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Efficiency of CaO Usage as a Pre-Clarification Adsorbent in Aqueous Stevia Extract

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Abstract

Stevia extract, when untreated, has a dark brown appearance, mainly because of the presence of impurities. CaO nanoparticles are effectively used as an adsorbent to purify the desired chemical compounds, decolorize the liquids, etc. in the food industry. In this study, the efficiency of CaO nanoparticle as a pre-clarification adsorbent was investigated for the removal of impurities in aqueous stevia extract before the purification process. For this purpose; dry stevia leaves were ground with distilled water (1:25, dry leaf:water, w/w) and kept in a water bath at 40 °C for 30 minutes. After the extraction process, the samples were centrifuged at 2900xg for 25 minutes at 25 °C. Then the extracts were treated with different concentrations of CaO. Steviol glycoside composition of the CaO-treated and untreated samples (control group) were analyzed by HPLC. According to the results, it was observed that the obtained stevia extracts were clearer (had lower turbidity values), especially when treated with CaO at the level of 0.15%, and no concentration loss occurred in terms of the glycosides analyzed in the extracts. Besides, the peak areas of both glycosides increased proportionally when CaO adsorbent was added to the main process. For further studies, investigation of other CaO-related parameters such as contact time, ambient conditions (pH, temperature, etc.) is recommended to be tried to increase the efficiency of this adsorbent in stevia processing.

1 INTRODUCTION

Food companies and consumers have particularly been interested in natural sweetening agents, which are generally nontoxic, having low calorific values, stable at different pH conditions and resistant to heat, also having no off-flavor after consumption. Thereby, the interest in stevia (*Stevia rebaudiana* Bertoni) plant which is accepted as a natural sweetener that is high in sweetness and harmless to the health in daily use increased in the last years. Also known as the ‘‘sweet herb of Paraguay’’ and “honey leaf”, the stevia plant is used for producing the natural sweetener of “Stevia” and exhibits anticarcinogenic (22, 25), antioxidant (24) and anti-hyperglycaemic (10) properties. The sweet flavor of this sweetener is attributed to its glycosides. The stevia leaves contain around 11 different steviol glycosides (3, 12). Among all, the main glycosides having higher sweetness intensity are Rebaudioside M (Reb M): 200–350 times more potent than sucrose (8), Stevioside tastes approximatively 300 times sweeter than 0.4% sucrose solution (7) is followed by Rebaudioside A (Reb A).

In general, water or organic solvents are used for the extraction of sweeteners from stevia leaves. The obtained extract is rich in sweetening glycosides but dark in color and turbid due to the suspended particles and plant materials such as color pigments, proteins, carbohydrates, and lipids; that infuse during extraction process (4). These suspended particles reduce the efficiency of the downstream operations for processing of stevia extract and provide impurity to the final products. Therefore, after extraction, clarification process is necessary for the removal of these particles from stevia extract for better consumer acceptance. For this purpose, different methods such as ultrafiltration (UF), microfiltration (MF), (5, 9, 16, 18) and coagulation with polyelectrolytes (2) are used. On the other hand, adsorption processes are widely employed in clarification (15, 20) because there is no necessity to use organic solvents at this stage. Many adsorbents have been examined to clarify the stevia extract such as alginate beads (1, 14), zeolites (15, 17, 20) and ion-exchange resins (6). In a study (19), a process was defined to obtain high purity Stevioside and Reb A by the usage of CaOH to remove impurities prior to decolorization process with

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amberlite beds. In another study (8), calcium oxide (CaO) was first being used for removal of the mechanical particles, proteins, polysaccharides, and coloring agents in stevia filtrate. CaO was also reported as a pH adjusting compound to increase membrane flux performance for stevia liquor together with ferrous sulfate ($FeSO_4$) coagulant (13) and as a precipitation aid to clarify the stevia extract (11).

When looking at the purpose of using CaO in stevia related studies; it is generally seen to be used as an auxiliary agent for the clarification process. Therefore, in this study, CaO was also considered as a pre-clarification agent and assessed its adsorbing performance to remove impurities in stevia extract without affecting steviol glycosides. In this experimental study, before clarification process, different concentrations of calcium oxide were implemented to the stevia extracts to observe clarification performance of this adsorbent. The optimal application dose was decided according to the increase in the concentration (according to the relative increase in the peak areas of the target steviol glycosides (Reb A and Stevioside) under analysis. After determining the optimal CaO amount, the stevia extracts were clarified with the combination of different agents (gelatin, bentonite and kieselsol) and the clarity efficiency of CaO pre-application was monitored with change in the turbidity values and in the concentrations of target steviol glycosides.

2 MATERIAL AND METHOD

2.1 Material

Dry leaves of (Levent 93) stevia variety was used as raw material for preparing the extracts. The fresh leaves were provided from the experiment area of the Field Crops Department located in the campus of Akdeniz University. Distilled water was used as solvent for the extraction process. Standard Reb A and Stevioside of 98% purity was obtained from M/s, Sigma-Aldrich, USA. Calcium oxide (analytical grade) was also obtained from a local laboratory chemical supplying company.

2.2 Methods:

2.2.1 Preparation of stevia extract

Dry stevia leaves were grinded with distilled water in a ratio of 1:25 (w/w). The extraction process was implemented at 40±1°C using a water bath for 30 minutes. Then the tubes were centrifuged at 25 °C for 30 minutes at the speed of 2900xg. The extract, cooled down to the room temperature, then was filtered through a filter paper. The filtered extract was used as the feed material for all the purification and clarification experiments.

2.2.2 Determination of the optimal adsorbing efficiency of CaO

CaO was added to the stevia extracts to adsorb impurities of other compounds. For this purpose, the optimal CaO concentration was determined. Accordingly; 100 mL of the filtered extracts were shed into the erlenmeyer flasks, different ratios (0.1-1.5%) of CaO were then added to samples. For each flask, the samples containing different concentrations of CaO, were mixed at 200 rpm in shaking incubator for 20 minutes. The supernatant was used for HPLC analysis of SGs. The extracts with no addition of CaO was considered as control group.

2.2.3 Clarification of CaO-added and non-added stevia extracts

In clarification process, three different clarifying agents (gelatin, bentonite and kieselsol) were used simultaneously. Stock solutions (5% for gelatin and bentonite, 15% for kieselsol) were prepared and added to the stevia extracts within the range of pre-determined concentrations (350 µL/70 mL for bentonite; 280-350-420 µL/70 mL for gelatin; 350-420-490 µL/70 mL for kieselsol) and then mixtures were incubated at 50 °C for 3 hours. At the end of the incubation period, the clarified extracts were separated into clean sample tubes and used for the analyses.

2.2.4 Determination of Steviol glycosides (SGs)

SGs present in the obtained aqueous extracts were identified and quantified by HPLC (Shimadzu, LC 20AD Series). Analysis conditions were as follows: Mobile phase: acetonitrile: 10mmol/L phosphate(sodium) Buffer (pH 2,6) (32:68) v/v; Analytical and protective column: Purospher® star Rp-18 endocapped (5µm), Injection volume: 10 µL, Flow Rate: 0,8 mL/min, Column temperature: 40 ºC, Detector: SPD-20A at 210nm (ambient temperature) (23). The efficiency of CaO treatment was evaluated according to peak area percentage of target SGs (Rebaudiside A: Reb A, Stevioside). For determining the optimal CaO concentration for adsorption efficiency, the peak area percentages of the samples were compared. It was calculated as the ratio of the peak area of the target glycoside to the total peak area of all other impurities detected in the chromatogram. For comparison of the effectiveness of CaO treatment on the clarified extracts, the quantitative amounts of SGs were calculated based on the external standard calibration technique.

2.2.5 Turbidity

Turbidity values of the clarified samples were determined in Nephelometric Turbidity Unit (NTU) value using a turbidimeter. After the calibration of the device using the standard turbidity solution, the samples transferred to the device-specific glass tubes were placed in the sample chamber of the turbidimeter and the hopper cover was closed. The measured values were recorded as the turbidity in NTU unit (21).

2.2.6 Statistical analysis

Data were analyzed using OriginPro 2019b statistical program. For comparison of all mean values, analysis of variance (ANOVA) test was used. This was followed by post hoc Tukey's HSD test to obtain multiple comparisons. The level of significance was set to be at $p < 0.05$.

3 RESULTS

3.1 Determination of the optimal CaO concentration prior to the clarification process

The peak areas of target SGs in the stevia extracts indicated that CaO treatment with varying concentrations was significantly effective to increase the concentration of SGs in the samples compared to the control group ($p < 0.05$).

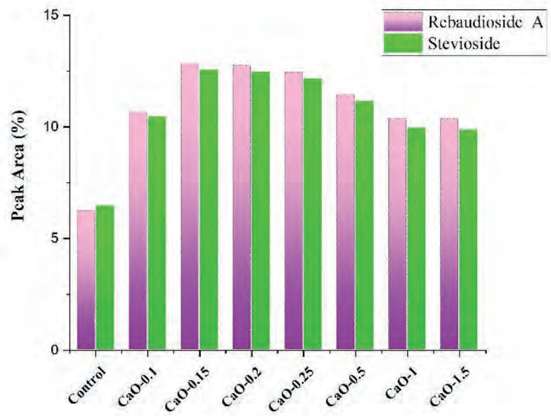

Figure 1. Variation in Reb A and Stevioside concentrations according to the added CaO amount in stevia extracts

On the other hand, the highest increase in the concentration of target SGs was observed in the treatment with 0.15% CaO for both Reb A and Stevioside (Figure 1). There was no difference between 0.15% and 0.2% CaO addition to the stevia extracts in terms of change in SGs concentration ($p>0.05$). The addition of 0.15% CaO to the stevia extract caused approximately 49% and 51% increase in the peak areas of Reb A (Control: 6.25%, 0.15% CaO: 12.85%) and Stevioside (Control: 6.45%, 0.15% CaO: 12.55%), respectively. Therefore, the optimal efficient adsorbing amount of CaO was determined as 0.15%.

3.2 Determination of the effective doses of the clarifying agents

The effectiveness of CaO pre-treatment before clarification process was monitored with turbidity values. Accordingly, only CaO application (CaO (SC): 17.90 NTU) alone was not enough to increase the clarity of the stevia extract. However, clarification process followed by CaO application was found more efficient to reach lower turbidity values (Table 1).

Table 1. Turbidity values of the stevia extracts during optimization of the clarification process

Samples				Sample Coding**	Turbidity (NTU)*
Control (*no CaO treatment and clarification*)				C	19.60^{b}±0.28
0.15% CaO (*no clarification*)				CaO	17.90^{c}±0.28
	Bentonite (µL/70 mL)	Gelatin (µL/70 mL)	Kieselsol (µL/70 mL)		
0.15% + Clarification	350	280	350	CaO-1	21.50^{a}±0.00
			420	CaO-2	15.65^{d}±0.07
			490	CaO-3	13.85^{f}±0.07
		350	350	CaO-4	12.60^{g}±0.00
			420	CaO-5	12.75^{g}±0.07
			490	CaO-6	8.02^{k}±0.01
		420	350	CaO-7	10.35^{i}±0.07
			420	CaO-8	8.72^{j}±0.01
			490	CaO-9	8.07^{k}±0.00
	Bentonite (µL/70 mL)	Gelatin (µL/70 mL)	Kieselsol (µL/70 mL)		
Control + Clarification	350	280	350	C-1	13.15^{g}±0.07
			420	C-2	13.65^{f}±0.07
			490	C-3	11.15^{h}±0.07
		350	350	C-4	15.50^{d}±0.14
			420	C-5	14.70^{e}±0.00
			490	C-6	13.95^{f}±0.07
		420	350	C-7	15.25^{d}±0.07
			420	C-8	10.50^{i}±0.14
			490	C-9	11.30^{h}±0.14

*: The values with different superscript letters in a column are significantly different ($p<0.05$)

With the adjustment of optimal doses for clarifying agents (350 µL/70 mL of bentonite; 350 µL/70 mL of gelatin, 490 µL/70 mL of kieselsol), the lowest turbidity value was observed in CaO-applied (0.15%) stevia extract (CaO-6: 8.02 NTU) compared to the other samples (Table 1). The results indicated that the usage of CaO adsorber as a pre-clarification treatment increased the efficiency of the clarification process.

3.3. The variation in the SGs concentration of CaO-applied and non-applied clarified stevia extracts

SGs concentrations and peak areas of the target SGs of the samples were analyzed statistically. According to the results, both CaO application and clarification process did not cause any loss in SGs concentration (for both Reb A and stevioside) ($p>0.05$; Table 2).

Table 2. Concentrations and peak areas of SGs in the clarified stevia extracts

Sample Coding*	Concentration of SGs (ppm)		Peak Areas of SGs (%)	
	Reb A	Stevioside	Reb A	Stevioside
C	479.83^{a}±5.23	482.67^{a}±5.09	6.43^{a}±0.00	6.47^{a}±0.00
CaO	462.70^{a}±16.09	463.26^{a}±16.89	12.79^{b}±0.12	12.54^{b}±0.28
CaO-6	458.04^{a}±10.78	449.83^{a}±0.82	13.16^{c}±0.05	12.73^{b}±0.01
C-6	473.86^{a}±0.66	473.57^{a}±1.45	6.50^{a}±0.07	6.52^{a}±0.01

*: The optimal doses of clarifying agents used for defined coded samples are as in Table 1
**: The values with different superscript letters in a column are significantly different ($p<0.05$)

In addition, when comparing the peak areas of SGs evaluated as an indicator of the removal of other impurities in the extract, it was observed that SGs in the stevia extract did not increase (Approximate increase in the peak areas were 1.09% and 0.77% for Reb A and Stevioside in "C" and C-6" coded samples) proportionally. This means that if stevia extract is clarified without CaO addition, there will be no additional purification for the target SGs. On the contrary, when the stevia extract was treated with CaO, the proportional peak area increased 98.9% for Reb A (calculated between "C" and "CaO" coded samples) and 93.8% for Stevioside (Table 2). Also, the compared data belonging to the CaO treated + clarified (CaO-6 coded) and only clarified (C-6 coded in Table 1) stevia extracts indicated that CaO application prior to the clarification process was more effective to remove other impurities. Namely, approximate increases in the peak areas of Reb A and Stevioside were observed as 101.7% and 95%, respectively. When the rates of peak areas increase were evaluated together (for C, CaO, C-6, and CaO-6 samples), it was seen that the CaO treatment contributed to the enrichment of the Reb A glycoside at a higher rate, although it enriched both glycosides analyzed. In conclusion, when applied before the clarification process, the Cao application contributes to the partial enrichment of these compounds in the extract by removing other impurities without causing any concentration loss in target steviol glycosides.

4 CONCLUSION

This study demonstrates that Calcium oxide (CaO) is an effective adsorbent for the enrichment of steviol glycosides by removing of other impurities in the extract. Stevia extract can be better clarified when CaO is used as a pre-clarification application. Also, CaO application may be recommended more where only Reb A glycoside is the main purpose to purify/isolate in the processes. However, although promising results were obtained in this study, potential future studies may be helpful to enhance the effect of this adsorbent more on stevia processing with some different process parameters such as the contact time of the CaO, and the effect of ambient conditions (pH, temperature, etc.).

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