

Effectiveness of Apical Pruning and Eco Enzyme Application on The Organoleptic Characteristics of *Stevia rebaudiana* Bertoni Leaves

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Abstract

Stevia is a natural sweetener with potential as a sugar substitute. Acceptance of stevia products is largely determined by organoleptic qualities such as sweetness, color, aroma, and aftertaste. This study evaluated the effect of pruning and eco enzyme on the sensory attributes of stevia. A factorial randomized block design was used with two factors: shoot pruning (0, 4, and 8 WAP) and eco enzyme (0, 1, 2, and 3 ml/L), each with three replications. Organoleptic evaluation employed the hedonic method with 100 panelists. Results showed that treatment combinations significantly affected sensory parameters. The highest sweetness (3.21) and neutral aroma (3.92) were obtained from unpruned plants treated with 3 ml/L eco enzyme, while pruning at 8 weeks with 3 ml/L eco enzyme gave the best color (3.79). Aftertaste remained moderately bitter but tended to improve in some treatments. Eco enzyme application enhanced sensory quality, supporting stevia's potential in healthy food products.

1. Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a natural sweetener plant with a sweetness level 200–300 times higher than sucrose (Rybchuk et al., 2021). Stevia leaves contain more than 30 types of steviol glycosides, with two main components, stevioside and rebaudioside A, as the principal contributors to sweetness. The stevioside content in the leaves is about 4–13% and rebaudioside A is 2–4% of the total glycosides (Pateliuk et al., 2021; Al-Fekaiki et al., 2021). The main advantage of stevia is that it does not increase blood glucose levels because steviol glycosides are not digested into glucose, so stevia intake does not raise acute blood sugar and can replace sucrose without adding calories (Ajami et al., 2020; Farhat et al., 2019). Stevia is now widely used in foods, beverages, and supplements, and its global market is projected to be worth more than USD 1 billion, continuing to grow as new products are launched each year (Samuel et al., 2018; Ciriminna et al., 2018). Demand is driven by consumers' desire to reduce sugar intake and avoid synthetic sweeteners, as well as their preference for sweeteners perceived as “natural” and zero-calorie (Putnik et al., 2020).

In addition to the content of active compounds, the sensory qualities of stevia such as sweetness level, infusion color, aroma, and aftertaste are crucial for consumer acceptance. One persistent challenge in the use of stevia is the bitter aftertaste, which can reduce liking. Stevioside is the main glycoside in stevia leaves and is known as the primary contributor to bitter and disturbing bitter aftertastes, thereby limiting its use at high levels (Medeiros et al., 2022). Rebaudioside A has higher sweetness potency and lower bitterness than stevioside, so extracts with a higher Reb A ratio generally provide a more preferable flavor profile. Stevioside more readily activates human bitter taste receptors hTAS2R4 and hTAS2R14, whereas rebaudioside A and M exhibit a sweeter taste and a cleaner aftertaste (Nasrullah et al., 2023). Therefore, cultivation strategies are needed that not only increase plant productivity but also improve the organoleptic quality of stevia leaves.

Pruning shoots is a simple cultivation technique that can affect vegetative growth, leaf production, and the accumulation of secondary metabolites in plants (Pala et al., 2013). This treatment has the potential

to improve leaf quality, including the content of natural sweetening compounds. At the same time, the use of eco enzymes produced by fermenting organic wastes such as fruit peels, brown sugar, and water is increasingly being investigated as a natural biostimulant. Eco enzymes contain enzymes, organic acids, and growth hormones that can improve soil fertility, stimulate plant metabolism, and enhance crop quality in an environmentally friendly way (Paendong et al., 2023). In stevia, eco enzyme fertilization has been shown to accelerate growth, increase leaf number, and raise fresh leaf yield compared with other organic fertilizers, thereby increasing production and farmers' income (Kriswantoro et al., 2022). Combining shoot pruning and eco enzyme application is therefore expected not only to improve stevia growth and productivity, but also to contribute to the organoleptic quality of the leaves by increasing steviol glycoside accumulation and overall leaf biomass (Hasanaklou et al., 2020; Abdullateef et al., 2011; Calapardo et al., 2024). Sensory evaluation is essential to determine how far these treatments can enhance sweetness, improve infusion color, increase aroma, and minimize undesirable aftertaste (Kaushik et al., 2010; Tarigan et al., 2024; Fatima et al., 2018).

2. Methodology

2.1. Study Area

This research was conducted on research land located at 03° 53' 09.7" North Latitude, 098° 64' 00.0" East Longitude with an altitude of ± 32 meters above sea level. The analysis was carried out at the Biotechnology and Pests Diseases Laboratory, Faculty of Agriculture, University of North Sumatra, at Jl. Dr. A. Sofian No.3, Padang Bulan, Kec. Medan Baru, Medan. This research was conducted from June to August 2024. The plant material used in this study was *Stevia rebaudiana* Bertoni obtained from local farmers in Kediri, East Java, Indonesia. The plants were cultivated under conventional agricultural conditions. Healthy plants with uniform morphological characteristics were selected and used as experimental materials for the apical pruning and eco-enzyme treatments. The tools used in this study included serving glasses, spoons, a teapot, a beaker, a digital scale, sample codes, an organoleptic assessment sheet, writing instruments, and a stopwatch.

2.2. Procedures

The design used in this research was a factorial complete randomized block design with 2 factors and 3 replications. The first factor is pruning (4 and 8 WAP), while the second factor is Eco Enzyme (0, 1, 2, and 3 ml/l water). Pruning is carried out in accordance with the research treatment, namely 4 WAP and 8 WAP on the fifth leaf sitting from the tip of the shoot., while Eco enzyme Eco enzyme is applied once every 2 weeks with a concentration determined according to the treatment, namely 0, 1, 2 and 3 ml/l water. Eco enzyme is given by spraying it on the entire plant canopy. To determine the volume of eco enzyme spray that will be used, calibrate first with a 1 liter handsprayer. Control of nuisance organisms is focused on minimizing pest attacks and prioritizing plant-based control. The pest many attacks include aphids (*Aphis* sp), armyworms (*Heliothis* sp.), grasshoppers and caterpillars/span caterpillars. This type of pest can be controlled by biological pesticide neem seed extract.

Stevia harvesting is carried out when the plants are 75 days old. The ideal time for harvesting is in the morning. Harvesting is done by cutting the stems about 10-15 cm from the ground using pruning shears. Post-harvest activities for stevia include picking the leaves attached to the stems and sorting them to separate the low-quality leaves. Next, washing is carried out to clean the leaves of dirt to maintain the quality of the stevia. For drying stevia leaves, use the air drying method in a dry, shaded place, which takes about one week. The dried leaves are yellowish-green in color with a maximum moisture content of 10%, a minimum stevioside content of 10%, and a maximum impurity content of 3% (Rukmana., 2003).

2.3. Sensory Evaluation

Sensory evaluation was conducted using a hedonic test to assess panelists' acceptance of the product. A total of 100 panelists evaluated sensory attributes including sweetness, color, aroma, and aftertaste. The assessment was based on a 5-point hedonic scale ranging from 1 to 5, where 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, and 5 = strongly like, following the method described by Setyaningsih et al. (2010), with modifications to the evaluated parameters.

The modified scoring criteria for each attribute were as follows:

1. Sweetness : 1 (very sweet), 2 (sweet), 3 (moderately sweet), 4 (slightly sweet), and 5 (not sweet).
2. Color : 1 (dark brown), 2 (light brown), 3 (yellow), 4 (slightly yellow), and 5 (greenish).
3. Aroma : 1 (very strong), 2 (strong), 3 (neutral), 4 (weak), and 5 (very weak).
4. Aftertaste : 1 (very bitter), 2 (bitter), 3 (moderately bitter), 4 (slightly bitter), and 5 (not bitter).

3. Result

The sensory evaluation of stevia leaves, assessed using four parameters, showed variable responses depending on treatment effects, with statistical significance determined using SPSS analysis. The sensory characteristics under shoot pruning and eco enzyme application treatments are presented in Figure 1.

3.1 Sweetness Level

The normality test results indicated that the combined treatment of shoot pruning and eco enzyme application produced p-values lower than 0.05, indicating that the sensory data for stevia sweetness were not normally distributed. Therefore, a non parametric Kruskal Wallis test was applied. The mean organoleptic scores for sweetness under different shoot pruning and eco enzyme treatments are presented in Fig. 1. As shown in Fig. 1, treatment P₀D₃ (no shoot pruning with 3 mL eco enzyme concentration) exhibited the highest sweetness level, with a score of 3.21, which was perceived as moderately sweet by the panelists. Overall, panelists' preference for stevia leaf taste showed relatively small variation, ranging from 2.67 to 3.21, corresponding to slightly sweet to moderately sweet perceptions. The Kruskal–Wallis test at a 95% confidence level revealed a significant effect of treatment on sweetness ($p = 0.004 < 0.05$), indicating significant differences among treatments. Pairwise

comparisons were subsequently performed using the Mann Whitney test to evaluate differences between treatment groups, and the results are presented in the following table.

Based on Table 1, the Mann–Whitney test results for sweetness indicated significant differences ($p < 0.05$) between several treatment pairs, namely P_0D_0 (control + 0 mL L⁻¹) and P_0D_1 (control + 1 mL L⁻¹), P_0D_1 (control + 1 mL L⁻¹) and P_0D_3 (control + 3 mL L⁻¹), P_2D_0 (8 MST + 0 mL L⁻¹) and P_2D_2 (8 MST + 2 mL L⁻¹), as well as P_0D_1 (control + 1 mL L⁻¹) and P_1D_1 (4 MST + 1 mL L⁻¹), indicating significant differences in sweetness levels among these treatments.

Treatment P_0D_3 (no shoot pruning with 3 mL eco enzyme application) exhibited a moderately sweet taste with a score of 3.21. This result suggests that the absence of shoot pruning combined with eco enzyme application may enhance the production of sweet compounds in stevia leaves, thereby increasing perceived sweetness. The increased leaf production under this treatment may contribute to higher concentrations of glycosidic compounds, particularly stevioside and rebaudioside, which are the primary constituents responsible for the sweetness of stevia leaves. Steviol glycosides are typically approximately 200–300 times sweeter than sucrose on a sweetness equivalence basis per unit dry weight (Hernandez *et al.*, 2021; Abubakar *et al.*, 2023). The total steviol glycoside content in stevia leaves usually ranges from about 4% to 20% of dry weight, depending on genotype and cultivation conditions (Orellana, 2023). Among the numerous steviol glycosides identified, the two predominant sweet constituents are stevioside, accounting for roughly 4–13% (w/w) of leaf dry weight, and rebaudioside A, accounting for about 2–4% (w/w) of leaf dry weight (Czinkóczy *et al.*, 2021; Khilar *et al.*, 2022). Stevioside and rebaudioside A generally contribute approximately 5–10% of leaf dry weight and represent around 90% of the total sweet steviol glycosides in conventional stevia cultivars (Lima *et al.*, 2021).

Table 1. Results of the Mann Whitney test analysis of the sweetness level of stevia

Treatment	<i>p</i> -value	Notes
$P_0D_0 - P_0D_1$	0,013	there are changes
$P_0D_0 - P_0D_2$	0,406	no difference
$P_0D_0 - P_0D_3$	0,259	no difference
$P_0D_1 - P_0D_2$	0,107	no difference
$P_0D_1 - P_0D_3$	0,000	there are changes
$P_0D_2 - P_0D_3$	0,052	no difference
$P_1D_0 - P_1D_1$	0,347	no difference
$P_1D_0 - P_1D_2$	0,231	no difference
$P_1D_0 - P_1D_3$	0,709	no difference
$P_1D_1 - P_1D_2$	0,713	no difference
$P_1D_1 - P_1D_3$	0,840	no difference
$P_1D_2 - P_1D_3$	0,823	no difference
$P_2D_0 - P_2D_1$	0,198	no difference
$P_2D_0 - P_2D_2$	0,013	there are changes
$P_2D_0 - P_2D_3$	0,550	no difference
$P_2D_1 - P_2D_2$	0,085	no difference
$P_2D_1 - P_2D_3$	0,365	no difference
$P_2D_2 - P_2D_3$	0,032	no difference
$P_0D_0 - P_1D_0$	0,788	no difference
$P_0D_0 - P_2D_0$	0,086	no difference
$P_0D_1 - P_1D_1$	0,002	there are changes
$P_1D_1 - P_2D_1$	0,129	no difference
$P_0D_2 - P_1D_2$	0,068	no difference
$P_1D_2 - P_2D_2$	0,807	no difference

$P_0D_3 - P_1D_3$	0,570	no difference
$P_1D_3 - P_2D_3$	0,193	no difference

3.2 Color Level

The normality test indicated that the combined treatment of shoot pruning and eco enzyme application produced p-values lower than 0.05, suggesting that the sensory data for stevia color were not normally distributed. Therefore, a non-parametric Kruskal–Wallis test was applied. The mean organoleptic scores for color under different shoot pruning and eco enzyme treatments are presented in Fig. 1. As shown in Fig. 1, treatment P_2D_3 (shoot pruning at 8 MST with 3 mL eco enzyme concentration) exhibited the highest color score (3.79). Overall, panelists' preference for stevia color showed relatively small variation, ranging from 2.88 to 3.79, with yellow coloration being more dominant. The Kruskal–Wallis test at a 95% confidence level revealed a significant effect of treatment on color response ($p < 0.05$), indicating significant differences among treatments. Pairwise comparisons were subsequently conducted using the Mann–Whitney test, and the results are presented in the following table.

Based on Table 2, the Mann Whitney test results for color response revealed significant differences ($p < 0.05$) among several treatment pairs. Significant differences were observed between P_0D_0 (control + 0 mL L⁻¹) and P_0D_2 (control + 2 mL L⁻¹), P_0D_0 and P_0D_3 (control + 3 mL L⁻¹), P_0D_1 (control + 1 mL L⁻¹) and P_0D_2 , and P_0D_2 and P_0D_3 . Similarly, significant differences were identified among treatments at 8 MST, including P_2D_0 (8 MST + 0 mL L⁻¹) compared with P_2D_1 (8 MST + 1 mL L⁻¹), P_2D_2 (8 MST + 2 mL L⁻¹), and P_2D_3 (8 MST + 3 mL L⁻¹), as well as between P_2D_1 and P_2D_3 and between P_2D_2 and P_2D_3 . In addition, significant differences were observed between P_0D_2 (control + 2 mL L⁻¹) and P_1D_2 (4 MST + 2 mL L⁻¹), P_0D_3 (control + 3 mL L⁻¹) and P_1D_3 (4 MST + 3 mL L⁻¹), and between P_1D_3 (4 MST + 3 mL L⁻¹) and P_2D_3 (8 MST + 3 mL L⁻¹). These results indicate that the treatments significantly affected the color attributes of stevia leaves.

Table 2. Results of the Mann Whitney test analysis of stevia color levels

Treatment	<i>p</i> -value	Notes
$P_0D_0 - P_0D_1$	0,181	no difference
$P_0D_0 - P_0D_2$	0,000	there are changes
$P_0D_0 - P_0D_3$	0,009	there are changes
$P_0D_1 - P_0D_2$	0,000	there are changes
$P_0D_1 - P_0D_3$	0,104	no difference
$P_0D_2 - P_0D_3$	0,000	there are changes
$P_1D_0 - P_1D_1$	0,218	no difference
$P_1D_0 - P_1D_2$	0,896	no difference
$P_1D_0 - P_1D_3$	0,423	no difference
$P_1D_1 - P_1D_2$	0,263	no difference
$P_1D_1 - P_1D_3$	0,669	no difference
$P_1D_2 - P_1D_3$	0,499	no difference
$P_2D_0 - P_2D_1$	0,045	there are changes
$P_2D_0 - P_2D_2$	0,047	there are changes
$P_2D_0 - P_2D_3$	0,000	there are changes
$P_2D_1 - P_2D_2$	0,849	no difference
$P_2D_1 - P_2D_3$	0,000	there are changes
$P_2D_2 - P_2D_3$	0,000	there are changes
$P_0D_0 - P_1D_0$	0,288	no difference
$P_0D_0 - P_2D_0$	0,372	no difference
$P_0D_1 - P_1D_1$	0,332	no difference
$P_1D_1 - P_2D_1$	0,171	no difference
$P_0D_2 - P_1D_2$	0,004	there are changes
$P_1D_2 - P_2D_2$	0,728	no difference

$P_0D_3 - P_1D_3$	0,012	there are changes
$P_1D_3 - P_2D_3$	0,000	there are changes

Sensory evaluation of stevia leaf infusion color indicated that treatment P_2D_3 (shoot pruning at 4 weeks after planting combined with 3 mL eco enzyme application) produced the highest color score (3.79), corresponding to a yellow color category. This result suggests that the treatment generated a more visually appealing infusion, which was likely preferred by the panelists. Panelists' preference for the color of stevia leaf extract showed relatively low variation, with scores ranging from 2.88 to 3.79, representing color intensity from slightly yellow to yellow. The yellow coloration observed under treatment P_2D_3 may be associated with increased concentrations of chlorophyll and phenolic compounds in stevia leaves resulting from shoot pruning and eco enzyme application. Stevia leaves contain a complex mixture of pigments, primarily chlorophyll and carotenoid derivatives, including β -carotene and xanthophylls such as α - and β -cryptoxanthin. Thin-layer chromatographic analysis reveals yellow spots with high R_f values, identified as β -carotene and cryptoxanthin, whereas chlorophyll a and b appear as blue-green and yellow-green spots rather than pure yellow, consistent with their spectral properties. Thus, the dominant yellow coloration is more appropriately attributed to carotenoids, while chlorophylls mainly contribute green to yellow-green tones (Fominykh *et al.*, 2020). In addition, flavonoids and tannins exhibit colors ranging from pale yellow to brown and can further influence the appearance of extracts, particularly in polar solvents such as ethanol or methanol (Nuryandani *et al.*, 2024; Singh *et al.*, 2025).

3.3 Aroma

The normality test indicated that the combined treatment of shoot pruning and eco enzyme application produced p-values lower than 0.05, suggesting that the sensory data for stevia aroma were not normally distributed. Therefore, a non-parametric Kruskal–Wallis test was applied. The mean organoleptic scores for aroma under different shoot pruning and eco enzyme treatments are presented in Fig. 1. As shown in Fig. 1, treatment P_0D_3 (no shoot pruning with 3 mL eco enzyme concentration) exhibited the highest aroma score (3.92), corresponding to a neutral aroma perception. Overall, panelists' responses to stevia aroma showed relatively low variation, with scores ranging from 3.21 to 3.92, indicating a generally neutral aroma profile. The Kruskal–Wallis test at a 95% confidence level revealed a significant effect of treatment on aroma response ($p < 0.05$), indicating significant differences among treatments. Pairwise comparisons were subsequently conducted using the Mann–Whitney test, and the results are presented in the following table.

Table 3. Results of the Mann Whitney test analysis of stevia aroma levels

Treatment	p-value	Notes
$P_0D_0 - P_0D_1$	0,133	no difference
$P_0D_0 - P_0D_2$	0,373	no difference
$P_0D_0 - P_0D_3$	0,005	there are changes
$P_0D_1 - P_0D_2$	0,307	no difference
$P_0D_1 - P_0D_3$	0,000	there are changes
$P_0D_2 - P_0D_3$	0,000	there are changes
$P_1D_0 - P_1D_1$	0,484	no difference
$P_1D_0 - P_1D_2$	0,012	there are changes
$P_1D_0 - P_1D_3$	0,079	no difference
$P_1D_1 - P_1D_2$	0,004	there are changes
$P_1D_1 - P_1D_3$	0,021	there are changes
$P_1D_2 - P_1D_3$	0,522	no difference
$P_2D_0 - P_2D_1$	0,071	no difference
$P_2D_0 - P_2D_2$	0,943	no difference
$P_2D_0 - P_2D_3$	0,178	no difference
$P_2D_1 - P_2D_2$	0,047	there are changes
$P_2D_1 - P_2D_3$	0,002	there are changes
$P_2D_2 - P_2D_3$	0,176	no difference
$P_0D_0 - P_1D_0$	0,231	no difference
$P_0D_0 - P_2D_0$	0,622	no difference
$P_0D_1 - P_1D_1$	0,791	no difference
$P_1D_1 - P_2D_1$	0,913	no difference
$P_0D_2 - P_1D_2$	0,050	there are changes
$P_1D_2 - P_2D_2$	0,045	there are changes

$P_0D_3 - P_1D_3$	0,041	there are changes
$P_1D_3 - P_2D_3$	0,833	no difference

Based on Table 3, the Mann–Whitney test results for aroma response revealed significant differences ($p < 0.05$) among several treatment pairs. Significant differences were observed between P_0D_0 (control + 0 mL L⁻¹) and P_0D_3 (control + 3 mL L⁻¹), P_0D_1 (control + 1 mL L⁻¹) and P_0D_3 , and P_0D_2 (control + 2 mL L⁻¹) and P_0D_3 . At 4 MST, significant differences were identified between P_1D_0 (4 MST + 0 mL L⁻¹) and P_1D_2 (4 MST + 2 mL L⁻¹), as well as between P_1D_1 (4 MST + 1 mL L⁻¹) and P_1D_3 (4 MST + 3 mL L⁻¹). Similarly, significant differences were found among treatments at 8 MST, including P_2D_1 (8 MST + 1 mL L⁻¹) compared with P_2D_2 (8 MST + 2 mL L⁻¹) and P_2D_3 (8 MST + 3 mL L⁻¹). In addition, significant differences were observed between P_0D_2 (control + 2 mL L⁻¹) and P_1D_2 (4 MST + 2 mL L⁻¹), between P_1D_2 (4 MST + 2 mL L⁻¹) and P_2D_2 (8 MST + 2 mL L⁻¹), and between P_0D_3 (control + 3 mL L⁻¹) and P_1D_3 (4 MST + 3 mL L⁻¹). These results indicate that the treatments significantly affected the aroma characteristics of stevia leaves.

Sensory evaluation of stevia leaf infusion revealed that treatment P_0D_3 (no shoot pruning with 3 mL eco enzyme application) produced the highest aroma score (3.92), corresponding to a neutral aroma category. This result suggests that the treatment generated a relatively more acceptable aroma profile, which may have been preferred by the panelists. Panelists' responses to stevia aroma showed limited variation, with scores ranging from 3.21 to 3.92, indicating a generally neutral aroma perception. The aroma profile observed under treatment P_0D_3 may be associated with increased availability of volatile compounds released during the infusion process. These compounds, derived from plant metabolic processes, contribute to the characteristic aroma of stevia leaf infusions. Aroma is a key dimension of sensory quality and a major determinant of consumer acceptance and food choice, particularly in beverages and other low-sugar products sweetened with stevia. Volatile compounds released from the food matrix enter the nasal cavity either orthonasally during sniffing or retronasally during mastication and swallowing, then dissolve in the mucus layer and bind to olfactory receptors, triggering neural signals to the brain (Pu *et al.*, 2022). Only a small fraction of the thousands of food volatiles are truly odor-active and define the specific aroma character of a given product (Diez *et al.*, 2019). Stevia essential oil, rich in terpenoids and phenolic compounds, shows antioxidant and antimicrobial activities and therefore has potential as a natural preservative that can simultaneously provide a mild herbal aroma in foods (Sharma *et al.*, 2025). To improve sensory acceptance, formulators frequently employ aroma-masking strategies, such as blending with congruent flavors (e.g., citrus or other fruit notes) or applying encapsulation technologies to modulate flavor release and attenuate undesirable aftertastes (Roohinejad *et al.*, 2025).

3.4 Aftertaste

The normality test indicated that the combined treatment of shoot pruning and eco enzyme application produced p-values lower than 0.05, suggesting that the sensory data for stevia aftertaste were not normally distributed. Therefore, a non-parametric Kruskal–Wallis test was applied. The mean organoleptic scores for aftertaste under different shoot pruning and eco enzyme treatments are presented in Fig. 1. As shown in Fig. 1, treatment P₀D₃ (no shoot pruning with 3 mL eco enzyme concentration) exhibited the highest aftertaste score (3.67), corresponding to a moderately bitter perception. Panelists’ responses to stevia aftertaste showed relatively low variability, with scores ranging from 2.78 to 3.67, indicating aftertaste intensity from slightly bitter to moderately bitter. The Kruskal–Wallis test at a 95% confidence level revealed a significant effect of treatment on aftertaste ($p < 0.05$), indicating significant differences among treatments. Pairwise comparisons were subsequently conducted using the Mann–Whitney test, and the results are presented in the following table.

Based on Table 4, the Mann–Whitney test results for aftertaste revealed significant differences among several treatments ($p < 0.05$), indicating that the applied treatments significantly influenced the aftertaste of stevia leaf infusions. Sensory evaluation showed that treatment P₀D₃ (no shoot pruning with 3 mL eco enzyme application) produced the highest aftertaste score (3.67), corresponding to a moderately bitter perception. This finding indicates that, although stevia leaves are widely recognized as natural sweeteners, specific treatments may influence the residual taste perceived after consumption. Panelists’ responses to aftertaste showed moderate variation, with scores ranging from 2.81 to 3.67, representing aftertaste intensity from slightly bitter to moderately bitter. The increased intensity of the moderately bitter aftertaste observed in the P₀D₃ treatment may be attributed to the accumulation of glycosidic compounds in stevia leaves. Although these compounds contribute substantial sweetness, they can also impart a bitter or licorice-like aftertaste, particularly when the proportion of stevioside is higher than that of rebaudiosides. In this context, the absence of pruning may promote the production of a larger leaf biomass, potentially increasing stevioside concentration and thereby enhancing the perceived bitter aftertaste. In addition, the application of eco enzyme as a liquid organic fertilizer can act as a growth stimulant and has been reported to improve yield and product quality in stevia cultivation, which may indirectly influence the taste profile of the leaves (Wahyuni *et al.*, 2025). Several steviol glycosides are known to elicit a pronounced bitter and lingering aftertaste, especially stevioside and certain rebaudiosides, whereas Reb D and Reb M exhibit minimal bitterness and a more rapidly decaying aftertaste (Tao *et al.*, 2020). Taste receptor assays have shown that steviol glycosides activate the human bitter taste receptors hTAS2R4 and hTAS2R14, explaining the bitter and off-taste sensations perceived by some consumers (Hellfritsch *et al.*, 2012; Acevedo *et al.*, 2016). Beyond steviol glycosides, leaf polyphenols, tannins, and pigments in less refined stevia extracts also contribute to bitter, astringent, and “herbal/medicinal” flavor attributes (Silva *et al.*, 2020).

Table 4. Results of the Mann Whitney aftertaste analysis of stevia

Treatment	<i>p</i> -value	Notes
$P_0D_0 - P_0D_1$	0,351	no difference
$P_0D_0 - P_0D_2$	0,001	there are changes
$P_0D_0 - P_0D_3$	0,000	there are changes
$P_0D_1 - P_0D_2$	0,000	there are changes
$P_0D_1 - P_0D_3$	0,000	there are changes
$P_0D_2 - P_0D_3$	0,110	no difference
$P_1D_0 - P_1D_1$	0,042	there are changes
$P_1D_0 - P_1D_2$	0,007	there are changes
$P_1D_0 - P_1D_3$	0,722	no difference
$P_1D_1 - P_1D_2$	0,219	no difference
$P_1D_1 - P_1D_3$	0,029	there are changes
$P_1D_2 - P_1D_3$	0,003	there are changes
$P_2D_0 - P_2D_1$	0,965	no difference
$P_2D_0 - P_2D_2$	0,016	there are changes
$P_2D_0 - P_2D_3$	0,000	there are changes
$P_2D_1 - P_2D_2$	0,036	there are changes
$P_2D_1 - P_2D_3$	0,000	there are changes
$P_2D_2 - P_2D_3$	0,010	there are changes
$P_0D_0 - P_1D_0$	0,080	no difference
$P_0D_0 - P_2D_0$	0,102	no difference
$P_0D_1 - P_1D_1$	0,016	there are changes
$P_1D_1 - P_2D_1$	0,004	there are changes
$P_0D_2 - P_1D_2$	0,002	there are changes
$P_1D_2 - P_2D_2$	0,624	no difference

$P_0D_3 - P_1D_3$	0,000	there are changes
$P_1D_3 - P_2D_3$	0,000	there are changes

4. Limitations and future prospects

This study has several limitations that should be considered when interpreting the findings. The sensory evaluation involved 100 panelists, which may not fully represent broader consumer preferences, and the use of untrained panelists may have introduced subjectivity and variability in the assessment. The evaluation was limited to selected sensory attributes, namely sweetness, color, aroma, and aftertaste, while other important parameters such as texture, mouthfeel, and overall acceptability were not included. In addition, the hedonic scale provided subjective preference data without detailed descriptive sensory characterization, and environmental conditions during testing as well as individual differences in taste perception may have influenced the results.

Future research should involve a larger and more diverse group of panelists to enhance the generalizability of the results. The use of trained sensory panels and descriptive sensory analysis methods is recommended to obtain more comprehensive product characterization. Further studies should also include additional sensory attributes, integrate sensory evaluation with physicochemical analyses and consumer preference studies, and examine product stability and acceptance under different storage conditions to support product optimization and broader application.

5. Conclusions

This study shows that shoot pruning and eco enzyme application significantly affect the organoleptic characteristics of *Stevia rebaudiana* Bertoni leaves. Treatment without pruning with 3 ml/L eco enzyme produced the highest sweetness level and neutral aroma, while pruning at 8 weeks post-planting with 3 ml/L eco enzyme produced the best color. The aftertaste still tended to be bitter, although it was reduced in some treatments. Overall, eco enzyme application plays a role in improving the organoleptic quality of stevia, thus potentially supporting its development as a natural sweetener in healthy food products.

Declarations

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Authors' Contributions

Riska Wahyuni designed the research concept, conducted the literature search, data extraction, and analysis, and drafted the initial manuscript. Chairani Hanum assisted in data analysis and interpretation. Lisa Mawarni contributed to literature review and manuscript editing.

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Data Availability Statement

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request

Ethics Statement

Ethical approval was waived by the Ethics Committee of the Faculty of Agriculture, Universitas Sumatera Utara, because the study involved voluntary sensory evaluation with minimal risk and no collection of sensitive personal data. All participants provided informed consent before participating in the sensory evaluation. The study was conducted in accordance with the ethical guidelines of Universitas Sumatera Utara.

Consent to participate

Informed consent was obtained from all individual participants involved in the study.

Plant Guidelines

The collection and use of *Stevia rebaudiana* in this study complied with institutional and national guidelines.

Permission to Collect Plants

The plant materials used in this study were obtained from cultivated sources and no special permission was required for their collection.

Competing Interest

The authors declare that there no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication or falsification, double publication or submission, and redundancy, were observed by the author

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Figures

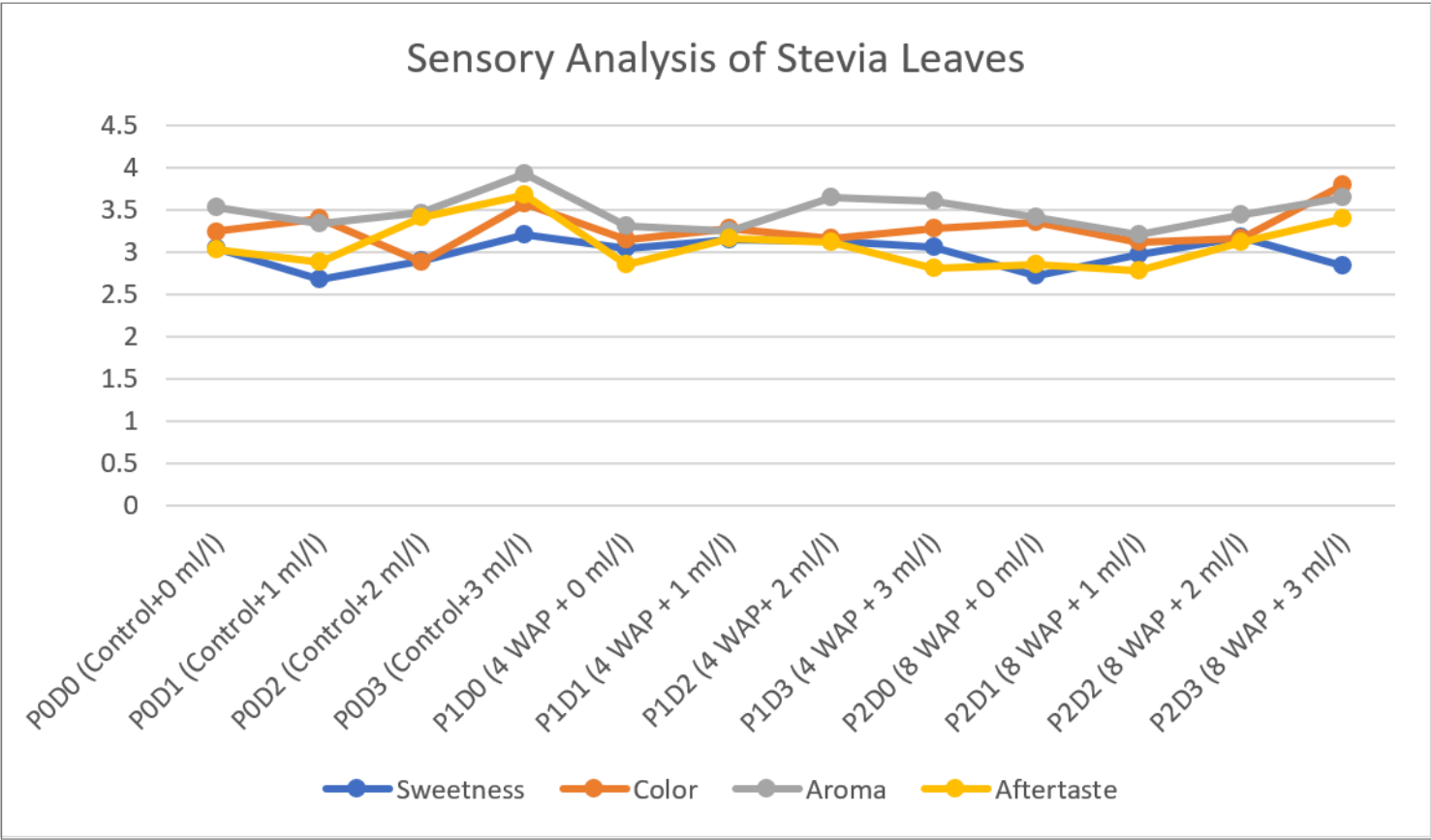


Figure 1

Sensory Analysis Results of Stevia Leaves