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Effects of enzymatic preparations on yield and antioxidant activity in must extraction from Cabernet Sauvignon

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ABSTRACT

Nine commercial preparations of pectinase were characterized by the activity of six different enzymes; pectinase, polygalacturonase, pectin methylesterase, pectin lyase, cellulase and xylanase, and evaluated their application to grape must extraction. Cv. Cabernet Sauvignon was treated at different temperatures (40, 50 and 60 °C) and different times (15 - 30 min), analyzing the total soluble solids (°Brix), extraction yield and reducing capacity. Hence, 3 preparations were selected and the enzyme concentration analyzed. The enzyme preparation Zimopec PX5® presented the best results for the evaluated parameters. The optimal condition was set to 50 °C for 30 min and 1.0 U of pectinase per gram of grape.

Keywords: Pectinase. Enzyme activity. Extraction. Grape must.

INTRODUCTION

Pectinolytic enzymes are a heterogeneous group of enzymes responsible for the degradation of pectin that are a structural polysaccharides present in the middle lamella of plants cell wall (Jayani, Saxena et al. 2005; Cabrera, Jang et al. 2009). Therefore, pectinases were one of the first enzymes to be used on juice and wine processes, due to the hydrolytic capacity of cell wall, that increase the yield and release of phenolic compounds as anthocyanins and flavonoids responsible for antioxidant activity (Kashyap, Vohra et al. 2001; Segade, Pace et al. 2015).

Pectinases combined with other enzymes could increase the pressing efficiency for juice or must extraction. Usually, commercial preparations of pectinase contain different enzymatic activities such as polygalacturonase, pectin methylesterase, pectin lyase, cellulase and hemicellulase (Pasha, Anuradha et al. 2013). Nowadays, there are several commercial preparations of pectinase indicated to application on juice and wine industries, being important to know the enzymatic composition of each preparation for a correct application (Tapre and Jain 2014; Dal Magro, Goetze et al. 2016).

The objective of this work was to compare the effects of nine different commercial enzyme preparations in the grape must extraction of Cabernet Sauvignon (*Vitis vinifera*). The variables time and temperature, as well as enzyme concentration were evaluated for extraction yield, total soluble solid and reducing capacity.

MATERIAL AND METHODS

The commercial pectinolytic preparations tested were: Pectinex Ultra SP-L® (1), Pectinex Ultra Color® (2), Pectinex Smash XXL® (3), Novozym 33095® (4), Pectinex Ultra Clear® (5), Pectinex BE XXL® (6), were kindly donated from Novozymes (Spain), Rohapect 10L® (7) was from Amazon group (Brazil), Lallzyme Beta® (8) from Lallemand Wine (France), and Zimopec PX5® (9) was from Veneto Mercantil (Brazil). Gallic acid, galacturonic acid, polygalacturonic acid, pectin from apple (ID 76282), xylan and Folin-Ciocalteu were from Sigma Aldrich (St. Louis, MO). Cabernet Sauvignon (*Vitis vinifera*) was kindly donated by Vitivinicola Jolimont (Canela, RS, Brazil).

ENZYMATIC ACTIVITIES

Pectinase (PE), polygalacturonase (PG), cellulase (CE) and xylanase (XLN) activity was determined by the reducing groups formed, using pectin, polygalacturonic acid, whatman n° 1 filter paper and xylan as substrate, respectively. The amount of reducing groups was estimated by the 3,5-dinitrosalicyclic acid (DNS) according to (Miller 1959). One unit of enzyme was defined as the amount of enzyme required to produce 1 μmol of reducing groups per minute. Pectin lyase (PL) activity was determined spectrophotometrically measuring the increase in absorbance at 235 nm, due to the formation of unsaturated products, as described by (Albersheim 1966). One PL unit was defined as the amount of enzyme that produces 1 nmol of unsaturated uronideo (ϵ = 5500 $M^{-1}.cm^{-1}$) per minute under the reaction conditions.

Pectin methylesterase (PME) activity was determined by titration of carboxylic groups liberated through de-esterification of citric pectin, as described by (Rouse and Atkins 1952). One PME unit was defined as the amount of enzyme which liberates 1 milliequivalent of carboxyl groups per minute under the reaction conditions.

MUST EXTRACTION AND ANALYSES

Grapes (50g) were gently crushed and incubated with each enzymatic preparation using 0.5 U of pectinase per gram of grape, under agitation for certain time and temperature. At the end, the must was pressed and analyzed. For all experiments a control, without enzyme, was performed. All the experiments were conducted in triplicate. Extraction temperature (40, 50 and 60 °C) and reaction time (15 and 30 min) were evaluated independently. Enzyme concentration (0.01 – 2.0 $U.g^{-1}$) was investigated.

Total Soluble Solids (°Brix) were measured using a refractometer at 20.0±0.5 °C. The reducing capacity (RC) of the samples was determined using the Folin–Ciocalteu method proposed by (Singleton and Esau 1968) with modifications. The measurements were conducted in triplicate and the average data were interpolated in a gallic acid calibration curve and the total phenolic content was expressed as equivalents of gallic acid ($mg.L^{-1}$). The extraction yield of each treatment was expressed as a percentage of the mass of must per initial mass of grape.

RESULTS AND DISCUSSION

Initially, nine commercial preparations were analyzed regarding six enzymatic activities (PE, PG, PME, PL, CE and XLN) and the results are shown in Table 1. The best preparations were 4, 5, 7 and 8, showing suitable values and ratios for all enzymatic activities.

However, Zimopec PX5® (9) presented the highest enzymatic activities to the main enzymes analyzed, PE, CE and XLN. Then, in the next experiments, we evaluated each enzymatic preparation on grape mash extraction, standardizing their activities on 0.5 $U.g^{-1}$ of total pectinase activity, and analyzing the yield and quality parameters.

Table 1: Enzymatic activities for each enzyme preparation.

	Activity ($U.mL^{-1}$)					
Enzyme preparation	PE	PG	PL	PME	CE	XLN
1 - Pectinex Ultra SP- L®	969.23	1649.31	20.12	280.00	19.45	854.56
2 - Pectinex Ultra Color®	1971.65	1484.57	173.41	1180.00	31.58	6436.67
3 - Pectinex Smash XXL®	1915.78	505.56	429.41	0.00	15.09	769.05
4 - Novozym 33095®	2212.12	1701.08	182.14	913.33	40.39	8098.17
5 - Pectinex Ultra Clear®	2275.66	2306.29	218.18	960.00	50.18	11395.77
6 - Pectinex BE XXL®	2064.22	1134.95	316.60	413.33	29.98	2699.78
7 - Rohapect 10L®	1720.22	1845.28	153.69	466.67	35.03	5956.38
8 - Lallzyme Beta®	3899.15	1313.17	94.06	0.00	975.89	7621.39
9 - Zimopec PX5®	9393.19	1482.52	93.57	0.00	1311.78	10831.14

The effects of temperature and time on grape mash proprieties for each preparation are shown in Table 2. The physicochemical properties of grape must present significant differences on temperature and time variation. Rising the temperature, it was observed an increase in °Brix and RC, however, at lower temperature (40 °C) a high yield was obtained. Thereby, 50 °C was chosen as the temperature for the further assays due the satisfactory ratio between the evaluated parameters.

Regarding the time, a significant difference is observed, with higher values to °Brix and RC, at 30 min, while the extraction yield is similar in both times. Therefore, it was determined 30 min as the extraction time for the subsequent analysis, due the best results for reducing capacity and °Brix.

Table 2: Analysis of yield, °Brix and reducing capacity of the grape mash treated with nine commercial enzymatic preparations.

EP	Yield (%)				° Brix				RC ($mg.mL^{-1}$)			
	40 °C^{A}	50 °C^{B}		60 °C^{B}	40 °C^{C}	50 °C^{B}		60 °C^{A}	40 °C^{B}	50 °C^{A}		60 °C^{A}
		15 min	30 min			15 min	30 min			15 min	30 min	
0	68.4	60.9	60.1^{c}	59.0	17.0	18.6	18.9^{b}	19.0	3.07	7.92	9.74^{b}	9.99
1	71.3	64.4	64.8^{b}	64.6	17.0	18.2	19.0^{b}	20.0	6.00	8.17	10.1^{b}	10.5
2	71.6	61.4	64.9^{b}	64.3	16.5	18.5	19.1^{b}	20.0	6.02	8.83	10.6^{b}	9.65
3	70.9	64.6	65.0^{b}	65.5	17.0	18.4	19.1^{b}	20.0	5.87	9.04	10.6^{b}	12.8
4	72.3	65.9	65.0^{b}	66.2	17.0	18.4	19.3^{ab}	20.0	6.66	9.37	11.1^{b}	13.8
5	73.3	64.9	64.4^{b}	66.9	17.0	18.6	19.5^{ab}	19.8	5.94	9.70	13.3^{a}	13.8
6	72.1	67.6	67.9^{a}	67.1	17.5	18.8	19.3^{ab}	19.8	6.90	10.9	12.5^{ab}	13.6
7	72.6	63.9	64.2^{b}	66.9	17.0	18.9	19.3^{ab}	21.0	5.09	9.63	10.5^{b}	13.5
8	72.1	64.2	65.3^{b}	65.1	17.2	18.9	19.5^{ab}	21.0	6.97	10.0	12.3^{ab}	11.6
9	73.1	66.7	68.5^{a}	65.3	18.1	19.2	19.8^{a}	21.0	7.22	10.8	13.7^{a}	14.3

* Same lowercase letters indicate that preparations are equal for each response. Same uppercase letters indicate that temperatures are equal for each response.

In order to determine the optimal amount of enzyme for application in grape must extraction, the enzyme concentration was evaluated on preparations 5, 8 and 9, which showed

the best results in previous analyzes. The concentration of pectinase ranged from 0.05 to 2.0 U per gram of grape. The highest yield was achieved using 1.0 $U.g^{-1}$ of PE for the preparation 9 (79.3 %) and 5 (79.13 %), while preparation 8 presented 75.4 %. However, there is no statistical difference for total soluble solid on enzyme concentration. The higher value of °Brix (20.0) was found for preparation 9, whilst preparations 8 and 5 showed °Brix of 19.5 and 18.4, respectively. Therefore, 1.0 $U.g^{-1}$ of PE was chosen as standard concentration for grape must extraction.

CONCLUSION

It can be concluded that all preparations are a mixture of different enzymes, and know their exact activities are necessary to make the right choice for the industry application. Extraction time and temperature showed changes in qualitative components of Cabernet Sauvignon grape must. Lower temperature and time are not enough to extract higher concentrations of total soluble solids and antioxidant compounds. Moreover, Zimopec PX5® presented the best results in the evaluated parameters, and the best conditions to improve yield and quality were 50 °C for 30 min with an enzyme concentration of 1.0 $U.g^{-1}$ of PE. At these conditions, it was possible to obtain a grape must with higher reducing capacity and better quality attributes.

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