



**ACID HYDROLYSIS OF CABERNET SAUVIGNON (*VITIS VINIFERA* L.) GRAPE
SKINS TO OBTAIN FERMENTABLE SUGARS**

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ABSTRACT

The reuse of agro-industrial by-products is important because it contributes to the proper disposal of these wastes in the environment, helping to reduce environmental pollution as well as adding value. This is particularly true of winemaking by-products. Because grape pomace is the main waste generated in winemaking, processes that involve the use of its components, such as skins, stems and seeds, tend to arouse the interest of the productive sector. The purpose of this study was to create a method of pretreatment and acid hydrolysis of grape skins that would yield sugars convertible into ethanol. To this end, acid or alkaline pretreatments followed by acid hydrolysis were carried out. Results this study showed, alkaline pretreatment and acid hydrolysis proved to be the best methods to release fermentable sugars, yielding 8.90 g L⁻¹ of reducing sugars and 6.76 g L⁻¹ of xylose in the condition of 4.5% NaOH in a treatment time of 45 min. As for glucose, although the best average yield was 2.26 g L⁻¹ of glucose in 30 min of treatment with 4.5% NaOH, the 15 min treatment with 4.5% NaOH can be adopted in order to increase the cost effectiveness of the process, since a yield of 2.02 g L⁻¹ of glucose was obtained in this treatment time.

Keywords: Grape Skins, Acid Hydrolysis, Xylose, Glucose, Fermentable Sugars

INTRODUCTION

Brazil ranks among the world's leaders in agricultural production, thus generating large quantities of agro-industrial wastes. The search for new methods of waste reuse has steadily increased, expressly for the purpose of reducing environmental pollution through the synthesis of new products [1, 2].

The wine industry is greatly interested in these technologies, because one of its most abundant waste is grape skins, a material that decomposes very slowly in the environment [3, 4]. This waste can be reused through the production of grape skin and seed meal, which has antioxidant properties [5], the recovery of phenolic compounds [6, 7], and the possibility of bioethanol production from the cellulose and hemicellulose contained in this lignocellulosic biomass [8, 4].

Two steps are required to transform these wastes into bioethanol. The first step involves the hydrolysis of polysaccharides contained in the lignocellulosic complexes into simple sugars, while the second step involves the fermentation of these sugars [9, 10].

Grape skins, as well as other lignocellulosic materials to be hydrolyzed, consists of cellulose, hemicelluloses and lignin [11]. Cellulose and hemicellulose are polysaccharides formed from hexoses and

pentoses, which can be hydrolyzed into monosaccharides (glucose, xylose) and eventually fermented into ethanol. Lignin can not be converted into ethanol, but its use as a source of energy in the process is recommended [12, 13].

There are various technologies for hydrolysis, including acid hydrolysis, which involves the use phosphoric, sulfuric acid, hydrochloric acid, acetic acid and peracetic acids in combination with heat treatment [14, 15, 16, 17]. Several recent studies indicate that the use of pretreatments may favor the hydrolysis process [18, 19]. There are several possibilities for pretreatment with different yields of glucose and xylose, such as treatments with acids, alkalis, autoclave, and steam explosion, aimed at removing the lignin and disrupting the fibers, which facilitate the subsequent hydrolytic attack [20, 21].

The purpose of this study was to obtain fermentable sugars from Cabernet Sauvignon grape skins by subjecting them to chemical acid or alkaline pretreatment combined with physical pretreatment in an autoclave, followed by acid hydrolysis.

MATERIAL AND METHODS

Raw Material and Reagents

Cabernet Sauvignon (*Vitis vinifera* L.) grape skins from the 2013 harvest were

kindly supplied by the winery Vinícola Bella Quinta of São Roque, SP, Brazil. The reagents used were: sulfuric acid (H₂SO₄), phosphoric acid (H₃PO₄) and sodium hydroxide (NaOH).

Acid or Alkaline Pretreatment

Acid or alkaline prehydrolysis was performed employing a ratio of 1:10 (10g

of grape skins to 100ml of solution) with phosphoric acid or sodium hydroxide. The prehydrolysis was performed at 121°C in a vertical autoclave (Phoenix AV75), using the reaction time and reagent concentrations listed in Table 1 [methodology adapted from 17 and 21].

Table 1: Reagent concentrations and reaction times employed in the experiments involving acid or alkaline pretreatment prior to acid hydrolysis

Experiments	Reagent concentration (%)			Reaction time (min)		
	2	4.5	7	15	30	45
1	+	-	-	+	-	-
2	+	-	-	-	+	-
3	+	-	-	-	-	+
4	-	+	-	+	-	-
5	-	+	-	-	+	-
6	-	+	-	-	-	+
7	-	-	+	+	-	-
8	-	-	+	-	+	-
9	-	-	+	-	-	+

Acid Hydrolysis

Ten grams of the prehydrolysate and 100ml of H₂SO₄ solution elevated by 2% were placed in a 125ml Erlenmeyer flask, which was placed in a vertical autoclave (Phoenix AV75) and treated at 121°C for 60min [methodology adapted from 16].

Analytical Methods

Concentration of reducing sugars

The concentration of reducing sugars was measured by DNS (3,5-dinitrosalicylic acid) [23] with different concentrations of xylose and glucose (Labsynth PA). Spectrophotometric readings were taken at 540 nm, and a calibration curve was plotted simultaneously. The results were expressed in g L⁻¹.

Xylose Concentration

The xylose concentration was determined by means of a clinical technique that measures the concentration of this pentose in the blood plasma or urine, adapted to samples of prehydrolyzed grape skins. To perform this analysis, phloroglucinol is dissolved in a solution of acetic acid and hydrochloric acid (9:1, v/v) up to a concentration of 36mMol/L. In an acid medium, pentoses form a complex colored with phloroglucinol, which can be determined by direct colorimetric reading [24, 25].

Glucose Concentration

The glucose concentration was determined by the colorimetric method (Doles TM) [26, 27, 28].

Analysis of the results

The experiments were performed in triplicate. The hydrolysis at were subjected to an analysis of variance (ANOVA) and the means were compared by the Tukey and Kramer tests, using the GRAPHPAD INSTAT program (Rutgers University, Camden, New Jersey). The treatments were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The concentration of 7% phosphoric acid (H_3PO_4) employed in the pretreatment of grape skins resulted in the highest mean concentrations of reducing sugars, showing a significant difference ($P < 0.05$) when compared to the lower concentrations (Table 2). As for the treatment time, the highest average of sugars was obtained in

15 min, although there was no significant difference of the averages obtained in the other tested times. Considering a reduction in the energy cost of the process, the best treatment time was found to be 15 min, using 7% acid.

Gómez et al. [17] achieved the best result of reducing sugars (23.1 gL^{-1} of reducing sugar) in the acid hydrolysis of sugarcane bagasse performed under the conditions of 4% of H_3PO_4 for 300 min at 122°C . This difference between those results and the ones obtained in the present study is attributed to the exposure time of the material to hydrolysis, as well as to the structural difference between the two materials.

Table 2 –Concentration of reducing sugars g.L^{-1} obtained after acid pretreatment and acid hydrolysis

Acid concentration (%)	Time (min)					
	15		30		45	
	M	SD	M	SD	M	SD
2.0	2.40	± 0.27	2.78	± 0.05	1.40	± 0.66
	A ¹ ab ²		Ab		Aa	
4.5	4.11	± 0.40	2.44	± 0.24	4.74	± 0.16
	Bb		Aa		Bb	
7.0	5.65	± 0.20	5.24	± 0.23	5.51	± 0.22
	Ca		Ba		Ba	

(1) Means followed by the same uppercase letter sin the same column do not differ, at a set time. (Tukey, $P > 0.05$)

(2) Means followed by the same lowercase letter son the same line do not differ, at a set concentration. (Tukey, $P > 0.05$)

As can be seen in Figure1, the best acid pretreatment condition for their lease of glucose was also 15 min at a concentration of 7%, releasing 0.66 g L^{-1} of glucose. A statistical analysis of the concentration of acid used here indicated that the experiments with 7% acid were

significantly more effective (Tukey; $P < 0.05$). In addition, a comparison of the treatment times revealed that the 15-min experiment did not differ statistically from the 30-min experiment ($P > 0.05$), which again highlights the potential savings in the process.

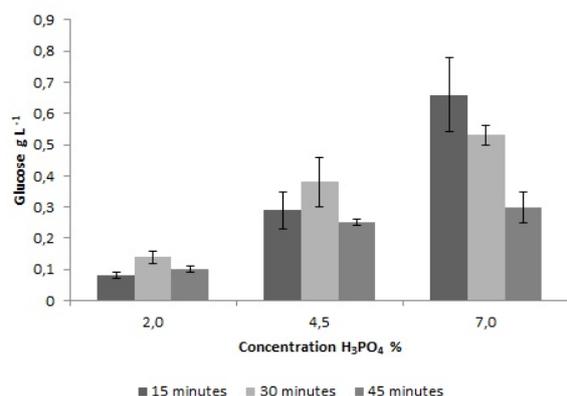


Figure 1: Concentration of glucose after acid Pretreatment and acid hydrolysis

Martin et al. [29] pretreated sugarcane bagasse with sulfuric acid, at 122°C, for 20 min, 40 min and 60 min, and obtained 2.1g, 3.7g and 4.0g L⁻¹ of glucose, respectively. Hence, the afore mentioned author applied longer incubation times in your experiments. Furthermore, in this work, we used phosphoric acid, which is as efficient as sulfuric acid, but cheaper and safer [17]. Schirmer-Michel et al. [30] investigated the effectiveness of acid hydrolysis on soybean hulls using 1.4% sulfuric acid, at 125°C for 1 hour, and obtained 0.38 g L⁻¹ of glucose. These results demonstrate that the yield obtained in the present study was 73.6% higher than that reported Schirmer-Michel et al. [30], respectively. The longer pretreatment times and lower acid concentrations employed in those two studies resulted in a lower sugar yield, when compared to the results in Figure 1. These data demonstrate that a higher concentration of acid was more effective in increasing the glucose yield than the

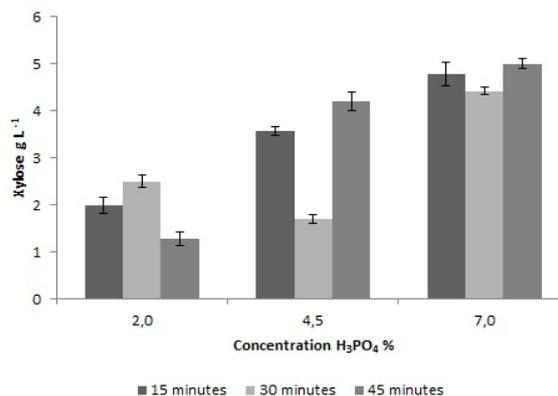


Figure 2: Concentration of xylose after acid pretreatment and acid hydrolysis

treatment time, and may represent a lower expenditure of energy in the process.

As for there lease of xylose, note that the highest average was 5.0 g L⁻¹ of xylose, resulting from test concentration of 7% phosphoric acid for 45min (Figure 2). Again, the increase in the acid concentration used in the grape skin pretreatment was associated with a significant increase (Tukey test; P<0.05) in sugar yield. However, increasing the dwell time of this material at high temperature did not statistically favor (Tukey test; P>0.05) the concentration of xylose, indicating that a 15 min dwell time would, again, be the most favorable one to reduce the energy cost of the process.

Upon analyzing the other results of the experiment, it is clear that the use of 7% phosphoric acid applied for 15 and 30 min yielded concentrations very close to the maximum found. The dwell time may be a key factor to identify the best condition, and the 15 min test proved to be the most

suitable one, since it stream lines the process and lowers its costs.

Egüés et al. [31] studied the self-hydrolysis of grape skin in an autoclave with a dwell time of 2 hours, and subsequent treatment with 2% sulfuric acid, and obtained a yield of 9.27g L⁻¹ of xylose. The higher sugar yield obtained by these authors indicates that self-hydrolysis may be a more efficient form of pretreatment for the release of sugar, but the long treatment time used by these researchers would increase the energy cost of the process.

Roberto, Mussato and Rodrigues [32] reported that the best result for the release of xylose was achieved at a concentration of 1.6% sulfuric acid, in 30 min pretreatment of the rice straw, which yielded 20.5g L⁻¹ of xylose. Those pretreatment times produced up to 4.1-fold better results than the ones achieved in this study, however, the authors utilize high pressure reactor. As for other acid hydrolysis experiments, Aguilar et al. [16], who used sugarcane bagasse as raw material and applied 2% sulfuric acid at 122°C, obtained 21.6g L⁻¹ of xylose and 3.0g L⁻¹ of glucose. These results suggest

that different types of raw materials and processes may have led to the differences in sugar yields described here.

When the grape skins has undergone an acid pretreatment is noted that the release of xylose was higher than that of glucose. According to Canettieri [33], the acid hydrolysis of biomass yields larger amounts of xylose than of glucose, because this pentose originates from xylan, which is easily degraded by acids owing to its amorphous structure. On the other hand, glucose derives from cellulose, which has a crystalline structure, so its degradation requires more severe process conditions [9, 34].

The highest average yield of fermentable sugars obtained with the alkaline pretreatment was achieved in 45 min with 4.5% NaOH, which yielded 8.90 g L⁻¹ of reducing sugars, although this average is not significantly higher than the averages achieved with the other treatment times (Table 3). However, Table 3 shows that fixing the time and increasing the alkali concentration employed in the process from 2.0% to 4.5% led to a significantly improved sugar yield.

Table 3: Concentration of reducing sugars (in g.L⁻¹) obtained after alkaline pretreatment and acid hydrolysis

Alkaline con-centration (%)	Time (min)			
	15		15	
	M	SD	M	SD
2.0	6.91 ± 0.12		7.76 ± 1.01	6.05 ± 0.04
	A ¹ a ²		Aa	Ba
4.5	7.45 ± 0.10		8.63 ± 0.09	8.90 ± 0.35
	Ba		Aa	Ca
7.0	7.12 ± 0.13		6.89 ± 0.52	5.13 ± 0.21
	Ab		Ab	Aa

(1) Means followed by the same uppercase letter sin the same column do not differ, at a given time. (Tukey, P > 0.05)

(2) Means followed by the same lowercase letter son the same line do not differ, at a given concentration. (Tukey, P > 0.05)

Among the alkaline pretreatment tests, the highest average glucose yield was obtained in 30 min at 4.5% NaOH, i.e., 2.26g L⁻¹ of glucose (see Figure 3). A statistical analysis of these data showed no difference (Tukey, P> 0.05) compared to the treatment performed in 15 min with a

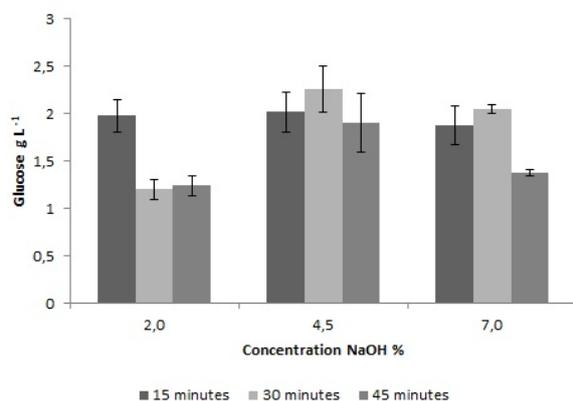


Figure 3 –Concentration of glucose after alkaline pretreatment and acid hydrolysis

The highest average release of xylose, i.e., 6.76 g L⁻¹, was achieved in 45min with 4.5% NaOH (see Figure 4). The xylose yield in this test differed statistically (Tukey, P<0.05) from all the others. However, considering that xylose is a secondary sugar in ethanol fermentation because it is not metabolically prioritized by yeasts, a 15 min treatment with 4.5% alkali could be adopted because, in terms of the yield of glucose and reducing sugars, it would not result in a statistically significant difference from the other treatments. Rocha et al., [35] it evidences than even in milder conditions, the utilized pretreatment alkaline cause a substantial cellulose loss. These results indicate that the concentration of reagent used in the pretreatment, as well

lower concentration of NaOH. Taking into account the cost-benefit ratio, this condition was favorable because it involves less time and a lower alkaline concentration, thus generating savings in the process.

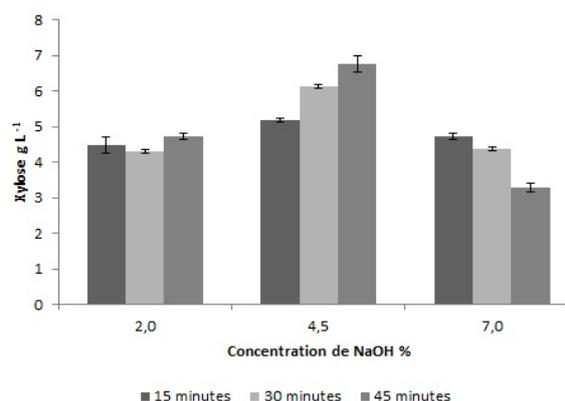


Figure 4 –Concentration of xylose after alkaline pretreatment and acid hydrolysis

as the conditions of hydrolysis and raw materials, are factors that directly affect the yield of fermentable sugars.

CONCLUSIONS

The alkaline pretreatment with 4.5% NaOH for 15 min was the best condition, in terms of cost-effectiveness, for the release of fermentable sugars in the acid hydrolysis of grape skins.

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