ENZYME MACERATION AND ANTIOXIDANT POTENTIAL OF EXTRACTS PRODUCED FROM GRAPE POMACE

Agnelli H. Oliveira[1,2], Sandra O. de Souza[3], Leda Maria F. Gottschalk[1], Renata Valeriano Tonon[1], Lourdes Maria Corrêa Cabral[1] and Ruann J. S. de Castro[2]

[1] Embrapa Agroindústria de Alimentos, Unidades Operacionais

[2] Universidade Estadual de Campinas, Departamento de Ciência de Alimentos

[3] Universidade Federal de Viçosa, Departamento de Fitotecnia

E-mail: agnelli.holanda@embrapa.br

ABSTRACT

Grape pomace (GP) from juice production is a source of bioactive compounds with antioxidant properties. This work aimed to study the antioxidant capacity in extracts produced from GP using a Central Composite Rotatable Design (CCRD) for evaluation of the effects of the temperature and pH during enzymatic hydrolysis with pectinase and cellulase. The best conditions for enzyme maceration and obtaining extracts with improved antioxidant properties were: application of pectinase (0.70%) plus cellulase (0.30%), 55 °C as incubation temperature and pH 4.5. At these conditions, the produced GPs extracts exhibited strong antioxidant capacity in ABTS, DPPH and FRAP methods, representing a source of bioactive compounds.

1. INTRODUCTION

Fruit juice processing results in large amounts of waste, such as skins, seeds, pomace, among others. Thus, the use of these residues as a source of bioactive compounds can be an interesting alternative in the production of food additives or supplements with high nutritional value.

Several extraction methods have been applied in the recovery of bioactive compounds, among these, the Enzyme Assisted Extraction (EAE). EAE has different advantages compared with non-enzymatic methods, including rapid extraction, maximum efficiency and low energy consumption (Tomaz et al., 2016).

In this way, the objective of this work was to study the production of extracts using grape pomace (GP) as substrate and to investigate the effects of the independent variables, such as pH, temperature

and combination between pectinase and cellulase in different proportions by experimental design, with the response variables being the antioxidant activities of GP determined by the ABTS, FRAP and DPPH methods.

2. MATERIAL AND METHODS

The GPs resulting from the production of grape juice, made from the varieties Isabel Early and BRS Violeta, were supplied by *Paluma Industry and Commerce* (Petrolina, PE, Brazil) in July 2018. To obtain the extracts with antioxidant properties, 5g of the dry material in forced air circulation (35 ± 4 °C for 2 days), followed by grinding in a blender for 10 min, were 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) plus Celluclast 1.5L (Novozymes, Copenhagen, Denmark), at different concentrations, and incubated under CCRD conditions (Table 1).

Table 1. Independent variables and levels used in the CCRD to study the effects of some parameters of process on the antioxidant properties of GP extracts

Factor	Unit	Coded Levels				
		(-1.68)	(-1)	(0)	(+1)	(+1.68)
Pectinex:Celluclast (X_1)	%	0.16:0.84	0.30:0.70	0.50:0.50	0.70:0.30	0.84:0.16
Temperature (X_2)	°C	41.6	45	50	55	58.4
pH (X_3)	-	4.2	4.5	5.0	5.5	5.8

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, lyophilized, 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). From the values obtained, the models were adjusted to the response variables and the regression coefficients were evaluated statistically ($p < 0.10$) by ANOVA, using *Protimiza* software (Campinas, SP, Brazil).

3. RESULTS AND DISCUSSION

Table 2 shows the CCRD with its independent variables and the results for antioxidant activities. The highest values obtained for ABTS, FRAP and DPPH were observed in run 13 (371.4 µmol TE/g), in run 4 (343.2 µmol TE/g) and in run 16 (145.2 µmol TE/g), respectively. The limited variability of the central points (runs 15-17) indicated good reproducibility of the experimental data.

The antioxidant properties of the extracts produced with GP were significantly affected by the independent variables X_1 (pectinase and cellulase ratio) and X_2 (temperature). On the other hand,

the variation of the parameter pH from 4.2 to 5.8 was not significant ($p \geq 0.10$) in the antioxidant properties of the GP extracts produced under the CCRD conditions.

Although the independent variables pectinase and cellulase ratio and temperature were significant ($p \leq 0.05$) in the antioxidant properties of GP extracts, it was not possible to obtain the Response Surfaces, where the models generated by this methodology had low Determination Coefficients ($R^2 < 0,75$). Therefore, the highest experimental values of antioxidant properties of GP extracts were detected under the following conditions: 1) ABTS method: enzyme ratio (pectinase:cellulase) (%) (0.50:0.50) at 50 °C and pH 4.2, 2) for FRAP method: enzyme ratio (0.70:0.30) at 55 °C and pH 4.5, 3) for DPPH: enzyme ratio (0.50:0.50), temperature at 50 °C and pH 5.0 (Table 2).

Table 2. CCRD experimental planning matrix for antioxidant capacity of grape pomace using different enzymatic preparations.

Runs	Independent variables			Antioxidant activity (µmol TE/g)*		
	X_1	X_2	X_3	Y_1 ABTS	Y_2 FRAP	Y_3 DPPH
1	-1	-1	-1	195.3	212.7	126.5
2	1	-1	-1	218.7	246.3	138.2
3	-1	1	-1	252.1	285.7	133.9
4	1	1	-1	346.3	343.2	142.8
5	-1	-1	1	224.8	239.8	133.0
6	1	-1	1	223.4	236.0	130.3
7	-1	1	1	281.5	287.0	134.8
8	1	1	1	299.1	290.7	142.3
9	-1.68	0	0	256.0	267.9	138.4
10	+1.68	0	0	267.0	279.6	141.5
11	0	-1.68	0	261.6	278.8	139.3
12	0	+1.68	0	281.1	306.4	138.8
13	0	0	-1.68	371.4	283.3	141.5
14	0	0	+1.68	271.4	283.4	136.6
15	0	0	0	307.5	289.0	140.5
16	0	0	0	296.7	287.6	145.2
17	0	0	0	326.6	289.7	142.9

X_1 = Ratio between Multifect Pectinase FE and Celluclast 1.5L.
X_2 = Temperature (°C).
X_3 = pH.
*µmol TE/g = Trolox Equivalent per g of dry grape pomace.

Increase in temperature leads to an increase in all responses. There was a slight increase in antioxidant capacity with increased amount of pectinase relative to the amount of cellulase in the

extracts. The solubility and diffusivity of some compounds enhance at high temperatures, because the viscosity of the shell extracts is reduced, and mass transfer and extraction process improved. However, temperatures above 60 °C can lead to thermal degradation of bioactive compounds (Lazar et al., 2016). Meini et al (2019) studied the optimization process in recovering phenolic antioxidants from grape pomace of the variety Syrah, where they found an increase in antioxidant capacity (TEAC) as a function of temperature (24 to 45 °C) and enzymatic units of tannase and cellulase, while the use of pectinase and the variation in pH (4.0 to 5.5) had no effect. However, in grape pomace from the variety Othello Black, pectinase hydrolysis favored increase in antioxidant activity evaluated by DPPH (Zambrano et al., 2018).

In order to select an appropriate condition of hydrolysis that favored the antioxidant properties of the GP extracts for the methods used in this study, the enzymatic hydrolysis using enzyme ratio (pectinase:cellulase) (%) (0.70:0.30), 55 °C as incubation temperature and pH 4.5 was defined as the most adequate.

4. CONCLUSION

The CCRD experimental design showed that temperature and enzyme ratio between pectinase and cellulase significantly affected the antioxidant properties of extracts produced from grape pomace. The best conditions for recovering of antioxidant compounds were obtained with pectinase: cellulase (%) (0.70:0.30), 55 °C as incubation temperature and pH 4.5. The results indicated that the GP extracts exhibited strong antioxidant capacity, representing a source of bioactive compounds that can be applied to obtain functional ingredients for food industry.

5. REFERENCE

Al-Duais, M., Müller, l., Böhm, V., Jetschke, G., 2009. Antioxidant Capacity and Total Phenolics of *Cyphostemma digitatum* before and after Processing: Use of Different Assays. Eur Food Res Technol. 228, 5, 813-821.

Lazar, L., Talmaciu, A.I., Volf, I., Popa, V.I., 2016. Kinetic modeling of the ultrasound-assisted extraction of polyphenols from Picea abies bark. Ultrason Sonochem. 32, 191-197.

Meini, M.R., Cabezudo, I., Boschetti, C.E., Romanini, D., 2019. Recovery of Phenolic Antioxidants from Syrah Grape Pomace through the Optimization of an Enzymatic Extraction Process. Food Chem 283, 257-264.

Tomaz, I., Maslov, L., Stupic, D., Preiner, D., Asperger, D., Kontic, J.K., 2016. Recovery of flavonoids from grape skins by enzyme-assisted extraction. Sep. Sci. Technol., v. 51, p. 255-268.

Zambrano, C., Kotogán, A., Bencsik, O., Pappa, T., Vágvölgyi, C., Mondal, K.C., Krisch, J., Takó, M., 2018. Mobilization of Phenolic Antioxidants from Grape, Apple and Pitahaya Residues via Solid State Fungal Fermentation and Carbohydrase Treatment. LWT - Food Sci. Technol 89, 457-465.