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Research Article

Efficiency of Tannin Reduction in Stevia Extract by Coagulation with Lime (CaO) and Adsorption with Cation Exchange Resin

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Abstract: Stevia, a plant commonly used as a natural sweetener, has the potential to be used as a sugar substitute because the stevioside content provides high sweetness and has a low caloric value. On the other hand, stevia contains tannins that can give it a bitter taste, complicating its use as a sweetener. This study investigates tannin reduction using coagulation with lime (CaO) followed by adsorption on a cation exchange resin. Lime was added to the stevia extract at concentrations of 0.5–6% (w/v) to form calcium-tannin precipitates, which were filtered and passed through resin columns. Tannin content was analyzed using spectrophotometry with Folin-Ciocalteu reagent, while sweetness was evaluated using % brix. Results indicate that 2% lime achieved a maximum tannin reduction of 99.8%, with a minimal sweetness reduction of 47.1%. Adsorption efficiency decreases over time, especially beyond 180 minutes, when efficiency drops to 67%. This two-step process effectively reduces tannin levels, improving stevia's flavor profile for use as a natural sweetener.

Keywords: cation exchange resin, coagulation, sweetness level, stevia, tannin

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a nutrient-rich natural sweetener plant from the *Asteraceae family*. Its leaves contain the diterpene glycosides stevioside, rebaudioside A-F, steviolbiosides, and glucosides, which impart sweetness. Stevia also has antioxidant, antimicrobial, antifungal, and anticarcinogenic activities, with fewer calories shows no side effects, and is non-toxic [1]. Therefore, stevia has great potential as a major source of low-calorie natural sweeteners to replace sugar [2].

The non-calorie value of stevia is suitable for people on a diet, diabetics, obese people, and phenylketonuria. In addition, the advantages of stevia leaves are high stability in acidic and alkaline media, heat stability (up to 200°C), light stability, and good solubility [2]. The content of steviol and the most glycosides extracted from *Stevia rebaudiana* leaves has a structural formula as shown in Figure 1.

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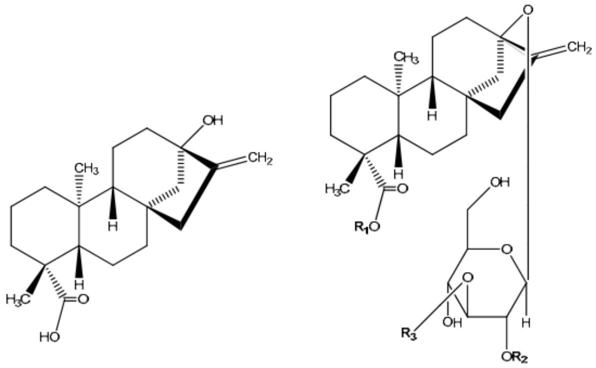

steviol steviol glycosides

Figure 1. Structure of Steviol Compounds and Glycosides [2]

On the other hand, stevia also contains tannins strongly present [1], which are polyphenolic compounds that can give food and beverages a bitter and astringent taste. The tannin content in stevia (*Stevia rebaudiana*) leaves varies depending on extraction methods, drying techniques, and environmental conditions. Previous research [2] extraction using 30% and 70% ethanol solvents obtained tannin levels of 0.0122% and 0.0123%. Another study reported that the tannin content of dried stevia leaves ranged from 5.2% to 6.0%, which was measured by the titrimetric method [3].

The presence of tannins in stevia can affect its flavor profile, making it less desirable in certain applications where a sweeter flavor without astringency is desired. This leads to research on methods to reduce the tannin content, such as using activated carbon for adsorption processes [2]. The tannin adsorption process using activated carbon has several disadvantages including being less effective for solutions with high concentrations, the effectiveness of adsorption is influenced by pH and temperature conditions and is highly dependent on the quality of activated carbon used. Repeated use of activated carbon also requires periodic replacement, which can increase operational costs [4] [5]. Other research in reducing tannin levels in sorghum found that alkaline solutions such as Na_2CO_3 (0.3%), can reduce tannins up to 77.46%[6]. The nature of tannins that can react with metal ions is the basis for the coagulation process using $Ca(OH)_2$ solution to precipitate tannin compounds dissolved in the extract.

Tannins are commonly found in compounds with a huge molecular weight of more than 1000 g/mol and can form complexes with proteins [3], [4]. The structure of tannin compounds consists of benzene rings that bind to hydroxyl groups (-OH). Tannins have a major biological role because of their function as protein precipitators and metal chelators [5]. Tannin contains phenolic hydroxyl groups (-OH) that can form hydrogen bonds or coordination complexes with metal cations such as Ca^{2+}. The nature of tannins that can react with metal ions is the basis for the coagulation process using $Ca(OH)_2$ solution to precipitate tannin compounds that are soluble in the extract. The -OH group interacts with Ca^{2+}, forming a coordination complex or chelate through a partial covalent bond. Calcium

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ions have a high coordination number (6-8), which allows multiple phenolic groups from one tannin molecule or multiple tannin molecules to coordinate with a single calcium ion . The adsorption reaction between Ca^{2+} metal ions and tannin is shown in Figure 2.

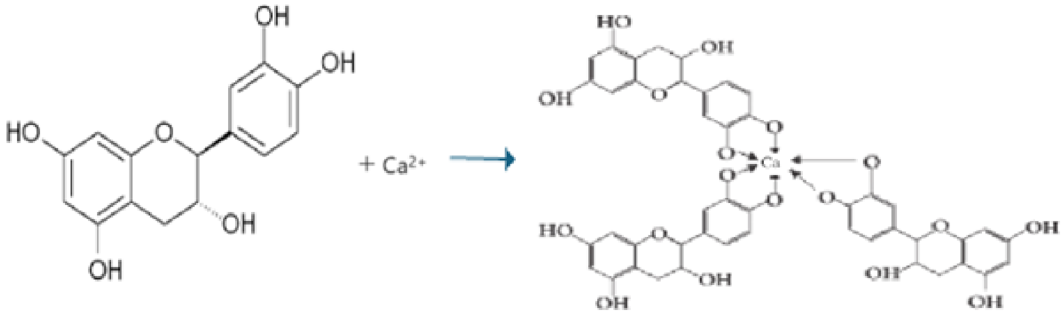

Figure 2. Coagulation Reaction of Tannins with Calcium Ions

The reaction of tannin with calcium ions involves the interaction of carboxylate (-COOH) and hydroxyl (-OH) groups of tannin with Ca^{2+} ions through the formation of ionic, coordination, or chelate complexes [7].

In contrast to previous studies, this study combines the coagulation method with $Ca(OH)_2$ and adsorption using ion exchange resins to provide more optimal results. This stage of the research involves adding lime (CaO) to the extract, which causes the dissolved Ca^{2+} ions to form a precipitate with the tannins. The tannin precipitate can be separated by decantation filtration. The next process is to bind the remaining calcium ions by an adsorption process using a cation exchange resin. Cation exchange resin is used to bind excess calcium ions that bind dissolved tannins in stevia extract.

This separation principle uses ion exchange chromatography technology to remove or reduce metal ions where the metal ions will be adsorbed into the resin [8]. Preliminary purification of the extract using ion exchange chromatography to give a primary extract of steviol glycosides of not less than 95% rebaudioside A [9]. This process involves the interaction between the resin and the calcium metal ions that bind to the tannin molecules, resulting in a decrease in tannin concentration in the extract. The adsorption study in the removal of condensed tannins in juice beverages suggests that the application of ion exchange resins in the removal of unwanted products can be considered as an alternative for tannin removal in beverages [10].

To measure the efficiency of reducing tannin levels through a combination of coagulation and adsorption processes, characterization of tannin levels before and after the process was carried out by spectrophotometric methods using folin ciocalteu reagent, as well as sweetness reduction tests by measuring the % brix value. The optimum condition of the process is the extract with maximum reduction in tannin content but minimum reduction in % brix.

Materials and Methods

Materials and instrumentation

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The tools used in this research are a glass column with an internal diameter of 3.0 cm and a height of 50 cm., a VIS spectrophotometer, and a brix refractometer.

The materials used are Simplicia/dried stevia leaves (*Stevia rebaudiana*), demineralized water, filter paper, lime (CaO), commercial ion exchange resin cation type DOW, Folin Ciocalteu phenol reagent, tannic acid p.a 20% sodium carbonate solution, and commercial stevia powder.

Extraction of Tannins in Stevia Leaves

Tannins cannot be extracted maximally if at low temperatures [7]. Stevioside extraction was carried out by boiling dry stevia leaves three times 10 minutes using demineralized water solvent with a ratio of dry stevia leaves: water, namely 1: 30 (w / v) at an extraction temperature of 100^0C for 30 minutes [11] [12].

Coagulation with $Ca(OH)_2$

A certain volume of stevia extract was added with lime at various concentrations: 0.5; 1; 1.5; 2; 2.5; 3; 4; 5; 6% w/v to the volume of extract, after stirring until homogeneous then allowed to stand for 12 hours. Filtering was done by decantation to separate the extract from the residue.

Calcium-tanine Adsorption with Resin

Cation exchange resin column preparation is done by first washing the resin with demineralized water 3-4 times until the pH is neutral. Then the resin was put into a glass column. Tannin extract from coagulation with filtered lime water is passed into the resin column, the filtrate is collected and then tested for tannin content and sweetness level.

Tannin Characterization

Determination of tannin content was carried out by visible spectrophotometric method using the Folin-Ciocalteu reagent. 0.1 mL of sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin Ciocalteu phenol reagent, 1 ml of 20% sodium carbonate solution, and diluted to 10 ml with distilled water. The mixture was homogenized and kept at room temperature for 30 minutes. As a standard solution, tannic acid (10, 20, 40, 60, 80, 100 mg/L) was prepared in the same way as the samples. The absorbance of the standard solution and sample was measured against the blank at 700 nm with a Visible spectrophotometer. [13].

Sweetness Level Test

Testing the degree of sweetness of the extract is done simply by measuring % brix using a brix refractometer.[14] [15].

Testing the degree of sweetness of a beverage/food is done by measuring % brix using a brix refractometer [11] [12]. Three drops of extract sample were dropped on a portable brix refractometer and the % brix was read.

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Results and Discussions

Stevia Extraction with Hot Water

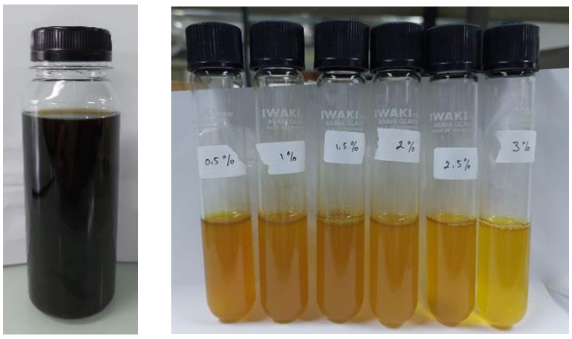
The extraction process is an important factor in the extraction process of stevia leaves. In preliminary research, gradual maceration showed that the level of stevia sweetness (%brix) decreased from the first fraction to the third fraction by 4.5; 1.3; and 0.8, respectively. In the fourth fraction, %brix was no longer readable so maceration was carried out three times with boiling for 10 minutes at a ratio of 1:30 (b/v).

Effect of Lime Concentration in Coagulation and Resin Column Adsorption Process on Tannin Content Reduction

Tannin secondary metabolite compounds from stevia can bind metal ions, due to the presence of hydroxyl groups that play a role in binding metal ions [16]. The lime used in this study is a CaO compound which when dissolved with extract stevia produces a calcium hydroxide base compound, $Ca(OH)_2$. The addition of $Ca(OH)_2$ increases the pH of the solution, which contributes to the precipitation of tannins.

$$CaO_{(s)} + H_2O \longrightarrow Ca(OH)_{2\,(l)}$$

$Ca(OH)_2$ is used as a coagulant in the processing of stevia extract through coagulation. The Ca^{2+} ions from calcium hydroxide will react with the tannins in the Stevia extract, producing a precipitate that can be removed from the solution by decantation or centrifuge filtration.



(a) (b)

Figure 3. View of Initial Stevia Extract After Boiling (a); View of Extract After Coagulation and Filtration (b)

After coagulation with lime water, the tannin content was reduced significantly where the higher the concentration of lime, the smaller the tannin concentration with the pH condition of the extract which is 10. The results of the extract after coagulation with lime and filtering are shown in Figure 3.

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(a) (b)

Figure 4. Resin chromatography column (a) and display of stevia extract after adsorption on resin column (b)

After passing through the adsorption process with the resin, the tannin content decreased quite sharply, a visual indication is shown by the disappearance of the yellow color of the tannin to colorless as shown in Figure 4 with the pH of the solution ranging from 6-8.

The initial stevia extract (1:30) has a tannin content of 1770.50 mg/L. The test was carried out by spectrophotometric method using tannic acid standard solution, the measurement was carried out at a maximum wavelength of 757 nm. In addition, the maximum wavelength of the stevia extract that has been coagulated with lime and the coagulated extract passed into the resin was also measured. The maximum wavelength of 1% stevia solution made from commercial stevia powder dissolved in water was also measured for comparison. It is shown in Table 1 that the wavelength of stevia extract that has been coagulated with lime is still close to the maximum wavelength of stevia extract, meaning that tannins are still contained in it. In contrast to the coagulated stevia extract that has been passed into the resin, the maximum wavelength is close to the maximum wavelength of the comparator stevia, meaning that the calcium ion-bound tannins have been adsorbed into the resin.

The results of the measurement of the tannin content of the coagulated extract in the variation of lime addition and then passed into the resin column are shown in Table 2. Measurements were made at a maximum wavelength of 757 nm.

Table 1. Comparison of Maximum Wavelength of Stevia Extract Before and After Coagulation/Adsorption

Sample	maximum wavelength (nm)
Stevia extract 1:30	757.03
Stevia extract after lime coagulation	721.97
Stevia extract after coagulation and adsorption	389.33
Stevia solution 1% b/v (commercial)	293.75

Table 2. Percentage Reduction of Tannin Content in the Initial Extract 1770.50 mg/L

Lime addition (%)	Tannin Content (mg/L) After coagulation	Percentage decrease of tannin content (%) after coagulation	Tannin Content (mg/L) After adsorption	Percentage decrease of tannin content (%) after adsorption
0.5	642.96	63.7	11.81	98.2
1	653.35	63.1	21.42	96.7
1.5	659.50	62.8	18.73	97.2
2	637.19	64.0	1.04	99.8
2.5	466.42	73.7	7.58	98.4
3	497.19	71.9	4.50	99.1
4	441.04	75.1	20.27	95.4
5	440.65	75.1	10.65	97.6
6	421.04	76.2	8.35	98.0

Based on **Table 2** and **Figure 5**, the extract that has passed through the resin column causes the percentage of tannin content reduction to reach 99.8%, namely in the addition of 2% lime.

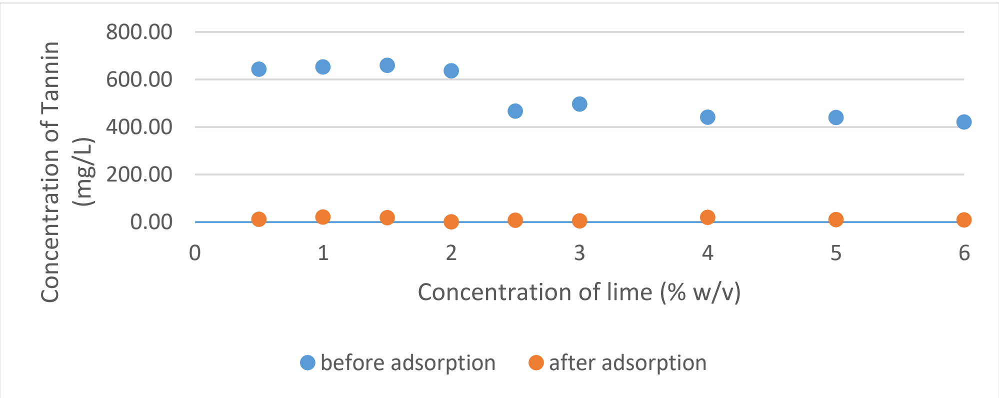

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Figure 5. Diagram of the Effect of Lime Concentration on the Decrease in Tannin Content Before and After Resin Adsorption.

Effect of Lime Concentration in Resin Column Coagulation and Adsorption Process on Sweetness Level Reduction

A small part of the stevioside content is adsorbed in the coagulation process. The optimum condition expected is a high decrease in tannin content but still contains stevioside which causes high sweetness. The initial extract before coagulation had a sweetness % brix of 1.4 and after coagulation with lime the % brix increased as tannins decreased, but at lime concentrations higher than 2%, the % brix value decreased. Testing the level of sweetness by looking at the decrease in %brix is shown in Table 3. This is in line with the % brix calibration test with commercial stevia powder standards showing that the higher the concentration of stevia, the higher the % brix.

Table 3. Effect of Lime Addition Concentration on % Brix Reduction Before and After Passing the Resin Column

% Brix	Lime addition (% w/v)	% brix		Percentage Decrease % brix (after coagulation and adsorption)
Before Coagulation (initial extract)		After lime coagulation	After resin adsorption	
1,4	0.5	1.4	0.7	50.0
	1	1.5	0.8	46.7
	1.5	1.6	0.8	50.0
	2	1.7	0.9	47.1
	2.5	1.8	0.8	55.6
	3	1.5	0.7	53.3
	4	1.3	0.6	53.8
	5	1.3	0.6	53.8
	6	1.3	0.6	53.8

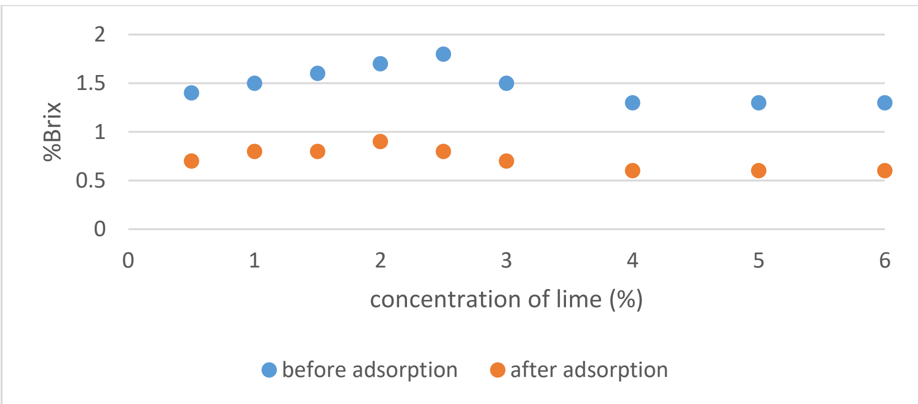

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Figure 6. Diagram of the effect of lime Concentration on % Brix Reduction Before and After Passing the Resin Column

It can be seen in Figure 6, that the addition of 2% lime water decreased the sweetness level by 47.1%, which is relatively small compared to other concentrations. Thus, the optimum concentration of lime addition in reducing tannin levels with a lower decrease in sweetness level is 2%.

Adsorption efficiency of calcium-tanine absorption by resin

Tannins in the extract form complexes with calcium ions during coagulation, which are later removed through ion exchange with the resin. The ion exchange resin adsorbs calcium-tannin complexes effectively due to the affinity between calcium ions and the functional groups of the resin. Adsorption efficiency decreases over time, especially beyond 180 minutes, when efficiency drops to 67% are shown in Figure 7. This is likely due to the saturation of resin active sites.

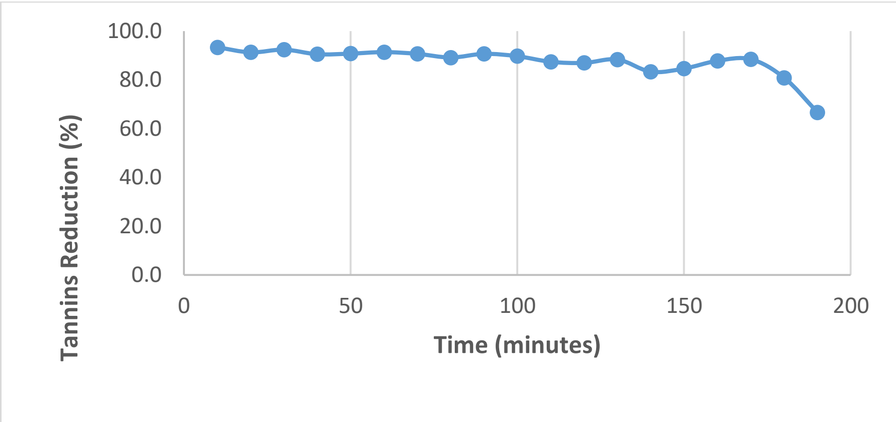

Figure 7. The Curve of Calcium-Tanine Reduction Efficiency Against Adsorption Time by Resin

The initial adsorption phase is rapid, suggesting surface adsorption as the primary mechanism. As time progresses, internal diffusion into the resin's structure could slow down the adsorption rate. The process likely aligns with the Langmuir adsorption model, where adsorption occurs at specific homogeneous sites on the resin until saturation.

The adsorption capacity is assessed by evaluating the reduction in tannin concentration. Maximum tannin removal efficiency is reported at 99.8% when 2% lime is used in the coagulation process. Beyond this optimal concentration, excess lime leads to inefficiencies due to decreased tannin concentration and lower interaction with the resin. The resin effectively reduces tannin content to a near-zero level after adsorption, with the final filtrate showing significantly reduced tannin levels and increased clarity.

Conclusion

The results showed that combining the coagulation method with lime water and adsorption with cation exchange resin can significantly reduce tannin content. This combination resulted in a tannin reduction efficiency of up to 99.8% with minimal retention of sweetness (% brix) of 47.1% at a lime (CaO) concentration of 2%. This approach offers a more effective solution for tannin removal while maintaining the quality of the final product, which has not been addressed in detail in previous studies.

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