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PRODUCTION OF GRAPE POMACE EXTRACTS WITH ANTIOXIDANT PROPERTIES: A STUDY BASED ON PROCESS OPTIMIZATION BY EXPERIMENTAL DESIGN

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ABSTRACT

This work evaluated the effects of different process parameters on recovery of antioxidant compounds from grape pomace (GP), using a Central Composite Design (CCD). The results obtained showed that the increase in the pectinase concentration applied in GP promoted a positive effect on the obtaining of extracts with best antioxidant properties evaluated by DPPH-radical scavenging, while the shortest reaction time positively affected the antioxidant activity measured by ABTS⁺ method. Increase in temperature leads to an increase in all responses. As for the increase in pH solution, there was an increase of antioxidant capacity by the DPPH and ABTS⁺ methods and a decrease by the FRAP. The best conditions for process optimization in order to increase the antioxidant properties of the extracts were found in the GPS treated with 2% pectinase, an incubation time of 1 hour, at 50 °C and pH 5. At these conditions, predicted values of antioxidant capacity determined by the ABTS⁺, DPPH and FRAP methods were 79.5, 24.2 and 108.9 µmol Trolox Equivalent per gram of dry GP, respectively. GPs exhibited strong antioxidant capacity, representing a source of bioactive compounds in obtaining functional ingredients in the food industry.

1. INTRODUCTION

Vine cultivation stands out in the Brazilian agribusiness with a production of 1.7 million tons of grapes, where 48.74% is destined for processing and 51.26% for the fruit trade in nature (ABF, 2018).

Despite the availability of grapes to agroindustry processing, its exploitation in the country is still limited in terms of waste management. Among the waste produced from fruit processing, grape

pomace (GP) has a potential economic interest. GP is composed of the seed, skins, rachis and the remains of the pulp, being the result of the crushed berries through of the juice separation process. This waste contains compounds with functional properties. In order to increase the overall yield and selectivity of antioxidant compounds in these residues, Enzyme Assisted Extraction (EAE) has been employed to cleave the polysaccharide chains of the cell wall.

EAE of antioxidant compounds in vegetable wastes is considered a useful and ecological alternative to conventional solvent extraction methods (Meini et al., 2019). Thus, the objective of this work was to evaluate the effects of some parameters of process on the recovering of antioxidant compounds from GP using an experimental design.

2. MATERIAL AND METHODS

The GPs were supplied by *Paluma Industry and Commerce* (Petrolina, Pernambuco, Brazil) in July 2018. To obtain the extracts with antioxidant properties, 5g of the dry and milled material was added to 45 mL of 100 mM phosphate-citrate buffer. The resultant solution was hydrolyzed by the addition of Multifect Pectinase FE (Genencor division, Rochester, USA) and incubated under CCD conditions (Table 1). After enzymatic hydrolysis, the reaction mixture was ice-cooled for 15 minutes, followed by centrifugation (17 000 x *g* for 10 minutes at 5 °C). The supernatant was collected, frozen, stored at -18 °C and used as extracts for further analyses. The antioxidant properties of the extracts were evaluated using the methods DPPH-radical scavenging, $ABTS^+$ cationic radical scavenging activity ($ABTS^+$), and iron-reducing antioxidant potential (FRAP) (Al-Duais et al. 2009). The results were analyzed using *Protimiza Experimental Design* online software (Campinas, SP, Brazil).

Table 1. Variables and levels used in the CCD for optimization of antioxidant capacity in grape pomace

Factor	Unit	Coded Levels (-1)	(0)	(+1)
Enzyme Concentration (X_1)	%	0	1	2
Reaction Time (X_2)	Hours	1	2	3
pH (X_3)	-	2	3.5	5
Temperature (X_4)	°C	40	45	50

3. RESULTS AND DISCUSSION

Table 2 and Figure 1 show Equations and Response Surfaces, respectively, of the antioxidant properties of GP extracts in function of the independent variables using CCD. The effects analysis showed that the enzyme concentration (pectinases) did not significantly ($p \leq 0.10$) affect the antioxidant capacity of the GP extracts measured by $ABTS^+$ and FRAP methods, however it positively influenced the responses for DPPH-radical scavenging.

Table 2. Polynomial equations containing the significant effects ($p \leq 0.10$) of the independent variables and coefficients of determination of the dependent variables (antioxidant properties)

Response	First Order Polynomial Equations	Coefficients of Determination	*p* -value
DPPH	$Y_1 = 22.92 + 0.41\ x_1 + 0.67\ x_3 + 0.67\ x_4 - 0.49\ x_3\ x_4$	$R^2 = 80.97\%$	< 0.05
ABTS$^+$	$Y_2 = 65.18 - 1.60\ x_2 + 8.09\ x_3 + 4.59\ x_4$	$R^2 = 88.08\%$	< 0.10
FRAP	$Y_3 = 107.32 - 5.66\ x_3 + 7.23\ x_4$	$R^2 = 80.95\%$	< 0.01

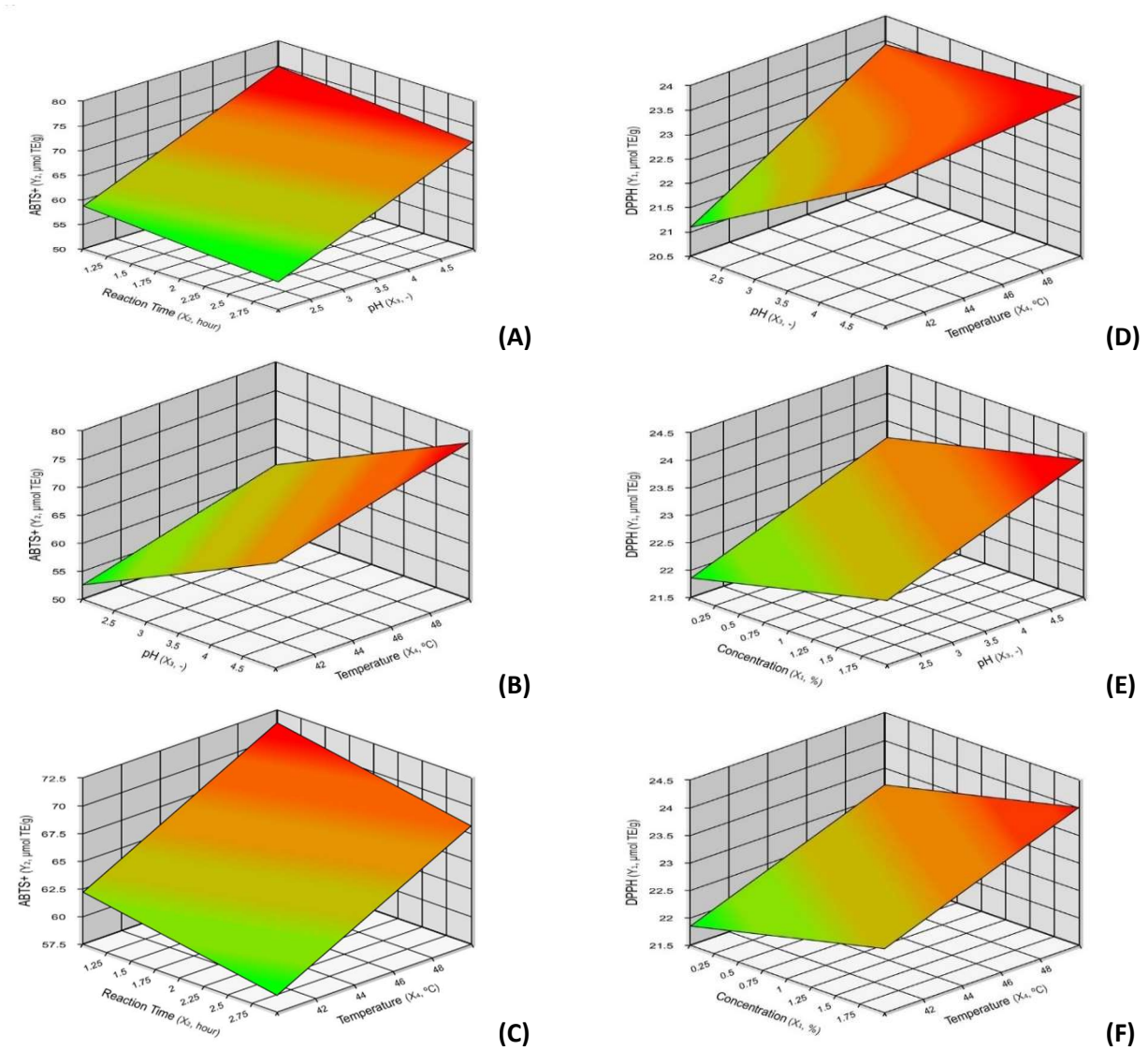

Figure 1. Response surfaces for antioxidant properties of the GP extracts produced and evaluated by ABTS$^+$ (A, B, C) and DPPH (D, E, F) methods: pH x temperature (A, D), pH x time (B), temperature x time (C), pectinase x pH (E) and temperature x pectinase (F)

Additionally, the reaction time did not significantly ($p > 0.10$) affect the antioxidant capacity of GP extracts evaluated by DPPH and FRAP methods, but it significantly affected the antioxidant activity measured by $ABTS^+$ method.

GPs treated with 2% pectinase, incubation time for 1 hour, at 50 °C and pH 5 were the best treatments in relation antioxidant capacity by the $ABTS^+$ and DPPH methods.

Regarding FRAP, the effects of pH on antioxidant capacity of GP extracts showed opposite results in relation to the responses obtained for DPPH and $ABTS^+$. On the other hand, the temperature presented similar effects on the antioxidant capacity of the extracts for the three methods. Therefore, regardless of pectinase concentration and incubation time, GPs treated at 50 °C and pH equal to two had an increase in the antioxidant capacity evaluated by FRAP method.

By the data set, the best conditions for process optimization in order to increase the antioxidant properties of the extracts were found in the GPs treated with 2% pectinase, a reaction time of 1 hour, at 50 °C and pH 5. At these conditions, predicted values of antioxidant capacity determined by the $ABTS^+$, DPPH and FRAP methods were 79.5, 24.2 and 108.9 µmol Trolox Equivalent per gram of dry GP, respectively. Despite finding an effect as a response from different variables, temperature and pH were the most important variables that affected the antioxidant capacity in the GP treated with pectinases. Meini et al (2019) observed an increase in antioxidant capacity of GP with increase of the incubation temperature, while the pectinase dosage and the pH variation had no significantly effects.

4. CONCLUSION

The best conditions for process optimization in order to increase the antioxidant properties of the extracts were found in the grape pomaces treated with 2% pectinase, incubation time for 1 hour, at 50 °C and pH 5. Extracts of grape pomace exhibited strong antioxidant capacity, representing a source of bioactive compounds in obtaining functional ingredients in the food industry.

5. REFERENCES

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