















Chemical composition of varieties of chaya (*Cnidoscolus aconitifolius* Mill. I.M. Johnst.) at different cutting ages

Composición química de variedades de chaya (*Cnidoscolus aconitifolius* Mill. I.M. Johnst.) a diferentes edades de corte

Composição química de variedades de chaya (*Cnidoscolus aconitifolius* Mill. I.M. Johnst.) em diferentes idades de corte

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Crop production

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Abstract

Cnidoscolus aconitifolius Mill. I.M. Johnst, is a plant distributed in tropical and subtropical, for its high nutritional value and adaptability to diverse environments. To evaluate the chemical composition of two varieties of chaya at different harvest ages. A completely randomized experimental design with a 2x4 factorial arrangement was used, with chaya varieties (Estrella and Mansa) and harvest age (60, 90, 120, and 150 days) as factors. The chemical composition and the presence of some secondary compounds (phenols, tannins, saponins, catechins and flavonoids) in chaya leaves were analyzed. The comparison of means tests for the interaction between variety and cutting time showed that the Mansa variety at 120 days had a higher fat content (7.04 %) and phenols (5.39 mg.g⁻¹), as well as a lower FDA content (15.12 %), unlike the Estrella variety, which at 90 days had a higher protein content (33.90 %). The Estrella variety had higher protein (29.90 %) and NDF (37.57 %) contents; however, the Mansa variety had higher total phenol content (4.78 mg) and ADF (16.90 %). The Estrella variety showed the highest protein content in the 90-day harvest, while NDF was higher in the first harvests (60 days) of the Mansa variety. Fat content was similar in both varieties. Phenols were more prevalent in the Mansa variety, although both showed high concentrations of tannins, saponins, catechins, and flavonoids, and low concentrations of triterpenes, amino acids, and reducing sugars.

Resumen

Cnidoscopus aconitifolius Mill. I.M. Johnst, es una planta ampliamente distribuida en regiones tropicales y subtropicales, reconocida por su alto valor nutricional y su adaptabilidad a diversos ambientes. El objetivo de esta investigación fue evaluar la composición química de dos variedades de chaya a distintas edades de cosecha. Se utilizó un diseño experimental completamente al azar con arreglo factorial 2x4, considerando como factores las variedades de chaya (Estrella y Mansa) y la edad de cosecha (60, 90, 120 y 150 días). Se analizó la composición química y la presencia de algunos compuestos secundarios (fenoles, taninos, saponinas, catequinas y flavonoides) en hojas de chaya. La prueba de comparación de medias de la interacción variedad vs edad de corte mostró, que la variedad Mansa a los 120 días presentó un mayor contenido de grasa (7,04 %) y de fenoles (5,39 mg.g⁻¹), así como una menor presencia de FDA (15,12 %), a diferencia de la variedad Estrella, que a los 90 días presentó un mayor aporte proteico (33,90 %). La variedad Estrella presentó un mayor contenido de proteína (29,90 %) y de FDN (37,57 %); sin embargo, la variedad Mansa mantuvo un mayor aporte de fenoles totales (4,78 mg) y de FDA (16,90 %). Se concluye que la variedad Estrella presentó el mayor contenido de proteína en la cosecha a los 90 días, y FDN fue mayor en las primeras cosechas (60 días) de la variedad Mansa, el contenido de grasa fue similar en ambas variedades. Los fenoles fueron más frecuentes en la variedad Mansa, aunque ambas mostraron altas concentraciones de taninos, saponinas, catequinas y flavonoides, y bajas concentraciones de triterpenos, aminoácidos y azúcares reductores.

Palabras clave: berza, nutrientes, digestión, compuestos secundarios

Resumo

Cnidoscopus aconitifolius Mill. I.M. Johnst, é uma planta amplamente distribuída em regiões tropicais e subtropicais, reconhecida por seu alto valor nutricional e adaptabilidade a diversos ambientes. O objetivo desta pesquisa foi avaliar a composição química de duas variedades de chaya em diferentes idades de colheita para alimentação animal. Foi utilizado um delineamento experimental inteiramente casualizado com arranjo fatorial 2x4, considerando as variedades de chaya (Estrella e Mansa) e a idade de colheita (60, 90, 120 e 150 dias) como fatores. A composição química e a presença de alguns compostos secundários (fenóis, taninos, saponinas, catequinas e flavonoides) nas folhas de chaya foram analisadas. O teste de comparação de médias da interação variedade x idade de colheita foi realizado. A análise do tempo de colheita mostrou que a variedade Mansa, aos 120 dias, apresentou maior teor de gordura (7,04 %) e de fenóis (5,39 mg.g⁻¹), além de menor presença de FDA (15,12 %), diferentemente da variedade Estrella, que aos 90 dias apresentou maior teor de proteína (33,90 %). A variedade Estrella apresentou maior teor de proteína (29,90 %) e FDN (37,57 %); entretanto, a variedade Mansa manteve maior contribuição de fenóis totais (4,78 mg) e FDA (16,90 %). Conclui-se que a variedade Estrella apresentou o maior teor de proteína na colheita aos 90 dias, e o teor de FDN foi maior nas primeiras colheitas (60 dias) da variedade Mansa; o teor de gordura foi similar em ambas as variedades. Os fenóis foram mais frequentes na variedade Mansa, embora ambas tenham apresentado altas concentrações de taninos, saponinas, catequinas e flavonoides, e baixas concentrações de triterpenos, aminoácidos e açúcares reductores.

Palavras-chave: berza, nutrientes, digestão, compostos secundários

Introduction

Population projections indicate that the global population will reach 9 billion over the next 25 years. However, feed is one of the most critical elements in livestock farming, given the high energy and protein demands of different animal species to maintain production levels (Airoboman and Onobhayedo, 2022). From a forward-looking perspective, shrubby plants have been recognized for their significant capacity to improve diets and produce large quantities of palatable biomass (Quiñones Chillambo *et al.*, 2020), among other benefits.

The criteria set forth by Gary and Anderson (2019) indicate that animal feed in stabled systems accounts for approximately 60-80 % of production costs, primarily due to the consumption of proteins, energy, carbohydrates, essential fats, vitamins, and minerals required to provide a balanced diet.

The nutritional value of food depends not only on its chemical composition and the digestibility of its components, but also on the maximum consumption by animals and on its impact on animal health, development, and production capacity (Leroy *et al.*, 2023).

In this regard, chaya (*Cnidoscopus aconitifolius* Mill. I.M. Johnst.) belongs to Euphorbiaceae family (Cifuentes *et al.*, 2010), it is a perennial shrub widely distributed in tropical and subtropical regions, primarily in low deciduous forest and xerophytic scrubland areas of Mexico (Kolterman *et al.*, 1984), where 20 of the 50 existing species originate (Kuri-García *et al.*, 2017). It has also been recognized in other Latin American countries and the southern United States for its high nutritional value and adaptability to diverse environments (Godínez-Santillán *et al.*, 2019), where it has been used not only as part of animal feed but also in human diets due to its ability to produce leaves rich in protein, vitamins, minerals, β -carotene, ascorbic acid, calcium, potassium, and iron, and other essential nutrients (Chin-Chan *et al.*, 2021).

In recent years, strategies have been developed to manage this crop more effectively, thereby improving the use of chaya in agriculture and animal production (Amaya *et al.*, 2020).

In the field of animal feed, the use of chaya as forage plays a fundamental role due to its potential to improve the quality of animal diets, especially in areas where feed resources are limited during certain seasons. However, the chemical composition of chaya can vary with factors such as genetic variety and harvest age, which influence its nutritional value and suitability as livestock feed (Rodrigues *et al.*, 2021).

Despite the extensive literature on the nutritional and medicinal properties of chaya, the impact of harvesting stages on different varieties of the plant has been little documented in Ecuador; however, within the environment, these studies are limited despite the widespread development of this crop, which is traditionally used as a living fence for the isolation of animal species. In this context, the objective of this study is to evaluate the chemical composition and nutritional value of two chaya varieties at different harvest ages for use as animal feed.

Materials and methods

Location

The field research and laboratory analyses were developed within the grounds of the Faculty of Agrosciences of the Technical University of Manabí, Ecuador, which is located in the *Ánima* site of the road that connects Chone with Boyacá, located in the city of Chone, in

the province of Manabí, geographically situated at the coordinates 00°41.248'S, 80°07.457'W, at an altitude of 10 msnm.

Obtaining and processing samples

For the collection of *C. aconitifolius* (Mansa and Estrella varieties), the harvest was conducted by collecting leaf blades from the lower, middle, and upper parts of the plant. In 20 plots of five plants each, planted 80 centimeters apart and one meter between rows, considering each plant as a repetition. Before harvesting, the plants were cut to ensure uniformity; the activity was completed on the same day, and plants were harvested at 60, 90, 120, and 150 days during 2024.

The process began in April 2024, when the chaya plants were equalized to take advantage of the rainy season and ensure adequate forage development. Harvesting was performed manually, making the cut 40 cm from the ground, and the samples were placed in jute sacks for transport to the laboratory. There, the plant material was dehydrated in a homemade electric tray dehydrator with hot-air flow at 55 °C for 24 hours.

Subsequently, the plant material was ground in an industrial mill to a particle size of 1mm and then placed in ziploc bags.

Soil characteristics

The soils in the Chone canton have textures ranging from clay loam to clay, with good water- and nutrient-retention capacity. They possess medium to high natural fertility, which favors crops such as corn, rice, cacao, plantains, pasture, and other tropical plants. Studies conducted in various sectors of Chone show variable organic matter content and pH levels, ranging from slightly acidic to neutral, depending on whether the soil is used for agriculture or forestry (Carrera *et al.*, 2014).

Chemical analysis

The samples were previously labeled according to the treatments and subsequently sent for analysis to the Bromatology Laboratory of the Faculty of Agricultural Sciences, where crude protein (%) was determined according to the method described by the NTE INEN-ISO 20483 standard, Kjeldahl Methodology; fat (%) was evaluated by the evaluation method of the NTE INEN-ISO 20483 standard; Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) by using the AOAC 962.09/ANKOM test method.

Preparation of extracts for phytochemical analysis

From the samples stored in amber bottles, one gram of powder was weighed and suspended in 100 mL of 100 % ethanol and shaken for 24 hours at room temperature. The extract was filtered through Whatman® No. 1 filter paper and concentrated using a rotary evaporator at 40 °C. The extract was then redissolved in one milliliter of ethanol-methanol for phytochemical analysis.

Analysis of total phenol content

The phenolic content was analyzed using the Folin-Ciocalteu test (Ardestani and Yazdanparast, 2007). The extracts were prepared after the samples were dehydrated. Alcohol (96 %) was used as the solvent, and the extracts were macerated under suitable conditions, free of moisture and sunlight.

Subsequently, the solvent concentration and removal were determined, and aliquots of the extract were prepared. These were mixed with the reagent, and a 7 % sodium carbonate solution was added to induce the characteristic blue color. The mixture was then incubated in the dark for 30 minutes under ambient conditions.

Total phenol content was measured using a Genesys UV spectrophotometer at a wavelength of 765 nm. Results were expressed in mg GAE.g⁻¹ of dehydrated leaf.

Phytochemical screening of the treatments under study

Phytochemical screening of samples was conducted for each species to detect secondary compounds. This was performed in triplicate, qualitatively, for Tannins: (FeCl₃ test, Vanilin-Hydrochloride test and alkaline test), Saponins: (Froth forming test), Catechins: Ferric chloride test, Flavonoids (Shinoda test and Zinc-Hydrochloride test), Triterpenes: (Liebermann test, Salkowsky test and Noller test), Amino acids: (Ninhydrin test), Reducing sugars: (Fehling's test).

The assessment of the presence of secondary metabolites was carried out through a qualitative evaluation with the following evaluation parameters: (-) Absence; (+) Slight presence; (++) Notable presence; (+++) Large presence.

Experimental design

The research employed a completely randomized design in a factorial arrangement, with chaya varieties (Mansa and Estrella) and cutting age (60, 90, 120, and 150 days) as factors. Each treatment had three replicates, with one experimental unit per replicate.

Statistical Analysis

The data were analyzed using InfoStat. Tukey's test was used to compare means at the 95 % confidence level. The following model was used for the ANOVA:

$$Y_{ij} = \mu + R + (Vch)_i + (Caj)_j + (Vch \times Caj)_{ij} + e_{ij}$$

Where: Y_{ij} = response variable, μ = constant common to all observations, $Vchi$ = Choya variety ($i=1, 2$), Caj = cutting age ($j=1, \dots, 4$), $Vch \times Caj_{ij}$ = combined effect of the i -th variety at the j -th cutting age, e_{ij} = random error $\sim N(0, \sigma^2_e)$.

Results and discussion

Crude protein analysis of chaya leaves, considering the effect of variety and cutting stage (Figure 1), shows that the Estrella variety at 90 contributed the most, accounting for 33.90 %.

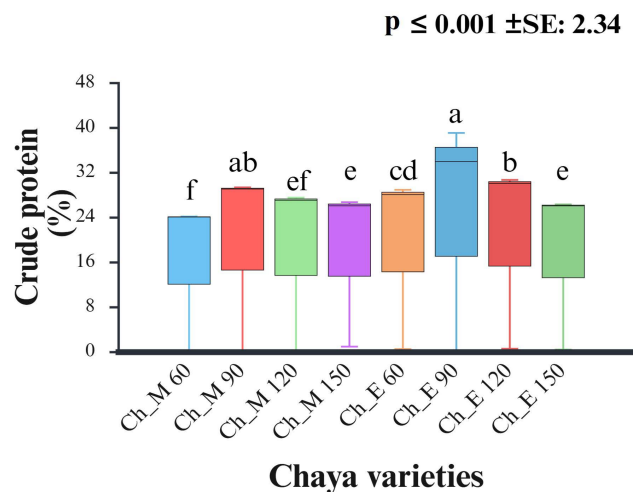


Figure 1. Crude protein content in leaves of *C. aconitifolius* varieties at different cutting ages. a, b, c, d, e, f. Different superscripts indicate significant differences according to Tukey's test at the 95 % confidence level. The vertical lines represent the standard deviation. $\pm SE$: Standard error of the mean. Ch_M: Chaya Mansa. Ch_E: Chaya Estrella. Cut-off age (60, 90, 120, and 150 days).

A similar pattern was observed in the leaves of the Mansa variety of chaya at 90 days, with a crude protein content of 33.9 %, which decreased as leaf maturity, coinciding with the appearance of new shoots on the foliage. However, the criteria presented by Ebel *et al.* (2019) indicate that the presence of this nutrient in chaya leaves is influenced by factors related to genetics and the growth cycle, with a higher concentration during the early stages of growth, as well as by the time of year, which includes environmental components that were not controlled.

The results of Alcívar Acosta *et al.* (2023), who evaluated the nutritional content of chaya leaves as an alternative for pig feed, report a favorable crude protein content of 31.73 %, comparable to the value reported in this study.

The results of the mean comparison analysis for the fat content variable (Figure 2) in the chaya varieties with the four cutting stages showed a significant behavior ($p=0.0214$), where the Mansa variety showed a superiority in the cut at 120 days with 7.04 %, while for the Estrella variety at 90 days, they were lower with 4.25 %.

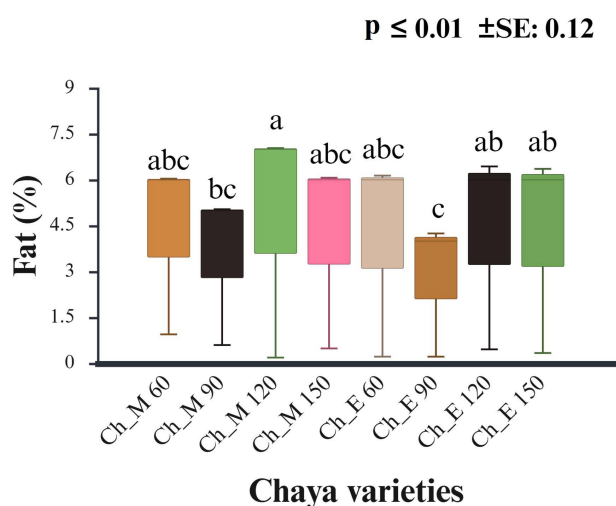


Figure 2. Fat content in leaves of *C. aconitifolius* varieties at different cutting ages. ^{a, b, c} Different superscripts indicate significant differences according to Tukey's test at the 95 % confidence level. The vertical lines represent the standard deviation. $\pm SE$: Standard error of the mean. Ch_M: Chaya Mansa. Ch_E: Chaya Estrella. Cut-off age (60, 90, 120, and 150 days).

Similarly, one can demonstrate that the cuts made at 60 and 150 days for the Mansa variety, and Estrella chaya at 60 days, show a good fat content in the analyzed samples, which could be related to sampling at an early age where this component is representative for this type of plant matter; probably with its relationship with some secondary components of the plant (Putri *et al.*, 2025); in responses to physiological or metabolic stress conditions in the region under study. This will constitute a new line of research to pursue to identify the reason for this close relationship.

Comparative studies between leafy plant species, including chaya, document variability in fat content, with values ranging from 5.20 to 8.61 % (Castellón *et al.*, 2024), which is within the range reported in this research.

The analysis of means for the NDF variable showed a significant difference ($p<0.001$) between the varieties and cutting ages used in chaya forage. The results showed that the Mansa variety at 60 days exceeded the NDF content by between 12-35 %, and the Estrella variety by between 7-32 % at 150 days, both compared to the rest of the treatments. While for ADF, the contents are relatively uniform. Observing the lowest values ($p<0.001$) in both varieties between 120 and 150 days (Table 1).

Table 1. Fibrous fraction of leaves of *C. aconitifolius* varieties at different cutting ages.

Variety	Cutting age (Days)	NDF (%)	ADF (%)
Mansa	60	45.62±0.59 ^a	19.56±0.61 ^a
Mansa	90	40.09±1.14 ^b	19.37±0.63 ^a
Mansa	120	32.15±0.93 ^c	15.12±0.55 ^d
Mansa	150	26.75±1.40 ^d	13.55±0.06 ^e
Estrella	60	38.51±1.30 ^b	19.77±0.14 ^a
Estrella	90	39.12±0.83 ^b	19.42±0.72 ^a
Estrella	120	29.25±0.45 ^{cd}	16.44±0.55 ^c
Estrella	150	43.39±1.40 ^a	17.86±0.06 ^d
$\pm SE$		12.5	6.4
p-value		0.001	

^{a, b, c, d} Different superscripts indicate significant differences according to Tukey's test at the 95 % confidence level. Number \pm standard deviation. $\pm SE$: Standard error of the mean.

The mean comparison analysis for the NDF variable showed a significant difference ($p<0.001$) between the varieties and cutting stages used in chaya forage. The results showed that the most representative values were achieved for the Mansa variety at 60 days and the Estrella variety at 150 days, with contents of 45.62 % and 43.39 %, respectively.

The NDF concentrations of the Mansa variety decreased from 60 to 150 days 41.36 %. Similar behavior is observed in the Estrella chaya leaf during the first 120 days, with NDF decreasing to 29.25 %. However, by 150 days, the values had increased significantly, a well-defined and characteristic feature of tropical forages. Likewise, this latter behavior is also related to an increase in the lignification processes of the leaf cell walls due to the physiological maturation processes of the leaves, which in turn can make them less digestible, a fundamental element when included in the diet of animal species (Guevara *et al.*, 2021).

High levels of NDF in plant species are associated with a lower percentage of digestibility and, consequently, a lower utilization of available nutrients (Alcívar Acosta *et al.*, 2023). These authors also reports NDF and ADF values of 23.78 % and 11.94 %, respectively, which are lower than those, reported in this study.

For the Mansa variety, the results show a greater decrease in this component with increasing harvest age, reaching an ADF contribution of 13.55 % by 150 days, the lowest value among the four harvests conducted for both varieties. In the case of the Estrella variety, the study results showed that ADF decreased for up to 120 days, and by 150 days, the indicator showed a slight upward trend.

ADF results are closely related to the amount of cell wall compounds that are not digestible within the digestive tract of animals. Hence, the presence of this compound determines forage digestibility, and the primary objective is to achieve lower values that favorably affect digestibility (Ledea-Rodríguez *et al.*, 2018).

Studies by Totakul *et al.* (2021), which analyzed the ADF content of chaya flour samples, reported 38.39 % for this indicator and 44.16 % for NDF, which are much higher than those found in this research, likely due to intra-species variability in Choya.

Analysis of total phenolic content across the studied treatments (Figure 3) indicated that the interaction between harvest age and variety significantly affected total phenolic content ($p < 0.001$). The highest values were recorded in the Mansa chaya variety at 120 days and in the Estrella variety at 60 days, with contents of 5.39 mg GAE.g⁻¹ and 5.32 mg GAE.g⁻¹, respectively.

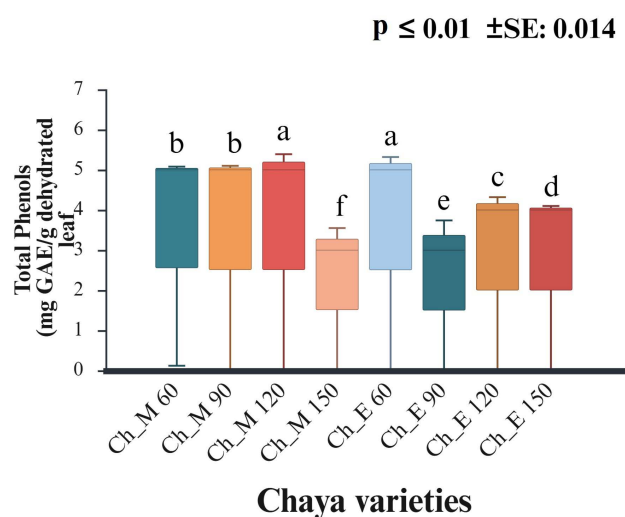


Figure 3. Total phenolic content in leaves of *C. aconitifolius* according to variety and cutting season. ^{a, b, c} Different superscripts indicate significant differences according to Tukey's test at the 95 % confidence level. The vertical lines represent the standard deviation. \pm SE: Standard error of the mean. Ch_M: Chaya Mansa. Ch_E: Chaya Estrella. Cut-off age (60, 90, 120, and 150 days).

The variable concentrations of total phenols induced by the combination of harvest age and chaya variety are related to the broad representation of phenolics and polyphenols in the secondary profile of this plant (Kuri-García *et al.* 2017). However, it is essential to highlight that the values presented here are ten times lower than those reported by (John and Opeyemi, 2015), who indicated contents of up to 15.17 mg GAE.g⁻¹ in fresh matter, while other studies considered in a bibliometric analysis by Panghal *et al.* (2021) indicated values similar to those obtained in the present study.

The results of the present study under discussion are satisfactory because, in addition to having corroborated the capacity to nourish animals from the richness and diversity of primary metabolites in leaves, the presence of functional groups of secondary metabolism allows us to infer in chaya, the capacity to control or reduce the emission of greenhouse gases, an aspect that was corroborated by Totakul *et al.* (2021) when considering chaya leaf pellets as a supplement in the reduction of greenhouse gas emissions.

The results of the phytochemical analysis of chaya leaves (Table 2) demonstrated that harvest age in both varieties influences the presence of compounds such as saponins, catechins, and flavonoids.

Table 2. Phytochemical screening in leaves of two *C. aconitifolius* varieties at different harvest stages.

Treatments	Varieties of <i>C. aconitifolius</i>							
	Mansa				Estrella			
	Cutting age (days)							
	60	90	120	150	60	90	120	150
Tannins	+	+	+	+	+	++	+	+
Saponins	+++	+++	++	+	++	++	+++	+
Catechists	+++	++	++	+++	+++	+++	+++	+++
Flavonoids	+	+++	++	++	+++	+++	++	++
Triterpenes	-	-	-	-	-	-	-	-
Amino acids	-	++	-	-	+	++	-	+
Reducing sugars	+	+	+	+	+	+	+	+

The table presents the results of qualitative phytochemical screening: (-) Absence; (+) Slight presence; (++) Notable presence; (+++) Large presence.

Comparative studies of the phytochemical profile between *Ficus capensis* and *C. aconitifolius*, conducted by Ezeigwe *et al.* (2020), indicated saponin and flavonoid contents in leaves of 5.88 % and 2.56 %, respectively, for *C. aconitifolius*, while the tannin content, at 5.70 %, was almost equal to that of saponins. Under the conditions of the present study, the qualitative evaluation suggested a low to slight presence of this compound.

In ruminants, moderate to high levels of dietary tannins have been reported to be beneficial, protecting nutrients, preventing ruminal degradation, and facilitating their passage to the lower digestive tract. Regarding saponins, research suggests that, at moderate concentrations, they can positively affect the control of pathogens in the digestive tracts of animal species. Conversely, consumption in high concentrations can cause intoxication (Sidana *et al.*, 2016).

The results of the catechin analysis suggest a variety of effects; similar results to those obtained for the Estrella variety were reported by Tinco-Jayo *et al.* (2024) in atomized extracts of leaves and stems of *C. diacanthus* (Pax. and K. Hoffm.). Triterpenes, on the other hand, were absent from the Chaya varieties in all four harvests. The results of Ngoc Thuy *et al.* (2025) differ from those presented in this study, which document a high level of presence in chaya leaves and stems. The presence of amino acids was only notable at early ages in both varieties.

Regarding reducing sugars, Alcívar Acosta *et al.* (2023), in characterizing the nutritional potential of various plant species, reported that chaya had a notable concentration, close to the descriptive value reported in this research.

Conclusions

The chemical composition analysis and phytochemical screening showed variability depending on the age and variety of chaya. The Estrella variety had the highest protein content at the 90-day harvest. Fat content was similar, and neutral detergent fiber (NDF) content was higher in the first harvests (60 days) for the Mansa variety. Phenols were more prevalent in the Mansa variety, although both showed high concentrations of tannins, saponins, catechins, and flavonoids, and low concentrations of triterpenes, amino acids, and reduce sugars.

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