Mixture design for the hydroalcoholic extraction of anthocyanins from purple elephant grass (*Cenchrus purpureus*)

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INTRODUCTION

Anthocyanins (ATC) are natural pigments belonging to the flavonoid class, widely recognized for their antioxidant activity and potential health benefits. These compounds have an increasing interest in the food, pharmaceutical, and cosmetic industries due to their bioactive properties and capacity to enhance the visual appeal of products[1]. Elephant grass (*Cenchrus purpureus*), traditionally valued for its biomass yield (> 250 $Mg.ha^{-1}.year^{-1}$), has recently attracted attention as a potential source of ATC. However, the characterization and optimization of extraction methods for these compounds remain underexplored[2].

This study focuses on optimizing the hydroalcoholic extraction of ATC from elephant grass leaves, emphasizing solvent composition and extraction time as key variables. Notably, the optimization process was conducted without the use of purified anthocyanin standards, relying instead on peak area as semi-quantitative responses to guide process development. To more accurately describe the relationship between solvent ratios and anthocyanin yield, non-canonical mixture design models were employed. These models enabled a more flexible and precise understanding of the extraction behavior, particularly under solvent conditions where traditional canonical models may fall short. Additionally, the optimal extraction time was accessed by UV-VIS spectroscopy, therefore this work provides a foundation for the valorization of elephant grass as a viable source of ATC and contributes to the design of efficient, scalable extraction protocols for functional product development.

RESULTADOS E DISCUSSÃO

Among the different mixture models tested, the non-canonical model

$$y' = b_1^* x_1 + b_2^* x_2 + b_{11} x_1^2 x_2 + b_{22} x_1 x_2^2 + b_{12} x_1^2 x_2^2$$

provided the best fit to the experimental data. This model exhibited no lack of fit, which was observed for classical canonical models; furthermore, this model outperformed other tested models by presenting the lowest RMSE and MAE, as evaluated using leave-one-out cross-validation, as well as the highest coefficient of determination (R^2). The model accurately captured the nonlinear interaction between water and ethanol in the extraction process reflecting the experimentally observed as can be seen in **Figure 1(B)**.

As shown in **Figure 1(A)**, the response surface for anthocyanin extraction highlights a distinct optimum region between 75% and 90% ethanol content, color and width stands for predicted area.

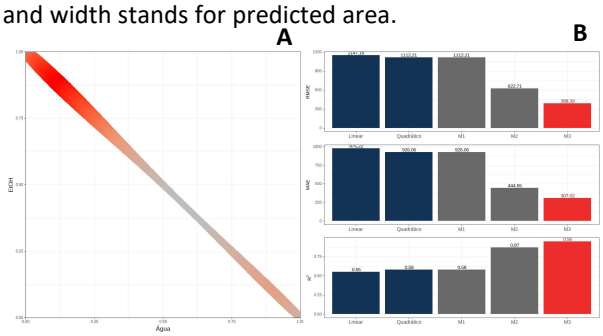

Figura 1. (A) Response curve for final non-canonical model. (B) Performance metrics for tested models

The predictive capability and agreement of the model with the experimental data reinforce its suitability for guiding solvent selection in anthocyanin extraction from elephantgrass, aiding in the development of efficient, targeted extraction strategies for bioactive-rich formulations.

CONCLUSÕES

Extraction was optimized using a non-canonical polynomial mixture model, which indicated 80–90% ethanol as the ideal solvent range. A 4-hour extraction optimized the rate, while 18 hours maximized anthocyanin content. These findings support future applications in ATC and provides a foundation for the valorization of elephant grass as a viable source of ATC.

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