BIORREFINERY, BIOECONOMY AND CIRCULARITY

RECOVERY OF PHENOLIC ACIDS FROM CORNCOB VIA EXTRACTION WITH ETHANOL/AMMONIUM SULFATE

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ABSTRACT

Phenolic acids are bioactive compounds for pharmaceutical and cosmetic applications, especially after esterification. Their production from natural sources is a hot research topic, and more efforts should be made to obtain them with high yield and purity. In this sense, the present study proposes aqueous two-phase systems (ATPS) ethanol/ammonium sulfate to recover and concentrate phenolic acids from corn cob via alkaline extraction. Conditions with greater amounts of ethanol and ammonium sulfate achieved higher recovery values but presented lower concentrations of phenolic acids in the top phase. The recovery of phenolic acids was indifferent to the alkaline extract dosage of up to 30% (w/w). Under optimized conditions (15% ethanol, 30% ammonium sulfate, and 30% extract; w/w), p-coumaric and ferulic acid recovery reached 90.5% and 89.7%, respectively. The post-ATPS top phase showed DPPH radical inhibition of 14.55%, surpassing the result of the initial alkaline extract (10.12%).

Keywords: p-coumaric acid. ferulic acid, alkaline hydrolysis, esterification.

1 INTRODUCTION

Progress has been made in using biomass of plant origin to obtain products with high added value over the years and, more recently, some for lignin. Lignin is a polyaromatic material expressed by plants to provide protection against biotic and abiotic stresses, and from an economic point of view, it can be treated as a solid fuel. The burning of lignin has been used to ensure energy self-sufficiency in industrial pulp and paper plants, and the surplus can be converted into electricity and sold to the grid[1]. Ferulic acid and p-coumaric acid are hydroxamic acids potentially used in the food, health, and cosmetics industries. In particular, these acids are recognized for their high antioxidant capacity, acting on skin damage, and reducing the occurrence of cancer. The compatibility of these acids in cosmetics and oils is improved after esterification, resulting in more hydrophobic compounds. Currently, these phenolic acids and derivatives are obtained from fossil sources and involve a series of chemical reactions with high carbon intensity. Although the route has been commercially consolidated for years, the origin of these phenolic acids conflicts with the global trend toward sustainability. In this scenario, lignin conversion could be the solution. Phenolic acids are associated with lignin by ether and ester bonds and can be depolymerized in an alkaline environment to release phenolic acids. This process is called alkaline extraction [2,3]. When derived from lignocellulosic biomass, the isolation of phenolic acids generally requires a series of steps. Removing unreacted lignin and xylan are among them, and conventional methodologies are non-trivial and time-consuming. Aqueous two-phase systems (ATPS) are well-known techniques in the biotechnology field and are more effective for recovering phenolic acids than adsorbents (resins or activated carbon). They also surpass adsorbents in terms of simplicity and speed of preparation. Short-chain alcohol/salt systems may be beneficial for esterification of phenolic acids. The system promotes the recovery and concentration of phenolic acid, while alcohols, such as methanol and ethanol, are the esterification reagents[4]. Therefore, if the alcohol/salt system is fed with an acidified stream, evaporation steps would be simplified, which could result in process intensification. In this sense, the present study investigated the generation and recovery of phenolic acids using ATPS. Corncob was used as raw material for alkaline extraction and ethanol and ammonium sulfate were chosen as phase formers. Different extraction operational conditions (chemical composition and percentage of extract) were evaluated on the recovery and concentration of phenolic acids.

2 MATERIAL & METHODS

Sodium hydroxide, acetic acid, sulfuric acid, ethanol, and ammonium sulfate were purchased from Synth (São Paulo, Brazil). Acetonitrile and (DPPH) were purchased from Sigma-Aldrich (MO, USA). Corn cobs were obtained from a corn processing farm in Lagarto, Sergipe. The corn cobs were first broken with a hammer before grinding in a TE-680 knife mill (TECNAL/Brazil) to a particle size of 20 mesh (~0.8 mm). The ground corncob sample was stored in plastic containers in a dry and dark place for future use. Phenolic acids were extracted from corn cobs using a methodology adapted from Maduzzi et al. (2024)[5]. In a 2 L beaker, 200 g of ground corn cob (on a dry basis) was mixed with 2 L of 4% (w/v) sodium hydroxide using a glass rod. The mixture was incubated in a thermostatic bath at 80 C with periodic shaking of the system. After 1 h of contact, the supernatant was collected by cloth filtration followed by vacuum filtration. Dilute sulfuric acid was added to the supernatant to adjust the pH to acidic (pH = 2) in order to remove lignin. Then, the pH was shifted to 7.0 with the addition of sodium hydroxide. Then, the concentration of

phenolic acids was evaluated by high-performance liquid chromatography (HPLC), and the supernatant was stored in amber vials in the refrigerator (- 4 °C). The preparation of ethanol/ammonium sulfate systems was carried out by mixing supernatant from the alkaline extraction of corncobs (pH 7.0), anhydrous ethanol, ammonium sulfate, and distilled water. The experiments were carried out in 15 mL conical tubes containing 5 g of total system mass, measured on an analytical balance. After weighing, the tubes were vortexed, and the phase volumes were recorded and collected. The recovery values of phenolic acids in the top phase, in percentage, were calculated by multiplying C_{ti} times V_t times DF times 100, divided by C_{ei} times V_e times DF. The terms C_{ti} and C_{ei} correspond to the concentrations of phenolic acid (p-coumaric acid or ferulic acid) in the top phase and the alkaline extract, respectively. The terms V_t e V_e correspond to the top phase and alkaline extract volumes, respectively. DF is the dilution factor used. Firstly, the phase composition was changed by combining different amounts of ethanol (15%, 20%, 25%, w/w), ammonium sulfate (15%, 17.5%, 20%, 22.5%, and 25%, w/w), while the supernatant was fixed at 10% (w/w). Notably, ammonium sulfate was prepared in a concentrated solution of 42% (w/w) (with the exception of some extract effect experiments). At another time, after choosing the best composition, the percentage of the extract was investigated in dosages from 5% to 55% (w/w) in order to avoid dilution of phenolic acids. Condition codes start with the use of the letter E followed by 2 digits. The first refers to the percentage (%v/v) of ethanol used, 1, 2 or 3, for 15%, 20% and 25%, respectively. The second digit indicates the amount of ammonium sulfate used (%w/w). Steps 2.5 for the amounts of ammonium sulfate was used. For E1 the quantities vary from 22.5% to 30%, with the code E11, E12, E13 and E14, respectively. The same logic applies to the other conditions. E2 ranging from 20.0% to 27.5% and E3 ranging from 17.5% to 25.0%. DPPH radical scavenging analysis was performed according to Padilha et al. (2021)[1]. Using ultrasound apparatus, a DPPH solution was prepared by dissolving 24 mg of DPPH (equivalent to 0.1 mM) in 100 mL of anhydrous ethanol. Furthermore, the solution was diluted again in anhydrous ethanol until the absorbance of the solution reached 0.4 at a wavelength of 517 nm. Aliquots of the DPPH solution (1 mL) were mixed with diluted samples of phenolic acids (200 μL) in test tubes, which were kept at room temperature for 10 min. Then, the absorbance was measured in a spectrophotometer at 517 nm. The phenolic acid samples comprise the initial alkaline extract and the top phase in the optimum condition and they were diluted 100 times in ethanol. The results were expressed in terms of percentage inhibition. The concentration of phenolic acids in the experiments was determined by HPLC analysis using CLC ODS C18 column (4.6 × 150 mm) and PDA detector. The analyses were carried out at an oven temperature of 35 °C and elution prepared with a gradient of 1% (v/v) acetic acid (A) and acetonitrile (B), according to Maduzzi et al. (2024)[5]. The calibration curves of p-coumaric acid (y (μg/mL) = 5.9067×106×area) and ferulic acid (y (μg/mL) = 9.9928×106×area) presented correlation coefficient (R2) values of 0.9998 and 0.9992, respectively.

3 RESULTS & DISCUSSION

Alkaline extraction proved effective in recovering phenolic acids from corn cobs, reaching 1.91 mg/mL for p-coumaric acid and 0.81 mg/mL for ferulic acid. Other phenolic compounds were also detected in the extract but in minor quantities. Some compounds detected were vanillin (0.09 mg/mL) and syringic acid (0.08 mg/mL). Regarding yield, p-coumaric acid and ferulic acid values correspond to 19.11 and 8.12 mg per gram, respectively. According to Padilha et al. (2024)[2], the alkaline environment (with ~4% sodium hydroxide) promotes the breaking of ester bonds between polysaccharides and lignin and ether bonds internal to lignin, ensuring the release of phenolic acids into the liquid medium. P-coumaric acid is associated with S units of lignin (abundant in cereal plants), while ferulic acid is responsible for the link between hemicellulose and lignin. Maduzzi et al. (2024)[5] suggest that the prior removal of polysaccharides (either by acid pretreatment or enzymatic hydrolysis) could lead to an extract with greater purity of p-coumaric acid at the expense of ferulic acid content. The phenolic acids were partitioned into the ethanol/ammonium sulfate system, and their composition effects are shown in Figure 1. In general, the recovery values of both phenolic acids increased with increasing tie-line length, i.e., with the increase in the amount of ethanol and ammonium sulfate in the system. The increase in ammonium sulfate favors the salting-out of solutes in the bottom phase, while the capture of phenolic acids is improved with the increase in the volume of the top phase. The recovery ranges of p-coumaric acid and ferulic acid were 73.50%-86.62% and 72.06%-85.47%, respectively. These values are in agreement with other reports in the literature on phenolic recovery by two-phase aqueous systems. Dhamole et al. (2014) applied cloud point extraction to alkaline corncob extract, and after optimization, they achieved p-coumaric acid and ferulic acid recovery results of 85% and 89%, respectively. Xavier et al. (2024)[6] reported phenolic acid recovery values between 67%-90% using systems with Tween 80 and choline chloride. Although recovery is undoubtedly a critical response, the subsequent fate of phenolic acids after a two-phase aqueous system demands a high solute concentration. These characteristics can be found in conditions with 15% (w/w) ethanol, specifically in condition E14. The condition offers a high concentration of phenolic acids (0.68 mg/mL for p-coumaric acid and 0.29 mg/mL for ferulic acid). At the same time, recovery values exceed 82%, showing no statistical differences concerning the highest average response values. Due to its distance from the single-phase region, the E14 condition also offers better stability than the other conditions with 15% (w/v) ethanol ethanol. For example, condition E11 did not form two liquid phases, so the extraction experiment was not performed (although it was in the region above the binodal curve).
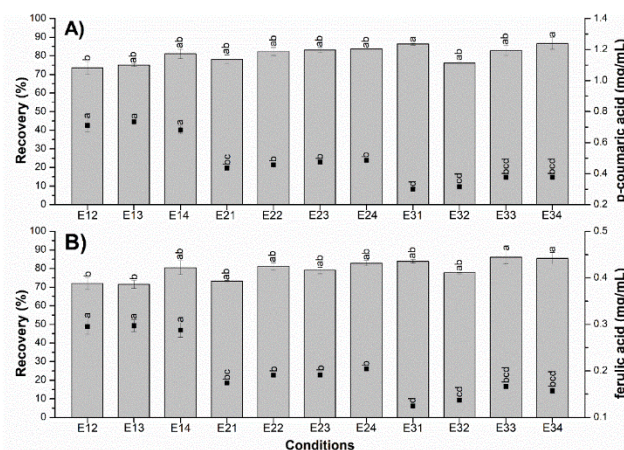
Figure 1 Effects of the composition of the ethanol/ammonium sulfate system on the recovery and concentration of p-coumaric acid (A) and ferulic acid (B) in the top phase of the ethanol/ammonium sulfate system. The experiments were carried out in conical tubes with a percentage of alkaline corncob extract of 10% (w/v), agitation time of 30 s, incubation time of 15 min, and room temperature (25 °C).

In order to improve the productivity of the process, different amounts of alkaline extract were tested. Figure 2 shows that the concentration of phenolic acids in the top phase directly correlated with the amount of extract added. For p-coumaric acid, the concentration rises from 0.36 mg/mL in 5% (w/w) extract to 3.31 mg/mL in 55% (w/w) extract. For ferulic acid, adding 55% (w/w) extract guarantees a solute concentration of 1.62 mg/mL, representing an increase of almost 10 times compared to the condition with 5% (w/w) extract. These data reinforce that the top phase, composed mainly of ethanol and water, offers a polar environment capable of easily solvating phenolic acids. Sugars and more hydrophilic compounds move to the salt-rich fraction, improving the purity of the phenolic acids in the top phase. The solubility of xylan is sensitive to the presence of organic solvents; therefore, it remains at the interface. Regarding recovery, choosing the condition with 55% (w/w) may represent solute loss, especially for p-coumaric acid. Recovery values reached a plateau of 90% in the range of 10%-30% (w/w) extract but declined to only 73% to 55% (w/w) extract. The excess solute in the top phase may have forced the exclusion of other solute molecules, as reported by Maduzzi et al. (2024)[5]. The top phase is mainly formed by ethanol and water, and the saturation values of p-coumaric acid are 10 mg/mL for anhydrous ethanol and only 0.2 mg/mL for water. These extract effects were less manifested for ferulic acid due to its lower content in the alkaline extract. In Figure 2B, it is possible to observe a decrease in the average recovery value of ferulic acid from 84.2% in the 30% (w/w) condition to 90.5% in 55% (w/w) extract, but without significant differences between the data ($p > 0.05$). Again, decision-making follows a balance between responses, and the 30% (w/w) extract condition meets these requirements.
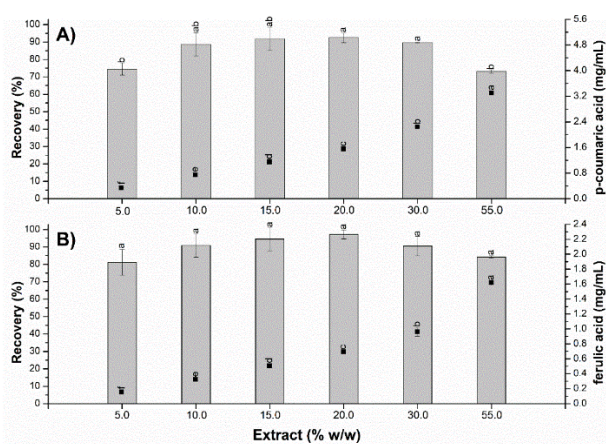

Figure 2 Effects of the percentage of alkaline corncob extract on the recovery and concentration of p-coumaric acid (A) and ferulic acid (B) in the top phase of the ethanol/ammonium sulfate system. The experiments were carried out in conical tubes with a composition of 15% (w/v) ethanol and 30% (w/w) ammonium sulfate (E14), agitation time of 30 s, incubation time of 15 min, and room temperature (25°C).

Antioxidant activity analyses were performed to confirm the migration and concentration of phenolic acids in the top phase. Using a 100-fold dilution in ethanol, the percentage of inhibition of the top phase reached 14.55%, while the response of the alkaline extract was only 10.12%. These results are attributed to the multiple hydroxyl groups in p-coumaric acid and ferulic acid, enabling the conversion of the DPPH radical into a stable reduced species. It is noteworthy that condition E14 used 30% (w/w) extract, yet the final concentration of phenolic acids in the top phase surpassed the initial alkaline extract. The perspective of future work is that more antioxidant activity tests will be carried out, mainly with lower dilution and after esterification of the acids.

4 CONCLUSION

Aqueous two-phase ethanol/ammonium sulfate systems have proven an effective way to recover and concentrate phenolic acids from corn cobs. P-coumaric acid and ferulic acid migrated to the top phase to the detriment of sugars and polysaccharides. The recovery values were still higher than 90% using low ethanol and higher extract dosages, which enables esterification processes to be carried out with the top phase.

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