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ELECTROCOAGULATION FOR D-PINITOL ENRICHMENT FROM CAROB EXTRACT: PLATE SELECTION

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Abstract

D-Pinitol (3-O-methyl-D-chiro-inositol), a bioactive compound holds promise for its anti-diabetic properties. However, synthesizing D-pinitol in laboratory settings is prohibitively expensive, prompting research to focus on extracting and purifying it from natural sources. This study introduces electrocoagulation (EC) as an innovative technique not previously explored for D-pinitol extraction from carob extract. EC facilitates the removal of charged compounds in colloidal suspensions by inducing collisions and neutralization with ions of opposite charges. The efficiency of this process largely depends on the types of plates used, as they vary in effectiveness based on the colloidal compounds in the solution.

This study evaluated aluminum (Al) and stainless-steel (SS) plates, commonly employed in EC research, to ascertain their effectiveness in the extraction process. Four distinct trials were designed, positioning each plate type at the anode and cathode ends within the EC system. The experiments maintained fixed operational parameters (Electrode interval: 3 cm, current density: 60 mA/cm^2, voltage: 30 V, and time: 60 min) in the carob extract from cultivated carob fruits grown in Antalya, Türkiye. The effectiveness of EC was assessed by determination of D-pinitol content (using HPLC separation and refractive index detection), colorimetric analysis of total phenolic content (using a spectrophotometer), color measurement (using a colorimeter), and turbidity measurement (using a turbidimeter) in the post-treatment

The study found that the Al (-) and SS (+) plate combination significantly enhanced D-Pinitol yield by 23%, increasing it from 17 to 42.36 g/100 g dry matter. Additionally, there were reductions in total phenolic content and turbidity and an improvement in color (L value).

The results indicate the success of EC as an intermediate process for D-Pinitol purification.

***Key words**: Carob, D-Pinitol, Electrocoagulation, Purification.*

1. Introduction

Electrocoagulation (EC) is a straightforward and efficient technology widely utilized in wastewater treatment to remove complex organic substances from liquids through adsorption, coagulation, sedimentation, and flotation mechanisms, as evidenced by studies from García-García *et al.*, [1], Inan *et al.*, [2], and Mollah *et al.*, [3]. The success of this process in purifying agrifood wastewater has been well-documented [4 - 9]. Despite the proven efficacy of EC in wastewater treatment, its direct application in the food industry has not been extensively explored. An exception is the pioneering study by Araya-Farias *et al.*, [10], which investigated electroflotation -a related process for apple juice clarification. Their findings indicate that the addition of gelatine to the electroflotation process significantly improves its efficiency, offering a more streamlined and effective clarification method that presents considerable benefits for the beverage industry. Complementing this, Qavami *et al.*, [11], studied an innovative approach with pulse electrocoagulation (PEC), marking a significant advancement in juice clarification technology. By fine-tuning the PEC parameters and employing minimal co-coagulants, their research achieved turbidity reduction to near-industrial standards, showcasing the potential of

EC technologies to revolutionize clarity and quality in the food industry. Similarly, Ogando *et al.*, [12], demonstrated that EC could be a sulfur-free alternative for sugarcane juice clarification, effectively removing phenolic compounds, turbidity, and color. Furthering this application, another study by Ogando *et al.*, [13], utilized aluminum electrodes in the EC process, achieving significant color and turbidity removal from sugarcane juice, which supports the use of EC as a cleaner alternative to traditional methods.

The enrichment and purification of bioactive compounds like D-pinitol from natural sources is a critical challenge in the pharmaceutical and food industries due to the high costs of synthetic production. Recognizing the need for an innovative and cost-effective purification method, this study introduces electrocoagulation (EC) as a promising technique for D-pinitol extraction from carob extract. The aim was to evaluate the effectiveness of EC, a method not previously explored for this purpose, and to determine the optimal electrode plate materials at fixed operational parameters. Utilizing aluminum and stainless-steel plates, the research meticulously controlled conditions such as electrode interval, current density, voltage, and time. The approach of this study was designed to be accessible to those unfamiliar with EC, explaining how the collision and neutralization of charged compounds could facilitate the purification process. The research tested whether EC could enhance D-pinitol yield, the impact of different electrode materials on extraction efficiency, and the identification of optimal EC parameters. The findings from this novel application of EC could potentially revolutionize the extraction and purification processes for bioactive compounds, making it a significant contribution to the field.

2. Materials and Methods

2.1 Research material: Fermented carob extract

Commercial carob concentrate was provided to produce the research material (fermented carob extract) for the study. The concentrate was obtained from a local organization that extracts cultivated carob fruits in Antalya province. Carob concentrate was first diluted to 5 °Bx value and then fermented using *Saccharomyces cerevisiae* (ATCC 36858) yeast, a special serotype that converts all the sugars in the medium into ethyl alcohol. The obtained fermented carob extract was then used in all the experiments for electrocoagulation plate selection. The fermentation environment was adjusted based on the conditions (Table 1) determined by Turhan *et al.*, [14]. The process was carried out in a bioreactor (Sartorius Stedim-Biostat C Plus, Goettingen, Germany) with a volume of 30 L.

Table 1. Compounds added to the fermentation medium

Compounds	Amount (g/L)
Yeast extract	6
Calcium chloride ($CaCl_2$ x $2H_2O$)	0.3
Ammonium sulfate ($(NH_4)_2SO_4$)	4
Magnesium sulphate ($MgSO_4$ x $7H_2O$)	1
Potassium dihydrogen phosphate (KH_2PO_4)	1.5

2.2 EC system design

An EC system has been designed for the EC of fermented carob extract. The system consisted of two interlocking chambers made of glass with a cooler inside except for the sample, as well as a chiller unit, a magnetic stirrer, a peristaltic pump, anode and cathode power cables, and a main power unit. The interior of the system is sized so that the surface area can accommodate fixed conductive plates. The linear current (DC) voltage and ampere values required by the system were provided by a rectifier operating between 0-200 Volts and 0 - 50 amperes. Since it was determined by preliminary trials that the samples heated up during the EC process, a cooling water bath was integrated into the peristatic pump to prevent volumetric sample loss. Thus, the temperature increase of the samples was prevented with the help of the refrigerant circulated from the cooling water bath with the help of a peristaltic pump.

2.3 Experiments for electrode plate selection in the EC process

An experiment was designed using different EC plates to determine the effect of the electrocoagulation process on the enrichment of D-pinitol in carob extract. Accordingly, aluminum (Al), and stainless steel (SS) electrode plates, which were observed to be used extensively in the studies of Muttaqin *et al.*, [15], and Mechelhoff *et al.*, [19], were decided to be used in the research. The decision on the fixed operating conditions (Electrode interval: 3 cm, current density: 60 mA/cm^2, voltage: 30 V, and time: 60 min) to be applied in the procedure was made by evaluating the results of the preliminary trials and the findings of the literature studies including various EC applications. Then, under these fixed conditions, EC experiments were performed in four different combinations [Al (+) / Al (-), SS (+) / SS (-), Al (+) / SS (-), Al (-) / SS (+)] as anode and cathode groups.

2.4 Analyses

2.4.1 Total dry matter

This analysis was performed to express the analyzed parameters on a dry matter basis. Accordingly, the liquid samples weighed in a volume of 10 mL were dried at 70 ^{0}C until they reached constant weighing in the oven [16].

2.4.2 Total phenolic content (TPC)

The spectrophotometric method defined by Spanos and Wrolstad [17], was used for the colorimetric determination of total phenolic content (TPC). For this purpose, 100 µL of samples were taken into a tube and 900 µL of distilled water was added to it. Then, 5 mL of 0.2N Folin-Ciocalteu solution and 4 mL of saturated Na_2CO_3 solution (75 g/L) were added, and the absorbance values were read at 765 nm wavelength in the spectrophotometer (Shimadzu UV-160A) by waiting for two hours after mixing the sample tubes. The TPC (mg/L) values were calculated using the previously prepared gallic acid standard curve.

2.4.3 Turbidity

The turbidity level in the samples was determined in terms of the Nephelometric Turbidity Unit (NTU) value using a turbidimeter (Hach 2100N). For this purpose, the samples were placed in the sample cell of the instrument and the cell cover was closed, then the measurement was made, and the reading value was recorded as turbidity [18].

2.4.4 Colour

Hunter Lab. UltraScan-VIS (Hunter Associates Laboratory Inc., USA) device was used to measure the color (L*a*b*) values of the samples. To evaluate the effectiveness of the EC process, the change in L values, representing darkness (L = 0) - lightness (L = 100), was observed [20].

2.4.5 D-pinitol

The amount of D-pinitol in the samples was determined by HPLC using the external standard method [21]. Shimadzu HPLC device (LC 20 AD), Transgenomic Nucleogel 87P column (3250x4.6 mm ID), and refractive index detector (RID) were used in the analysis. Milli-Q water (isocratic) was used as the mobile phase. The moving phase flow rate is set at 0.6 ml/min. Samples were injected after filtering through a 0.45 µm filter. The injection volume is set at 20 µL, the column furnace temperature is 85 ^{0}C, and the detector (RID) cell temperature is set at 60 ^{0}C.

2.4.6 Statistical analyses

The data of three repetitions per EC process were assessed by using the OriginPro 2019b statistical program (OriginLab Corporation, Northampton, Massachusetts). Analysis of Variance (ANOVA) and Tukey's HSD test (when necessary) were used at a significance level, P = 0.05 to interpret the average (Mean ± standard deviation) values.

3. Results and Discussion

As the criterion for determining the most effective electrode plate combination in the EC process, it was aimed to obtain the sample having the highest D-pinitol content, the lowest TPC and turbidity values, and the closest appearance to colorlessness (high L value) simultaneously.

The highest D-pinitol content (23.03 g/100 g Dry Matter - DM) was reached with Al (+) - Al (-) electrode plate combination compared to the non-electrocoagulated fermented carob extract (control group ($P < 0.05$) (Figure 1). However, since D-pinitol content was not the only criterion in determining the effectiveness of the electrocoagulation process, the TPC, turbidity, and L values of the samples were evaluated simultaneously with D-pinitol.

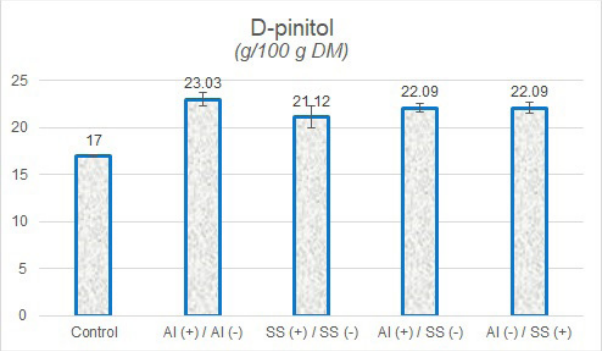

Figure 1. Electrode-induced variability in D-Pinitol content during electrocoagulation

Accordingly, since the electrocoagulation process facilitated the separation of impurities as flocs without affecting the D-pinitol content of fermented carob extract, the Al (-) / SS (+) electrode plate combination was determined as the best, providing the lowest turbidity (37.55 NTU) and highest L (L = 42.36) values, together with approx. 85% decrease in TPC (114.66 mg gallic acid/100 g DM) and higher D-pinitol content (22.09 g/100 g DM) compared to control (Figure 2).

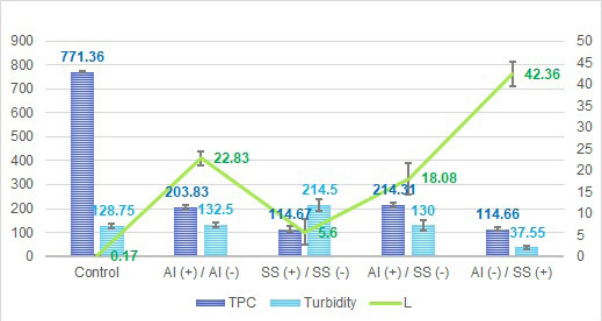

Figure 2. Variations of total phenolic content, turbidity, and L values depending on different electrode plates applied in electrocoagulation

In addition, in fermented carob extract treated with the Al(-)/SS(+) electrode plate combination, there was a 29.94% increase in D-pinitol (from 17 g/100 g DM to 22.09 g/100 g DM) concentration with the removal of non-D-pinitol impurities.

The findings of the current study, which utilized an Al(-)/SS(+) electrode plate combination for the extraction of D-pinitol from carob extract, are indicative of the broad applicability of electrocoagulation (EC) in the food industry. This is in line with the work of Araya-Farias *et al.*, [10], who employed electroflotation for apple juice clarification and found that additives could significantly improve the process. Similarly, Qavami *et al.*, [11], demonstrated the effectiveness of pulse electrocoagulation (PEC) in juice clarification, achieving substantial turbidity reduction. Ogando *et al.*, [12], further validated EC as a sulfur-free alternative for sugarcane juice clarification, effectively removing phenolic compounds and turbidity, a finding that was supported by their subsequent study in 2021 [13] using aluminum electrodes for significant color and turbidity removal. These studies collectively affirm the efficacy of EC in enhancing the quality and purity of food products, paralleling the current study's successful application of EC in purifying bioactive compounds, and suggesting a promising direction for future research and industrial applications.

The findings of this study not only corroborate the established benefits of EC in wastewater treatment but also extend its application to the extraction and purification of bioactive compounds. The Al(-)/SS(+) electrode plate combination presents a promising alternative to traditional methods, offering a cost-effective and environmentally friendly solution. However, it is important to acknowledge the limitations of the study, such as the scale of the experiment and the reproducibility of results on an industrial level. Future research should focus on scaling up the process and evaluating its economic viability for commercial applications, exploring the long-term stability of the EC process, the use of different electrode materials, and the operational parameters that could influence extraction efficiency.

In conclusion, the application of EC using the Al(-)/SS(+) electrode plate combination for D-pinitol extraction from carob extract represents a significant advancement in the field, with the potential to set a new standard for the purification processes within the industry and enhance the quality and purity of bioactive compounds in existing systems. The study contributes novel insights into the application of EC, paving the way for future research and industrial practice advancements.

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4. Conclusions

- This study has established electrocoagulation (EC) using the Al (-) / SS (+) electrode plate combination as a novel and effective method for the extraction of D-pinitol from carob extract. The evidence presented demonstrates that this approach not only improves the yield of D-pinitol but also enhances the purity of the extract by significantly reducing turbidity and total phenolic content. These findings address the initial problem of finding a cost-effective and efficient purification method for bioactive compounds, which has implications for both the pharmaceutical and food industries.
- The significance of this research lies in its potential to offer a more sustainable and economically viable option. However, questions remain regarding the scalability of the process and its application in different industrial settings. Future directions may include exploring alternative electrode materials, operational parameters, etc. to fully harness the benefits of EC for extraction of bioactive compounds.
- In summary, the study contributes a practical solution to bioactive compound purification and opens avenues for further innovation in the field.

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