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## Alternative extraction solvents and their protective effect of polyphenols in red grape pomace

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### Abstract

Currently, economic and ecological aspects represent the main objectives of the agri-food sector. Thus, the valorization of by-products resulting from processing represents an important solution both for increasing revenue and for reintegrating compounds with bioactive properties into the food matrix.

Red grape pomace (RGP) is a by-product that, due to its high polyphenol content with remarkable on health properties has attracted growing interest in terms of their recovery and utilization.

The main objective of the present study was to optimize the ultrasound-assisted extraction process of TPC from RGP using the response surface methodology. The independent variables studied for ethanol extraction were temperature, extraction time and ethanol concentration, respectively temperature, extraction time and solvent volume for natural deep eutectic solvents (NaDES) extraction.

The highest total polyphenol content (TPC) obtained in the ethanol-based experiment was  $465.81 \pm 1.28$  mg gallic acid equivalents (GAE) /100 g DW, at 35°C, for 22.5 minutes, and an 70% ethanol. For the NaDES extraction, the highest TPC value was  $414.04 \pm 0.80$  mg GAE /100 g DW, at 60°C, for 60 minutes and 10 mL solvent.

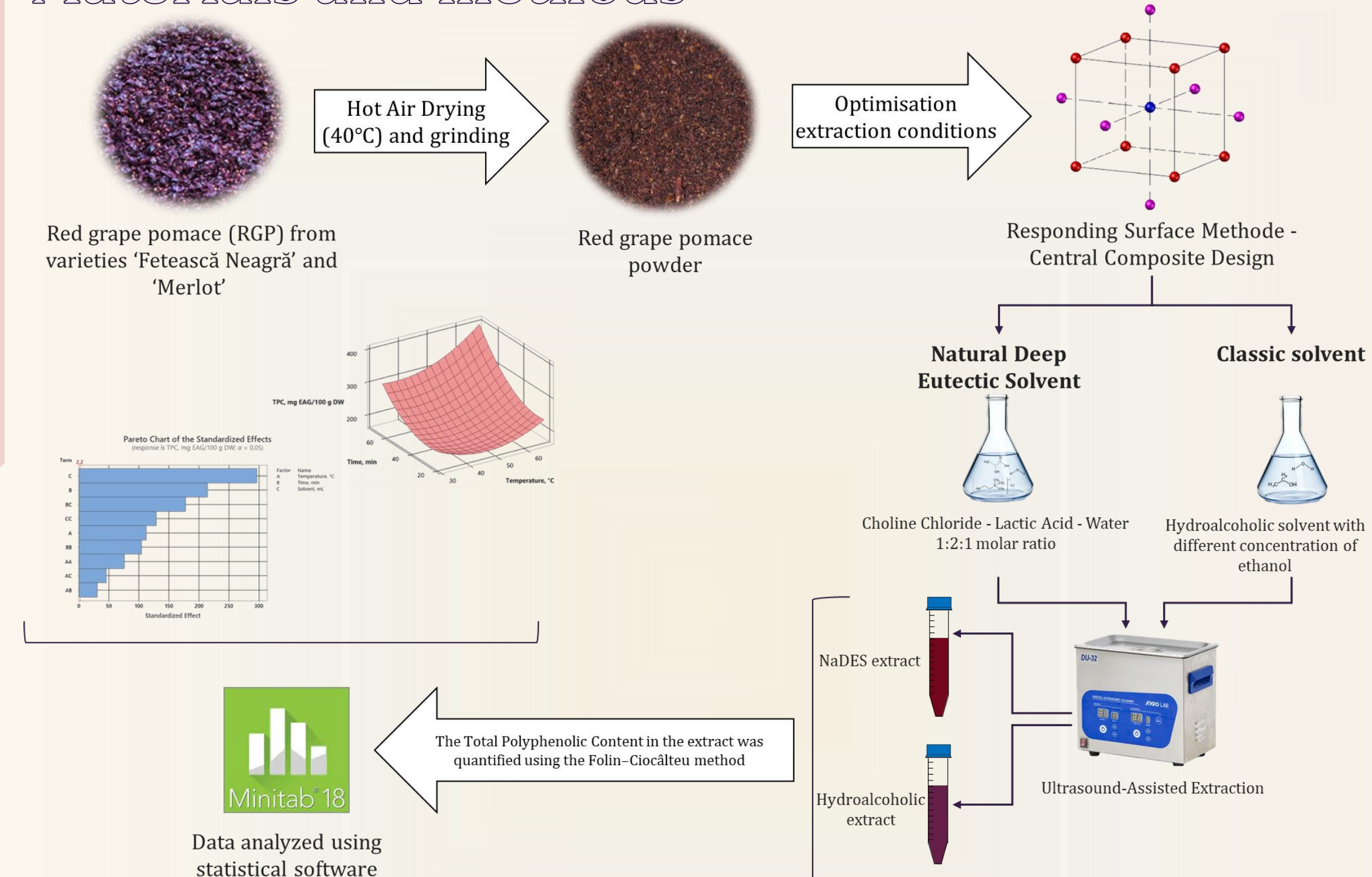
Although the temperature and extraction time were significantly higher for NaDES, the solvent comprising a mixture of choline chloride, lactic acid and water in a 1:2:1 molar ratio, provided superior protection for polyphenols under high thermal conditions. In contrast, in the case of extraction with 70% ethanol at 51.8°C and a short extraction time of 22.5 minutes, the polyphenol content significantly decreased to  $300.2 \pm 2.60$  mg GAE /100 g DW. The results are valuable in terms of establishing the optimum parameters for extraction, to enhance the bioactive concentration and extraction yields.

### Introduction

The valorization of red grape pomace (RGP) through the extraction of polyphenolic compounds represents both a challenge and an opportunity. Introducing these extracts in food matrices can lead to the development of sustainable, economical, high value-added products and provide health [1].

This study aims to identify the optimal conditions for developing a green ultrasound-assisted extraction method for polyphenolic compounds from the plant matrix, by applying a Central Composite Design (CCD), using a natural deep eutectic solvent (NaDES), with conventional ethanol extraction serving as the reference method.

### Materials and methods



#### Determination of Total Polyphenol Content (TPC)

The total polyphenol content of the extract was determined using the method described by Serea *et al* [2]. 0.2 mL extract, 15.8 mL of ultrapure water and 1 mL of Folin-Ciocalteu reagent were added in a test tube. After 10 minutes, 3 mL of a 20% (w/v) Na<sub>2</sub>CO<sub>3</sub> solution was added. The samples were then incubated in the dark at room temperature for 90 minutes. Absorbance was measured at 765 nm using a Biochrom; Libra 22 UV/Visible Spectrophotometer. The results were expressed as gallic acid equivalents per 100 g of dry weight (mg GAE/100 g DW).

### Conclusion

This study aimed to evaluate the advantages of using NaDES as an alternative to ethanol extraction, in the context of developing a green extraction approach. The two solvents used for the extraction of TPC from RGP exhibited significantly different behaviors under varying time and temperature conditions, differences that can be explained to their distinct compositions. Therefore, replacing ethanol with a NaDES represents a sustainable and efficient alternative, due to its comparable ability to extract high levels of polyphenols from the plant matrix. These findings support the potential application of NaDES in environmentally friendly processes for the valorization of agro-industrial by-products.

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### Results and discussion

- As can be seen in **Figure 1**, the particularities of the solvent influenced the extraction of polyphenolic compounds from RGP. The differences between the solvents used for polyphenolic compounds extraction from RGP showed a significant effect on the extraction efficiency.
- The maximum of TPC obtained during the hydroalcoholic extraction was recorded at a temperature of 35°C and a time of 22.5 minutes. Extraction conditions exceeding these values led to a decrease in the content of phenolic compounds, most likely due to their thermal degradation and oxidation.
- According to the Pareto Chart (**Fig. 2**), temperature (A), extraction time (B), solvent (C), interactions between these factors (AB, AC, BC), as well as their square terms (A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup>) significantly influenced the extraction of TPC in the case of the extract obtained with NaDES ( $p < 0.05$ ).

- In contrast, for the hydroalcoholic extract, temperature (A) and solvent (C) ( $p > 0.05$ ) did not have a significant effect on the extraction of these compounds from the plant matrix.
- It can also be observed that the interaction between the independent variables time (B) and solvent (C) exerted the most significant influence on TPC extraction in both extracts. This effect can be explained that the solvent at a constant temperature for a long time extraction leads to a reduction in its viscosity, thereby facilitating the prolonged migration of polyphenols from PGR.

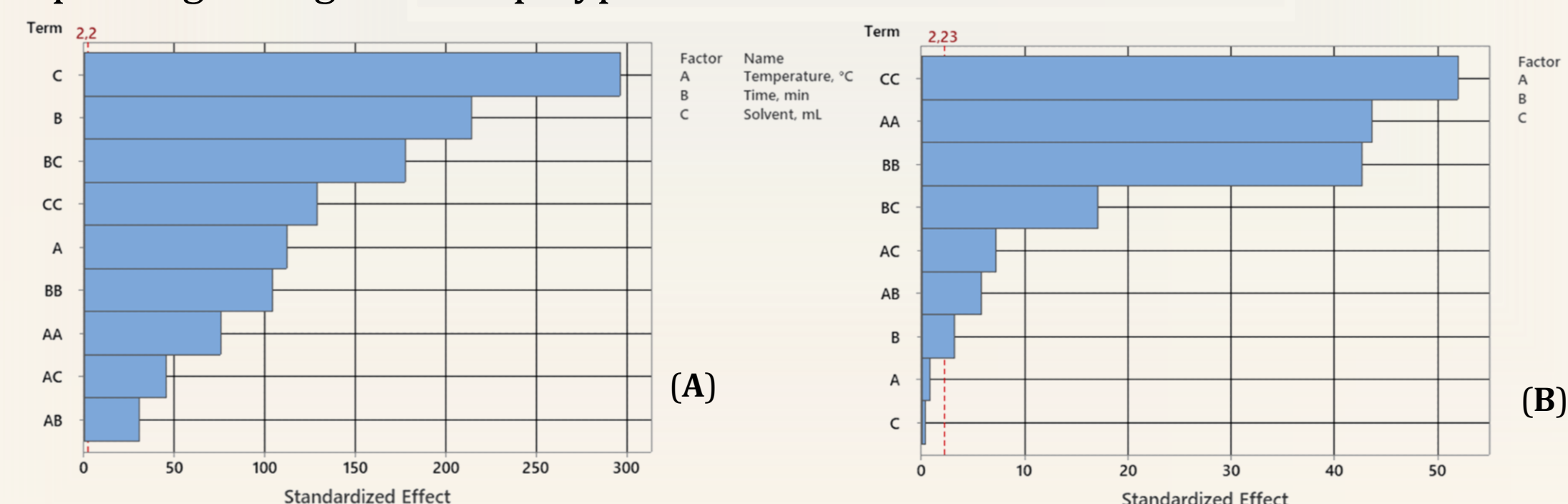


Figure 2. Pareto Chart of Standardized Effects – Response: TPC (mg GAE/100 g DW),  $\alpha = 0.05$  for: (A) NaDES extract, (B) Hydroalcoholic extract

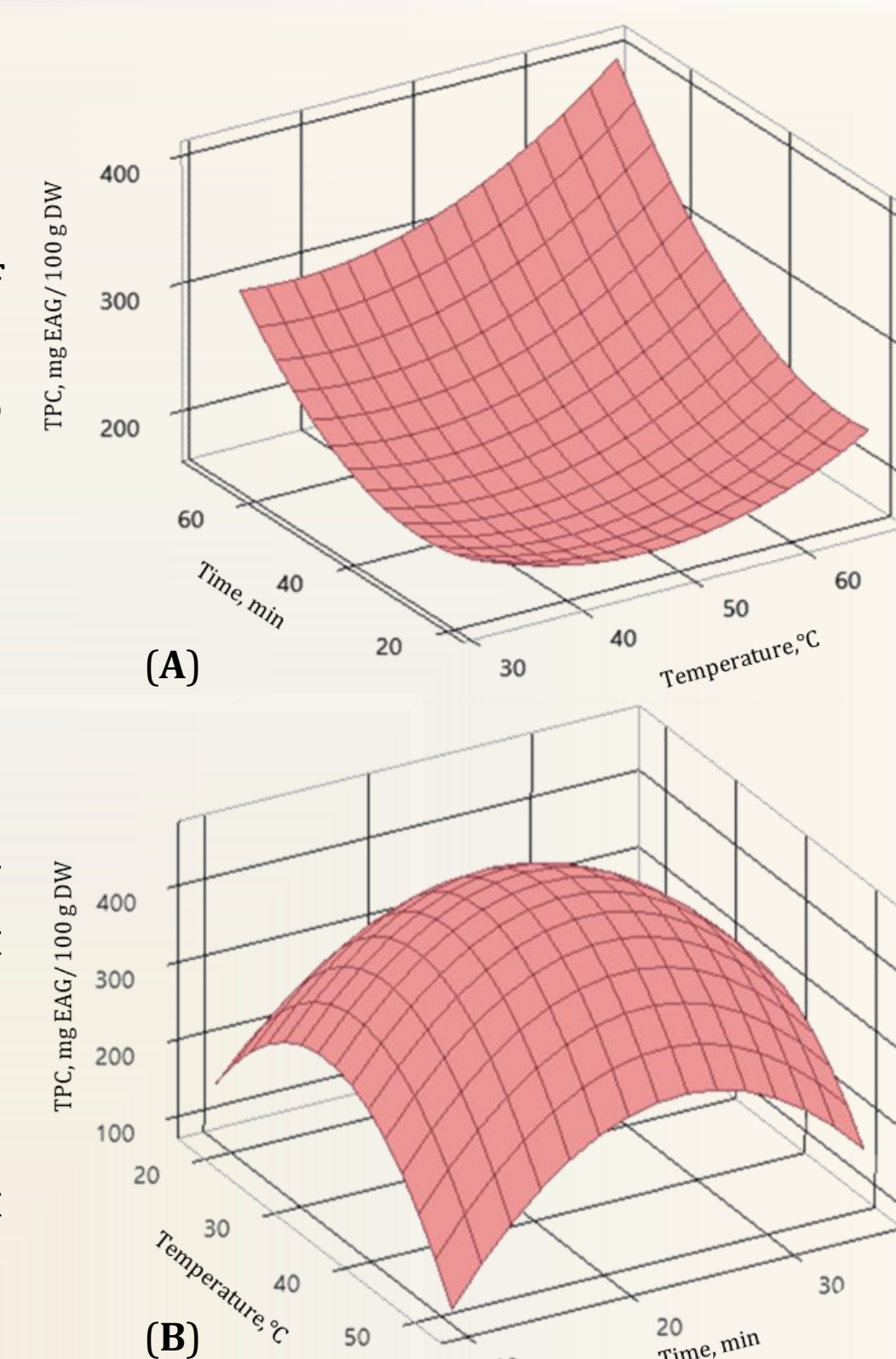


Figure 1. Three-dimensional response surface for analyzing the interaction effect between time and temperature on the dependent variable (TPC) for (A) – NaDES extract, (B) – Hydroalcoholic extract