



**BAYBURT
ÜNİVERSİTESİ**



ICADET

**4th INTERNATIONAL
Conference on Advanced
Engineering Technologies**

PROCEEDINGS

28-29-30 September 2022

BAYBURT / TURKEY

*Güvenli Şehrin
Huzurlu Üniversitesi*  www.bayburt.edu.tr

CONSULTATIVE COMMITTEE

Mutlu TÜRKMEN

Rector of Bayburt University

Metin UÇURUM

Dean of Engineering Faculty

ORGANIZATION COMMITTEE

Conference Chair

Ayla ARSLANER

Vice Chairs

Özlem YILMAZ

Track Chairs

Hamid YILMAZ	Özlem YILMAZ
İbrahim Hakkı KARAKAŞ	Recep ÇATAR
Naciye KUTLU KANTAR	Yunus KAYA
Özlem ÇAKIR	

Members

Aybike KAMILOĞLU	Kamil Emre GERÇEKASLAN
Didem GÜLERYÜZ	Murat ÇOLAK
Engin ERBAYRAK	Mustafa Alptekin ENGİN
Erhan SULEJMANI	Sara ALTUN GÜVEN
Duygu TEKİN	Şeyma EMEÇ
Gülşah ÇALIŞKAN KOÇ	Tariq AZİZ
	Tuğba ELBİR

Conference Secretaria

Duygu TEKİN	Tuğba ELBİR
Sara ALTUN GÜVEN	Yunus Emre ASAN
Şeyma EMEÇ	

SCIENTIFIC COMMITTEE

- Abdelkrim REBIAI**
El-Oued University
- Abdellah SALHİ**
University of Essex
- Abdulkadir Cüneyt AYDIN**
Atatürk University
- Abdulkerim DİLER**
Atatürk University
- Abdullah BAŞÇI**
Atatürk University
- Âdem AKSOY**
Atatürk University
- Âdem DEMİR**
Sakarya University of Applied Sciences
- Âdem KAYA**
Atatürk University
- Amjad Khansaheb BALANGE**
India CIFE
- Ahmad ABUSHATTAL**
Al Hussein Bin Talal University
- Ahmet AKKÖSE**
Atatürk University
- Ahmet ATASOY**
Sakarya University of Applied Sciences
- Ahmet BİRİNCİ**
Karadeniz Technical University
- Ahmet BUDAK**
Atatürk University
- Ahmet DODOĞLU**
Atatürk University
- Ahmet CANSIZ**
İstanbul Technical University
- Ahmet DUMLU**
Erzurum Technical University
- Ahmet KOLİP**
Sakarya University of Applied Sciences
- Ahmet Semih UZUNDUMLU**
Atatürk University
- Ahmet Şahin ZAİMOĞLU**
Atatürk University
- Ahmet TORTUM**
Atatürk University
- Ahmet YARTAŞI**
Çankırı Karatekin University
- Bülent ÇAVUŞOĞLU**
Atatürk University
- Burak EFE**
Necmettin Erbakan University
- Can HAŞİMOĞLU**
Sakarya University of Applied Sciences
- Cansu Ekin BONACINA**
Ankara University
- Caner OKUTAN**
Doğuş University
- Ali MORTAZAVI**
İzmir Democracy University
- Alırıza KALELİ**
Samsun University
- Alper Erdem YILMAZ**
Atatürk University
- Alperen TOZLU**
Bayburt University
- Amina RAKIDA**
Azerbaijan National Academy of Science
- Amine ASSOUGUEM**
Sidi Mohamed Ben Abdellah University
- Arzu Kavaz YÜKSEL**
Atatürk University
- Aslı İŞÇİ YAKAN**
Ankara University
- Aybike KAMİLOĞLU**
Bayburt University
- Ayla ARSLANER**
Bayburt University
- Ayşe DALOĞLU**
Karadeniz Technical University
- Ayşegül ABUŞOĞLU**
İstanbul Technical University
- Ayşe Nur YÜKSEL**
Kahramanmaraş İstiklal University
- Azra Skender**
University of Bihac
- Bahar BAYRAK**
Atatürk University
- Barbaros ATMACA**
Karadeniz Technical University
- Barış ŞİMŞEK**
Çankırı Karatekin University
- Baybars Ali FİL**
Balıkesir University
- Bilge DEMİR**
Karabük University
- Binnur GÜRÜL**
Gelişim University
- Beraat ÖZÇELİK**
İstanbul Technical University
- Burak DİKİCİ**
Atatürk University
- Erhan SULEJMANİ**
University of Tetova
- Ergün YILDIZ**
Atatürk University
- Erman Kadir ÖZTEKİN**
Bayburt University
- Ertan YILDIRIM**
Atatürk University
- Erol İSKENDER**
Karadeniz Technical University

Cemal KÖSE*Karadeniz Technical University***Cengiz TOKLU,***Beykent University***Charis GALANAKIS***Galanakis Laboratories***Cüneyt GÜLER***Mersin University***Çağatay TEKE***Bayburt University***Çağlar YÜKSEL***Atatürk University***Deniz Serkan Celalettin TAPKIN***Bayburt University***Deniz Turan KUNTER***Wageningen University***Derya DIŞPINAR***Foseco R&D***Didem GÜLERYÜZ***Bayburt University***Dursun ÖZYÜREK***Karabük University***Elif Feyza TOPDAŞ***Atatürk University***Elif DAĞDEMİR***Atatürk University***Elisângela Elena Nunes Carvalho***Federal University of Lavras***Emel ÖZ***Atatürk University***Emin MERCAN***Bayburt University***Emine ORHAN***Atatürk University***Emin USLU***Bayburt University***Emrah ÖZAHİ***Gaziantep University***Emre DEMİRER DURAK***Van Yüzüncü Yıl University***Engin DEMİRAY***Pamukkale University***Engin ERBAYRAK***Bayburt University***Ensar OĞUZ***Atatürk University***Erdal ÖNER***Bayburt University***Erdem CÜCE***Recep Tayyip Erdoğan University***Hakan TEMUR***Atatürk University***Hamdy Abdel-Hady ZAHRAN***Egypt NRC***Hamid YILMAZ***Bayburt University***Hanene AKROUT***Waste Water and Environment Laboratory***Erol ŞADOĞLU***Karadeniz Technical University***Erol YILDIRIM***Atatürk University***Ertuğrul ÇAM***Samsun University***Fatih DADAŞOĞLU***Atatürk University***Fatih ÇALIŞKAN***Sakarya University of Applied Sciences***Fatih Mehmet ÖZKAL***Atatürk University***Fatih ÖZ***Atatürk University***Fatih YILMAZ***Bayburt University***Fehim FINDIK***Sakarya University of Applied Sciences***Filiz Nuran ACAR***Atatürk University***Filiz YANGILAR***Erzincan Binali Yıldırım University***George Karatzas***Technical University of Crete***German Francisco de la Fuente***CSIC- CSIC-University of Zaragoza***Gökay AKKAYA***Atatürk University***Göker AKSOY***Işık University***Gökhan ÖZTÜRK***Atatürk University***Gülşah Çalışkan KOÇ***Uşak University***Gürsel ÖZKAN***Atatürk University***Güzin KABAN***Atatürk University***Hacer KAYA***Gümüşhane University***Hacı Süleyman GÖKÇE***Bayburt University***Hafize FİDAN***University Of Food Technology – Plovdiv***Haluk Çağlar KAYMAK***Atatürk University***Hakan Alper KAMİLOĞLU***Bayburt University***Mehmet ÇOPUR***Bursa Technical University***Mehmet Emin ARZUTUĞ***Atatürk University***Mehmet ERTUĞRUL***Atatürk University***Mehmet GAVGALI***Atatürk University*

Hasan ÖZCAN*Ankara Yıldırım Beyazıt University***Heba Hassan Abd El-Azim SALAMA***Egypt NRC***Huriye KADAKAL***Bayburt University***Hüseyin GENÇCELEP***19 Mayıs University***Hüseyin SERENCAM***Trabzon University***Hüseyin Yıldırım DALKILI***Erzincan Binali Yıldırım University***İbrahim Hakkı KARAKAŞ***Bayburt University***İbrahim Yücel ÖZBEK***Atatürk University***İhsan BAKIRCI***Atatürk University***İlyas UYGUR***Düzce University***İsmail DANABLI***İstanbul Technical University***İsmail ÖNDEN***TÜBİTAK TÜSSİDE***Ismayadi Bin İSMAİL***Universiti Putra Malaysia***Jacek SELEJDAK***Czestochowa University of Technology***Javier CAMPO***University of Zaragoza***Kağan Koray AYTEN***Erzurum Technical University***Kâmil Emre GERÇEKASLAN***Hacı Bektaş Veli University***Kübra ÇİNAR TOPÇU***Bayburt University***Luis Alberto Aguerol LAMBAN***University of Zaragoza***Mahmoud DarAssi***Princes Sumaya University for Technology***Mahir KADAKAL***Bayburt University***Maksym Grzywiński***Czestochowa University of Technology***Mehmet Ali KURT***Mersin University***Mehmet ÇELEBİ***Uşak University***Mehmet ÇINAR***Bayburt University***Mustafa YAŞAR***Karabük University***Mükerrem KAYA***Atatürk University***Nabil ADRAR***İstanbul Technical University***Naciye Kutlu KANTAR***Bayburt University***Mehmet Kerim GÜLLAP***Atatürk University***Mehmet Mutlu YENİSEY***İstanbul University-Cerrahpaşa***Mehmet ÖZYAZICIOĞLU***Atatürk University***Mehmet Sinan YILDIRIM***Celal Bayar University***Melek EKİNCİ***Atatürk University***Meltem Kızılca ÇORUH***Atatürk University***Memnune ŞENGÜL***Atatürk University***Meral OLTULU***Atatürk University***Metin UÇURUM***Bayburt University***Mohd Nizar HAMİDON***University of Putra Malaysia***Mourad HEBALI***Mustapha Stambouli University***Muhammet Vefa AKPINAR***Karadeniz Technical University***Muhammad SULAİMAN***Abdul Wali Khan University***Muhammed Yasin ÇODUR***Erzurum Technical University***Muhittin TURAN***Bayburt University***Murat ÇOLAK***Bayburt University***Murat KARAOĞLU***Atatürk University***Murat YAYLACI***Recep Tayyip Erdoğan University***Musa ARTAR***Bayburt University***Musa BUTE***Gaziantep University***Mustafa Alptekin ENGİN***Bayburt University***Mustafa Fatih ERTUGAY***Erzincan Binali Yıldırım University***Mustafa ACARER***Selçuk University***Mustafa ŞENGÜL***Atatürk University***Mustafa TAN***Trakya University***Ravish CHOUDHARY***ICAR-Indian Agricultural Research Institute***Recep ÇATAR***Bayburt University***Recep BONCUKÇUOĞLU***Cerrahpaşa University***Rıza POLAT***Atatürk Üniversitesi*

- Neşe Erol ERTUGAY**
Erzincan Binali Yıldırım University
- Neşe Şahin YEŞİLÇUBUK**
İstanbul Technical University
- Nur ERTEK TOSUN**
Atatürk University
- Serkan ŞENOCAK**
Atatürk University
- Oğuz Akın DÜZGÜN**
Atatürk University
- Okan Tarık KOMESLİ**
Atatürk University
- Osman KOLA**
Adana Alparslan Türkeş Sci. and Tech. Uni.
- Osman Nuri ŞARA**
Bursa Technical University
- Osman Ünsal BAYRAK**
Atatürk University
- Ömer CAN**
Bayburt University
- Ömer Faruk EFE**
Bursa Technical University
- Ömer YÜKSEK**
Karadeniz Technical University
- Özge ŞAKIYAN DEMİRKOL**
Ankara University
- Özgür ANIL**
Gazi University
- Özlem BARIŞ**
Atatürk University
- Özlem ÇAKIR**
Bayburt University
- Özlem YILMAZ**
Bayburt University
- Pier Paolo ROGGERO**
University of Sassari
- Rabi KARAALİ**
Bayburt University
- Rafet ASLANTAŞ**
Osmangazi University
- Ramazan ÇAKMAKÇI**
Onsekiz Mart University
- Ramazan KAÇAR**
Karabük University
- Ramazan YILMAZ**
Sakarya University of Applied Sciences
- Rajka BOŽANIĆ**
University of Zagreb
- Ravi PANDISELVAM**
ICAR-CPCRI
- Şule Akbaş BAŞAR**
Anadolu University
- Tariq AZİZ**
Jiangsu University
- Tamer Mohamed Ali El-Messery**
Egypt NRC
- Taner TEKİN**
Atatürk University
- Remzi ŞAHİN**
Atatürk University
- Rohini KARUNAKARAN**
AIMST University
- Sabriye Banu İKİZLER**
Karadeniz Technical University
- Safiye Nur DİRİM**
Ege University
- Said BROUMİ**
University Hassan II
- Saliha ERENTÜRK**
Atatürk University
- Salim ASLANLAR**
Sakarya University of Applied Sciences
- Seifeddine JOMAA**
Helmholtz-Centre for Environmental
- Samet ÇELİK**
Atatürk University
- Sebahattin NAS**
Pamukkale University
- Selda ÖRS CIRİK**
Atatürk University
- Senem Kamiloğlu BEŞTEPE**
Uludağ University
- Serkan BAYAR**
Atatürk University
- Serkan ŞENOCAK**
Atatürk University
- Servet KARASU**
Recep Tayyip Erdoğan University
- Sevinç GÜLSEÇEN**
İstanbul University
- Sezai ERCİŞLİ**
Atatürk University
- Sinan YAPICI**
İnönü University
- Songül ÇAKMAKÇI**
Atatürk University
- Süleyman GÜNDÜZ**
Karabük University
- Şahset İrdemez**
Atatürk University
- Şaziye Canan BÖLÜKBAŞI AKTAŞ**
Atatürk University
- Şenol GÜRİSOY**
Karabük University
- Şenol KÖSE**
Van Yüzüncüyıl University
- Şeref ORUÇ**
Karadeniz Technical University
- Ümmüğülsüm ERDOĞAN**
Bayburt University
- Vecihi AKSAKAL**
Bayburt University
- Veli SÜME**
Recep Tayyip Erdoğan University
- Volkan KAHYA**
Karadeniz Technical University

Tayfun DEDE*Karadeniz Technical University***Teodor ILIEV***University of Ruse***Tolga AKCAN***9 Eylül University***Tomas NECAS***Mendel university in Brno***Tuğba GENÇ KESİMCİ***Iğdır University***Türkay KOTAN***Erzurum Technical University***Ufuk AYDIN***Atatürk University***Uğur Cem HASAR***Gaziantep University***Ümit GİRSEL***Kahramanmaraş Sütçü İmam University***Ümit YILDIRIM***Bayburt University***Xinan Yang***University of Essex***Yahya TÜLEK***Pamukkale University***Yavuz GÜL***Sivas Cumhuriyet University***Yeliz TEKGÜL***Aydın Adnan Menderes University***Yunus KAYA***Bayburt University***Yusuf TUNÇTÜRK***Van Yüzüncüyıl University***Zekai TARAKÇI***Ordu University***Zeynep DURLU GÜL***Atatürk University***Zeynep Neşe KURT ALBAYRAK***Atatürk University*

CONTENTS

Keynote Speakers

Recent Applications in Thick Film Technology <i>Mohd Nizar HAMIDON</i>	1
R&D and Innovation in the Food Industry <i>Osman KOLA</i>	2
Mühendislik ve Teknoloji Felsefesi <i>Durmuş Günay</i>	3
The use of Biodiversity in Horticulture <i>Sezai ERCİŞLİ</i>	4
Mechanical property comparison of Al11Si wheels grain refined by Ti, Nb and Nucleant 1582 <i>Derya DİŞPİNAR</i>	9
Strain Engineering of Autochthonous <i>Saccharomyces Cerevisiae</i> by Genome Shuffling <i>Devarajan THANGADURAI, Ravichandra HOSPET, Jeyabalan SANGEETHA</i>	10
Concrete Road Design and Application <i>Muhammet Vefa AKPINAR</i>	11

Participants

Combustion Synthesis of NaSrVO ₄ : Nd ³⁺ Nano Powders with Enhanced NIR 1.056 µm Luminescent Performance for Solid State Laser and Bio- Imaging Applications <i>Kamni PATHANIA</i>	12
Uses of Black Rosehip in the Food Industry <i>Hojjat PASAZADEH, İlkey KOCA</i>	13
Change in Bioactive Properties During Drying of Black Rosehip Fruit <i>Hojjat PASAZADEH, İlkey KOCA</i>	14
Catalytic Activity of Clay Supported Nickel Catalyst for Toxic Dyes Reduction in Effluent of Chemical Industries <i>Şakir YILMAZ</i>	15
Evaluation of Processed Sausage Products Sold in the Market in Terms of Carcinogenic Volatile N-nitrosamines <i>Sena ÖZBAY</i>	21
An AI Based Formulated Feedback-System for Interpreting Conclusive Emotions for a Group of People <i>Ankita DIXIT, Mohit VASHISHTA, Karan GULERI</i>	28
Case Study of Demand Prediction in the Food Industry <i>Aslı BORU İPEK</i>	29
Tunnel-Soil-Structures Interaction Effect due to Seismic Loading <i>Abderrahim ACHOURI, Mohamed Nadir AMRANE</i>	36
A Nonthermal Emerging Technology for Reducing the Oil Absorption in Deep-Fried Foods: Ultrasound <i>Anahi Guadalupe COZAIN MONTIEL, Hilal TOMBULOĞLU, Sadettin TURHAN</i>	37
The Role of the Government Incentives in Remanufacturing <i>Mehmet ALEGÖZ</i>	42
Towards a Sustainable Future: Innovative Solutions for Food Systems <i>Ayşe Burcu ATALAY</i>	43
Impact of Different Ambient Temperatures on Egg-Laying and Hatching Parameters in Japanese Quail <i>Hüseyin Baki ÇİFTÇİ</i>	44
Efficiency of CaO Usage as a Pre-Clarification Adsorbent in Aqueous Stevia Extract <i>Ahmet HACIOĞLU, Hatice Reyhan ÖZİYİCİ, Mustafa KARHAN</i>	45
Effect of Substitution of Crushed Firebrick Wastes as a Partial Replacement for Natural Fine and Coarse Aggregate in Concrete <i>Mohammed KHATTAB, Samya HACHEMI, Hicham BENZETTA</i>	51
Open-circuit Faults and Short-circuit Faults for DC-DC Boost Converters <i>Fatma KHATER, Abderrezak AIBECHE, Sid Ali FELLAG</i>	57



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

Ahmet HACIOĞLU¹, Hatice Reyhan ÖZİYCI*², Mustafa KARHAN¹

¹ Akdeniz University, Faculty of Engineering, Department of Food Engineering, Konyaaltı, Antalya, Türkiye

² Antalya Bilim University, School of Tourism, Department of Gastronomy and Culinary Arts, Döşemealtı, Antalya, Türkiye

Keywords:

Stevia
CaO
Purification
Nanoparticle
Adsorption

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 approximately 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

*hatice.oziyaci@antalya.edu.tr

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 (FeSO₄) 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 °C, 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 ($p < 0.05$).

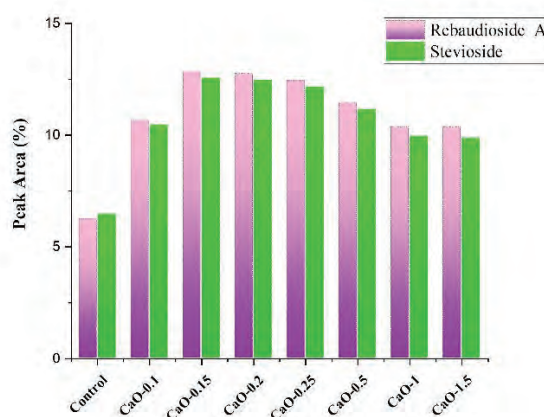


Figure 1. Variation in Reb A and Stevioside concentrations according to the added CaO amount in stevia extracts

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)*	
<i>Control (no CaO treatment and clarification)</i>			C	19.60 ^b ±0.28	
<i>0.15% CaO (no clarification)</i>			CaO	17.90 ^c ±0.28	
<i>0.15% + Clarification</i>	Bentonite ($\mu\text{L}/70\text{ mL}$)	Gelatin ($\mu\text{L}/70\text{ mL}$)	Kieselsoil ($\mu\text{L}/70\text{ mL}$)	CaO-1	21.50 ^a ±0.00
			350	CaO-2	15.65 ^d ±0.07
			420	CaO-3	13.85 ^f ±0.07
	350	350	490	CaO-4	12.60 ^e ±0.00
			350	CaO-5	12.75 ^e ±0.07
			420	CaO-6	8.02 ^k ±0.01
	420	420	490	CaO-7	10.35 ⁱ ±0.07
			350	CaO-8	8.72 ^j ±0.01
			420	CaO-9	8.07 ^k ±0.00
<i>Control + Clarification</i>	Bentonite ($\mu\text{L}/70\text{ mL}$)	Gelatin ($\mu\text{L}/70\text{ mL}$)	Kieselsoil ($\mu\text{L}/70\text{ mL}$)	C-1	13.15 ^g ±0.07
			350	C-2	13.65 ^f ±0.07
			420	C-3	11.15 ^h ±0.07
	350	350	490	C-4	15.50 ^d ±0.14
			350	C-5	14.70 ^e ±0.00
			420	C-6	13.95 ^f ±0.07
	420	420	490	C-7	15.25 ^d ±0.07
			350	C-8	10.50 ⁱ ±0.14
			420	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 $\mu\text{L}/70\text{ mL}$ of bentonite; 350 $\mu\text{L}/70\text{ mL}$ of gelatin, 490 $\mu\text{L}/70\text{ mL}$ of kieselsoil), 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.).

Acknowledgments

One of the authors of this study, Ahmet Hacıoglu, is supported by the Scientific and Technological Research Council of Turkey (TUBITAK-Domestic PhD Scholarship Program) and the Council of Higher Education Board (YOK-100/2000 PhD Scholarship Program).

References

- [1] F. S. Arakawa, C. R. Mahl, S. P. de Oliveira, G. da Igreja, M. R. Simões, and C. F. da Silva, “Clarification of Aqueous Stevia Extract Using Alginate Beads—Evaluation by Factorial Design Methodology”, *Adsorption Science & Technology*, vol. 30(2), pp. 147-158, 2012.
- [2] É. J. Bunhak, E. S. Mendes, N. C. Pereira and S. C. Costa, “Aplicação de polieletrólitos sintéticos na clarificação do extrato aquoso de estévia”, *Acta Scientiarum Technology*, vol. 24, pp. 1643-1648, 2002.
- [3] M. Carakostas, I. Prakash, A. D. Kinghorn, C. D. Wu and D. D. Soejarto, “Steviol glycosides”, in *Alternative Sweeteners*, Marcel Dekker, 2012, pp. 159–180.
- [4] C. S. Chhaya, "Primary clarification of stevia extract: a comparison between centrifugation and microfiltration", *Separation Science and Technology*, vol. 48, pp. 113–121, 2013.
- [5] C. S. Chhaya, “Clarification of Stevia extract by ultrafiltration: Selection criteria of the membrane and effects of operating conditions”, *Food and Bioproducts Processing*, vol. 90, pp. 525–532, 2012.
- [6] W. S. Fuh, and B. H. Chiang, “Purification of steviosides by membrane and ion exchange processes”, *Journal of Food Science*, vol. 55(5), pp. 1454-1457, 1990.
- [7] J. M. Geuns, (2003), “Stevioside”, *Phytochemistry*, vol. 64(5), pp. 913-921, 2003.
- [8] I. Prakash, A. Markosyan and C. Bunders, “Development of next generation stevia sweetener: Rebaudioside M”, *Foods*, vol. 3(1), pp. 162-175, 2014.
- [9] J. C. Martínez-Alvarado, B. Torrestiana-Sánchez and M. G. Aguilar-Uscanga, “Isolation of steviol glycosides by a two-step membrane process operating under sustainable flux”, *Food and Bioproducts Processing*, vol. 101, pp. 223-230, 2017.
- [10] P. B. Jeppesen, S. Gregersen, K. K. Alastrupp and K. Hermansen, “Stevioside induces antihyperglycaemic, insulintropic and glucagonostatic effects *in vivo*: Studies in the diabetic goto-kakizaki (GK) rats”, *Phytomed.*, vol. 9, pp. 9–14, 2002.
- [11] R. Kaushik, P. Narayanan, V. Vasudevan, G. Muthukumar and A. Usha, “Nutrient composition of cultivated stevia leaves and the influence of polyphenols and plant pigments on sensory and antioxidant properties of leaf extracts”, *Journal of Food Science and Technology*, vol. 47(1), pp. 27-33, 2010.
- [12] A. D. Kinghorn, N. C. Kim and D. H. L. Kim, “Terpenoid glycoside sweeteners”, in *Naturally Occurring Glycosides*, John Wiley & Sons, 1999, pp. 399–429.
- [13] X. Liu, N. Graham, T. Liu, S. Cheng and W. Yu, “A comparison of the coagulation performance of PAFC and FeSO₄ for the treatment of leach liquor from Stevia processing”, *Separation and Purification Technology*, vol. 255 (117680), pp. 1-12, 2021.

- [14] C. R. Mahl, S. P. de Oliveira, B. B. da Silva, G. da Igreja, M. R. Simões and C. F. Silva, "Effect of pH on the clarification of Stevia rebaudiana Bertoni extract using alginate beads", *Adsorption Science & Technology*, vol. 28(2), pp. 189-194, 2010.
- [15] I. C. C. Mantovaneli, E. C. Ferretti, M. R. Simões and C. Silva, "The effect of temperature and flow rate on the clarification of the aqueous Stevia-extract in a fixed-bed column with zeolites", *Brazilian Journal of Chemical Engineering*, vol. 21, pp. 449-458, 2004.
- [16] S. Mondal and S. De, "Prediction of ultrafiltration performance during clarification of stevia extract", *Journal of Membrane Science*, vol. 396, pp. 138-148, 2012.
- [17] E. P. Moraes and N. R. C. Machado, "Clarification of Stevia rebaudiana (Bert.) Bertoni extract by adsorption in modified zeolites", *Acta Scientiarum*, vol. 23(6), pp. 1375-1380, 2001.
- [18] M. H. M. Reis, F. V. Da Silva, C. M. G. Andrade, S. L. Rezende, M. R. Wolf Maciel and R. Bergamasco, "Clarification and purification of aqueous stevia extract using membrane separation process" *Journal of Food Process Engineering*, vol. 32(3), pp. 338-354, 2009.
- [19] S. Purkayastha, A. Markosyan and M. Malsagov, "Process for manufacturing a sweetener and use thereof", *US Patent* 8,337,927 B2, 2012.
- [20] F.V. Silva, R. Bergamasco, C. M. G. Andrade, N. Pinheiro, N. R. C. F. Machado, M. H. M. Reis, A. Alberto de Araújo and S. L. Rezende, (2007) "Purification process of Stevioside using zeolites and membranes". *International Journal of Chemical Reactor Engineering*, vol. 5(1).
- [21] S. Tajchakavit, J. Boye, D. Belanger and R. Couture, "Kinetics of haze formation and factors influencing the development of haze in clarified apple juice. *Food Research International*", vol. 34(5), pp. 431-440, 2001.
- [22] K. Toyoda, H. Matsui, T. Shoda, C. Uneyama, K. Takada and M. Takahashi, "Assessment of the carcinogenicity of stevioside in F344 rats", *Food and Chemical Toxicology*, vol. 35(6), pp. 597-603, 1997.
- [23] K. Turgut, M. Karhan, A. Hacıoglu, M. Yildiz and N. Unal, "Effects of different harvest times and leaf positions on steviol glycosides content in *Stevia rebaudiana bertoni* under mediterranean climate conditions", *Fresenius Environmental Bulletin*, vol. 30(9), pp. 10522-10529, 2021.
- [24] Y. Xi, T. Yamaguchi, M. Sato and M. Takeuchi, "Antioxidant mechanism of *Stevia rebaudiana* extract and antioxidant activity of inorganic salts", *Nippon Shokuhin Kagaku Kogaku Kaishi*, vol. 45(5), pp. 317-322, 1998.
- [25] A. Yamada, S. Ohgaki, T. Noda and M. Shimizu, "Chronic toxicity study of dietary stevia extracts in F344 rats", *Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi)*, vol. 26(2), 169-183_1, 1985.