, 1997).

Table 6 Nutritional value per serving (Serving size: 20 g)

Nutritional compound	Control	Formula 3
Energy (Kcal)	8.69	0.58
Carbohydrate (mg)	2,130.00	140.00
Sugar (mg)	201.2	16.00
Fiber (mg)	160	121
Protein (mg)	41.000	5.00
Fat (mg)	6.99	n.d.
Phosphorus (mg)	n.d.	0.02
Calcium (mg)	0.02	0.12
Iron (mg)	0.01	0.01
Sodium (mg)	0.57	0.55
Potassium (mg)	0.04	0.13
Magnesium (mg)	0.01	n.d.
Vitamin C (mg)	0.02	0.10
Carotenoids (μg)	n.d.	0.05

n.d.: the Nutritional compound was found in very trace amounts

Conclusion and Suggestions

This study successfully developed and evaluated sugar-free jellies enriched with Chaya (*Cnidoscolus aconitifolius*) leaves. The Chaya leaf powder exhibited safe cyanide levels, appropriate water activity, and acceptable moisture content. The jellies displayed a green-yellow color and a suitable texture for chewing. Notably, higher Chaya leaf powder content resulted in increased phenolic and flavonoid content, indicating enhanced antioxidant properties. Formula 3, with its balanced cost-effectiveness and nutritional benefits, was selected for further investigation. Its low-energy profile aligns with established guidelines for nutrition and health claims. These findings highlight the potential of Chaya-infused sugar-free jelly as a promising health food option. Future research should focus on the long-term stability of active compounds and consumer acceptability to ensure effective market penetration and cater to diverse consumer preferences.

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Author Contributions

PJ designed the study and performed experiments on jelly formulas, and physicochemical properties. PS evaluated antioxidant activities. JN performed nutritional studies. CJ prepared Chaya leaf powder and detected cyanide. SM analyzed data. All authors wrote and edited the manuscript to submission for publication.

Conflict of Interests

All authors have no conflicts of interest.

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