flour: a green alternative to conventional methods for adding value to agricultural

by-products

Running title: Production of xylooligosaccharides from Brazilian *Syrah* grape pomace

flour

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Abstract

BACKGROUND: The aim of this work was to determine the most favorable

conditions for the production of xylooligosaccharides (XOS) from Brazilian Syrah

grape pomace. Chemical processes were performed using a rotatable central composite

design, where the concentration of sulfuric acid or concentration of sodium hydroxide and grape pomace flour: solvent mass ratio were the dependent variables. Enzymatic production was also evaluated using xylanase produced by *Aspergillus niger* 3T5B8 and Viscozyme® enzymatic commercial cocktail.

RESULTS: Chemical extraction allowed to recover 21.8 to 74.6% and 5.2 to 96.3% of total XOS for acid and alkaline processes, respectively. Enzymatic production using xylanase extracted up to $88.68 \pm 0.12\%$ of total XOS and up to $84.09 \pm 2.40\%$ with Viscozyme®.

CONCLUSION: The present study demonstrated different feasible methods to produce high added value molecules, the xylooligosaccharides, from *Syrah* grape pomace flour, valorizing this major by-product. The use of enzymatic cocktails demonstrated to be an alternative to the conventional methods, allowing to obtain an eco-friendly and sustainable grape pomace extract.

Keywords: biomass; grape pomace; xylooligosaccharides; enzymatic production, xylanase

1. Introduction

Grapes are one of the most cultivated fruit crops worldwide, around 67 million tons annually, from which more than 70% is intended to wine industry that generates up to 20% of wasted biomass in the form of grape skin, seeds, stems and residual pulp, known as grape pomace [1-3]. Syrah is a vigorous grape variety with a spreading growth habit and a tendency to produce long, trailing shoots. Growth can be excessive on deep, fertile soils and with high-vigor rootstocks. A versatile variety, Syrah is well adapted to a wide range of vinicultural temperature regions, winery uses, and wine styles [4].

As a lignocellulosic feedstock, grape pomace is mainly constituted by polysaccharides, arranged as hemicellulose and cellulose cross-linked to lignin, but there are also other components such as proteins, fat and ash [5, 6]. Grape pomace is reported to be rich in high added value compounds such as non-digestive polysaccharides that constitute the dietary fiber, structural proteins and phenolic compounds [6, 7].

More recently, the use of different solvents and enzymes to partially hydrolyze the polysaccharides into oligosaccharides is in evidence due to their diverse application and market evaluation. The composition and the structure of xylooligosaccharides (XOS) depend on the source and the production process [8, 9]. XOS can be prepared from different vegetal sources, such as from xylan rich agricultural residues by autohydrolysis process, which does not eliminate undesirable components such as soluble lignin and monosaccharides, generates oligosaccharides with a high degree of polymerization and it requires extensive purification processes [8]. Alternatively, as an improvement from the method, acid or alkaline solvents or enzymes can be added aiming better results in terms of oligosaccharides recovery [10]. Enzymatic hydrolysis

is attractive because it does not produce undesirable byproducts, results in lower production of monosaccharides and its hydrolytic action is specific for each biochemical group present in the biomass [11]. Extraction of XOS is improved by endoxylanases with less or no amount of β-xylosidase activity. While endoxylanases form XOS from xylan, β-xylosidase degrades XOS to xylose [11-13].

Recently, extraction of XOS from lignocellulosic feedstocks using alternative methods has become very common. Acidic and enzymatic extractions were applied to obtain XOS from tobacco stalk, corn cob and wastewaters of viscose fiber mills [8,14,15]. Enzymatic extractions using xylanases have been the most common method to extract XOS from widely different biomasses such as corn cob, sugarcane bagasse, oil palm fronds, cotton stalk, sunflower stalk, wheat straw or rice hull [13,16-18]. Nevertheless, there are no works referring to XOS production using grape pomace.

Therefore, the aim of this work was to valorize a major agricultural by-product through the extraction of XOS from the Brazilian *Syrah* grape pomace by the use of different methods, namely chemical and enzymatic hydrolysis.

2. Experimental

2.1. Raw Material

Syrah grape pomace (*Vitis vinifera L. cv. Syrah*) from red sparkling production, harvested in January 2016, was provided by Ouro Verde Winery (Miolo Wine Group), located at Vale do São Francisco, Bahia, Brazil. The pomace was oven-dried at 45 ºC for 24 hours and the flour was obtained by milling and sieving the dried pomace in a Bonina 0.25 df depulper (Itametal, Itabuna, Bahia, Brazil). Since the seeds already have a well-established technological route for recovery of grape seed oil, which is a well-

established value-added ingredient, they were retained in the sieve and the pomace flour was packed under vacuum and stored in a desiccator, at room temperature, until use.

Pomace flour was analyzed, in triplicate, for moisture content, ash, protein and total dietary fibre through AOAC methods [19-21], and for fat content through AOCS method [22]. Determination of cellulose and hemicellulose content was carried out according to NREL methodology [23].

2.2. Chemical production of XOS

Chemical production of XOS were performed in an autoclave at 120 ºC for 90 minutes, using 5 g of pomace flour. Sulfuric acid (acid extraction) or sodium hydroxide (alkaline extraction) were used as solvents at different concentrations. After cooling at room temperature, samples were filtered under vacuum with Whatman filter paper No. 1.

The best parameters of acid and alkaline extractions were determined by response surface methodology, according to a 2^2 central composite design, using the XOS extract yield as response. Two factors were analyzed as independent variables: S: L ratio (X_1) and concentration of sulfuric acid or sodium hydroxide (X_2), being evaluated in five levels, according to Tables 1 and 2. The following polynomial equation was fitted to data:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 \tag{1}$$

Where βn are constant regression coefficients, y is the response (XOS yield), and x_1 and x_2 are the coded independent variables (S:L ratio and H_2SO_4 or NaOH concentration, respectively).

After the definition of the best production conditions, the kinetics of chemical extraction was determined for 120 minutes.

2.3. Enzymatic production of XOS

Enzymatic extractions were performed as alternative to chemical treatment, using an enzymatic cocktail with xylanase activity, produced at our lab, and commercial Viscozyme® L cellulolytic enzyme mixture from Novozymes (Bagsvaerd, Denmark).

2.3.1. Xylanase production

Aspergillus niger 3T5B8 conidia, stored in sterile soil at -18 °C, were transferred for agar medium and incubated at 32 °C for 7 days. Conidia were then removed with 20 mL of sterilized Tween 80 0.3% and 1 mL of spore suspension was transferred for inoculation in 4.6 g of corn cob enriched with 6 mL of aqueous solution containing: 22.8 μL of $KHPO_4$ 20% (w/w), 0.118 mg $ZnSO_4$, 0.138 mg $Fe_2(SO_4)_3$, 0.3 μg $MnSO_4$, 0.0015 μL H_2SO_4 95% and 336 mg peptone, and finally incubated at 32°C for 5 days. The initial moisture content, determined gravimetrically, was adjusted to 60%. All liquid added to the flask was taken into consideration for calculating the moisture content.

Solid state fermentation was conducted in columns, using wheat bran as substrate. Briefly, 100 g of substrate were supplemented with 60 mL of $(NH_4)_2SO_4$ 0.91% in HCl 0.1 N, sterilized and conidia were inoculated at final concentration of 10^7 spores/ g. Inoculated media was transferred to the fermentation columns, which were incubated in a fermentation bath at 32 °C with adjusted aeration.

To extract the enzyme, fermented matter was mixed with citrate buffer (0.05 M, pH 4.8) at a solid/liquid ratio of 1 g initial dry substrate/ 2.5 ml buffer. The mixture was incubated at 32 °C for 60 min. Subsequently, solids were separated from the extract by centrifugation at 13.500×*g* for 15 min. The supernatant was filtered through Whatman

No. 1 filter paper to obtain a clear extract and assayed for xylanase activity by the method of Gomes *et al*. with slight modifications [24].

2.3.2 Determination of enzyme activity

The release of reducing sugars in 30 min at 50 °C, pH 5.0 (0.2 M sodium acetate buffer) was measured as xylose equivalents using dinitrosalysilic acid method, using 1% birchwood xylan solution as substrate [24,25]. The method was performed in triplicate. One unit of xylanase activity (U) is defined as the amount of enzyme liberating 1 μmol of xylose/min, under assay condition.

2.3.3. Production of XOS

Enzymatic production of XOS was performed with the enzymatic cocktail obtained in 2.3.2 section and with Viscozyme®, with final xylanase activities of 10 and 100 UI/ g each, following the method described by Sabiha-Hanim *et al*. (2011) and Goméz-García *et al*. (2012) [17,26]. Briefly, the enzyme mix was diluted in sodium acetate 0.2 M buffer, in the desired enzymatic activity, and added to 100 mg of grape pomace at S:L ratio of 1:18. The pH was adjusted to 5.0 and incubated at 40 °C with shaking at 200 rpm for up to 6 h. The reaction was stopped by heating the test tubes to 100 °C for 5 min and the supernatant was filtered through Whatman No. 1 filter paper to obtain a clear extract.

2.4. Determination of total reducing sugars

Reducing sugars were analyzed through Dinitrosalicylic Acid (DNS) method [25]. DNS was added to 200 mL of NaOH 2N solution with a final concentration of 50 g/ L,

homogenized and then 500 mL of Rochelle salt 0.6 g/ mL were added. The mixture was heated at 40 ℃ and diluted to final volume of 1000 mL.

2.5. Quantification of monomeric and oligomer sugars: *High Performance Liquid Chromatography* (HPLC)

Prior HPLC analysis, the acidic and alkaline extracts were neutralized with sodium hydroxide 20 M or sulfuric acid 0.01 M, respectively, and all samples were re-filtered with Millipore Millex syringe filter 0.22 μm. Identification and quantification of xylan-derived sugars (xylose, xylobiose, xylotriose, xylotetraose and xylopentose) were performed in Waters 600 HPLC equipped with Refractive Index Detector at 45 ℃ (Waters Corporation, Milford, MA, USA), with an Agilent Carbohydrate 5 μm (4.6 x 250 mm) column at 30 ℃ (Agilent, Santa Clara, USA), and based on the isocratic method described by Macrae [27]: the mobile phase was established by acetonitrile 70% with a flow rate of 1 mL/ min. Compounds were identified by the comparison to the retention times of pure standards, as for xylose and xylobiose (Sigma, St. Louis, USA), and xylotriose, xylotetraose, xylopentose, and xylohexaose (Megazymes, Wicklow, Ireland), and quantified through external standard calibration.

Percentage of XOS extraction yield was determined using equation (2).

(2) Production of XOS (%) = Concentration of XOS (g/ 100 g)/ Total xylan in grape pomace flour (9.61 g/ 100 g) x 100

2.6. Statistical analysis

Statistical analysis was performed with IBM SPSS statistic program v 23.0 (Illinois, USA), using t-student test for independent samples and analysis of variance

(ANOVA) with Bonferroni post hoc test. Differences were considered to be significant at a level of $p < 0.05$. For the central composite design, the analysis of variance (ANOVA), test for the lack of fit, determination of the regression coefficients and the generation of surface responses were carried out using the Statistica 7.0 software (StatSoft, Tulsa, USA).

3. Results and Discussion

3.1. Characterization of *Syrah* grape pomace flour

Syrah grape pomace was oven-dried at 45 ºC for 24 hours and seeds were separated from the grape pomace during the milling and sieving processes to obtain a flour with a particle size inferior than 300 μm, which was further analyzed for moisture content (95.9 g/ kg), ash (38.0 g/ kg), total protein (38.5 g/ kg), fat (13.4 g/ kg), sugars (543.0 g/ kg) and dietary fiber (271.2 g/ kg).

Structural carbohydrates were determined through acidic hydrolysis [23], and revealed high contents of glucose and xylose (119.5 and 84.6 g/ kg of pomace, respectively), a low concentration of arabinose (5.8 g/ kg of pomace) and no traces of mannose or galactose. As a lignocellulosic feedstock, grape pomace was also analyzed, through the NREL method [23], for its cellulose, hemicellulose and lignin content which were 132.7, 102.6 and 307.7 g/ kg, respectively. Grape pomace is usually rich in different classes of polysaccharides, and consists of 30% of neutral polysaccharides, including glucan, xylan, galactan, and mannans, depending on the grape variety [28, 29]. It also comprises ca. 20% of acidic polysaccharides, such as rhamnogalacturonans, arabinogalactans and pectins [28]. Due to the absence of mannose and galactose in the monosaccharide profile, and to the insignificant concentration of arabinose all the

observed xylose was considered to be related to the xylan's structure. Therewith, total xylan was determined based on xylose concentration and adjusted, corresponding to 96.1 g/ kg of pomace.

The carbohydrates profile is in accordance with the results reported by Zheng *et al.* [30] for red grape pomace (cellulose 14.5 wt % and hemicellulose 10.3 wt %), but lower than the values reported by Mendes *et al.* [31] for *Touriga Nacional* (cellulose 20.8% and hemicellulose 12.5%). The protein content is in accordance with different pomace varieties such as *Cabernet Sauvignon* (31 g/ kg), *Callet* (27 g/ kg), *Manto Negro* (32 g/ kg), *Merlot* (38 g/ kg) and Syrah (33 g/ kg) [27]. Fat content in grape pomace is mainly provenient from seeds oil. In this study, once the seeds were separated from the pomace using a depulper, a low fat composition was found, in accordance with previously reported data [6]. Dietary fiber and carbohydrate content had higher values than the other macromolecules, as expected for a vegetal matrix.

3.2 Chemical production of XOS

Total sugars present in the extracts obtained by chemical extraction varied from 174.4 to 825.7 g/ kg of pomace flour for acid extraction and from 96.8 to 361.2 g/ kg of pomace for alkaline extraction. Regarding XOS production, the extraction yields ranged from 21.83% to 74.58% for acid treatment and from 5.15 to 96.28% for alkaline treatment, as shown in Table 3. Strong acids, such as sulfuric acid used in the experiment, allow higher degree of polysaccharide hydrolysis and therefore, more simple sugars are produced. However, this hydrolysis is dependent on the acid concentration, which explains the high variable concentration of sugars present in the acid extracts when compared to the alkaline extracts.

These results are in accordance with the results obtained by Chapla *et al.* [11], who reported that dilute alkali extraction method was the most suitable method for the production of xylan from raw corncobs. Alkali causes the swelling of lignocellulosic feedstocks, leading to a decrease in the degree of polymerization and crystallinity, separation of structural linkages between lignin and carbohydrates, and final disruption of lignin, helping to achieve a simple recovery of xylan from lignocellulosic feedstocks [32,33]. Dilute acid method extracted relatively less amount of xylan when compared to alkaline process.

Table 4 shows the regression coefficients for the coded polynomial equations, the F values and the determination coefficients (R^2). Some non-significant terms were eliminated and the resulting equations were tested for adequacy and fitness by the analysis of variance (ANOVA). The fitted models were suitable, showing significant regression, low residual values, no lack of fit and satisfactory determination coefficients.

According to Figure 1, mass ratio between pomace flour (solute) and solvent was the parameter with the highest impact on the XOS production yield, for both acid and alkaline extractions. The use of higher volumes of solvent involves higher mass transfer gradient, resulting in higher production of XOS. In the case of alkaline extraction, XOS production showed a linear behavior and, thus, the axial points were not used in the model. In contrast, the acid extraction showed a significant curvature and therefore, in this case, the axial points were considered.

Acid concentration showed a slight effect on XOS production (Figure 1a), while the increase of NaOH concentration led to the increase on the amount of recovered XOS, which can be a result from the more intense hydrolysis. This result could be explained by the concentration range of NaOH used in the alkaline hydrolysis (0.2 −

9.8%, w/v), which was higher than the concentration range of H_2SO_4 used for acid hydrolysis (0.1 – 4.9%, w/v), resulting in more pronounced effects on the XOS production. The results from acid hydrolysis are in accordance with the results obtained by Akpinar and co-workers, who produced higher amounts of XOS with lower concentrations of H_2SO_4 (0.125 and 0.250 mol/L) than with 0.5 mol/L H_2SO_4 [8]. The results obtained by Sun and colleagues, who extracted XOS from perennial shrub using alkaline solvent, KOH, are in accordance with our results, as they produced more XOS when using higher concentrations of alkali [34].

Modelling and analyzing the surface response for XOS recovery allowed to conclude that the most adequate operational conditions was achieved with NaOH at concentration of 8.4% and a flour: solvent ratio of 1:18, with a yield of 96.28% for total extraction of XOS.

The specific extraction of each XOS is shown in Figure 2. Chemical extraction only allowed the production of xylotetraose (X_4) and xylopentaose (X_5) from the pomace flour and an overlook to the results indicates that both methods extracted a majority of X_4 and X_5. In the acid extraction, trials 7, 8 and 9 (trial 9 corresponding to the average of extractions of XOS in the central point) had a significantly higher ($p < 0.05$) extraction of X_4, indicating that a middling mass ratio (1:14 in these cases) may be useful to control the degree of hydrolysis of xylan. On the other hand, trials 1 to 4 produced more X_5 but no significant differences were found between them, except for trial 2, which significantly produced more X_4 ($p < 0.05$). Alkaline extraction presented a more heterogeneous XOS recovery: trials 2, 4, 8 and 9 produced more X_5, while trial 6 extracted more X_4 ($p < 0.05$).

Although it is known that degree of polymerization of XOS affects their prebiotic effect, to be studied in the near future it is not so well studied how specific strains

degrade and use XOS as carbon source. Efficient degradation of XOS by bacteria requires different enzymes, including β-xylosidase, α-glucuronidase, α-L-arabinosidase, or acetyl xylan esterase, thus degradation of XOS vary between strains [35]. Gullón and co-workers studied the use of XOS by different Bifidobacteria and concluded that xylotriose was the most consumed, followed by xylobiose, xylotetraose and xylopentaose [36]. However, many authors use mixtures of XOS with different polymerization degrees (2 – 6) as carbon source for *in vitro* fermentations of Bifidobacteria [37,38]. Considering all these variables, the best extraction condition was considered the one that extracted more amount of total XOS and not a specific xylooligosaccharide.

As all extractions were performed for 90 minutes, an additional kinetics assay was performed in the selected best extraction condition (8.4% NaOH and S:L ratio of 1:18), in order to analyze the influence of extraction time on XOS produce. Results are presented in Figure 3, which shows that XOS extraction continuously increased up to 90 minutes and did not show significant increase after 120 minutes of extraction ($p > 0.05$). This means that the optimal time of extraction would be 90 minutes, as used in the factorial planning, and the best condition for XOS extraction was 8.4% NaOH and 1:8 of S:L ratio.

Although chemical treatments at high temperatures are efficient in breaking the ultrastructure of the cell wall, generating partial hydrolysates of polysaccharides, there are also undesirable reactions, such as the formation of monosaccharides that can easily originate toxic compounds like furfural and 5-hydroxymethylfurfural, requiring purification steps to remove them, and lately increasing the process costs [39,40]. Thus, another alternative for obtaining XOS from plant material is the enzymatic treatment. Enzymatic hydrolysis using endoxylanases, in turn, prevents formation of toxic

byproducts due to low temperature and high specificity, so it has been considered a good alternative for XOS produce [13,39,40].

3.3. Enzymatic extraction

3.3.1 Production of xylanases and determination of enzymatic activity

Production of XOS from various sources of xylan, such as sugarcane bagasse or cotton stalks, using commercial xylanases have been reported in different works [13,39]. However, fairly few attempts have been made for production of XOS using indigenously produced xylanases. In order to make the process cost effective and economic, xylanase used under the present study was produced with a low cost technique under optimized conditions using wheat bran as a substrate under solid state fermentation, as mentioned [24].

Viscozyme® (Novozymes, Bagsvaerd, Denmark) is an enzymatic cocktail with (endo- 1, 3 (4)-) beta-glucanase, xylanase, cellulase and hemicellulase activities, produced by *Aspergillus aculeatus*. It was chosen as control for its hemicellulase and xylanase activities. Xylanase activity determined for the xylanase produced from *A. niger* 3T5B8 and for Viscozyme® were 28.77 ± 0.79 and 116.41 ± 4.27 IU/ mL, respectively. Data sheet of Viscozyme reports an activity of ca. 100 FBG/ g, which is in accordance with the results obtained. Although the produced enzyme is suitable for the extraction of XOS, it has a significantly lower xylanase activity than the enzymes produced by Chapla *et al.*, 9200 ± 78.5 IU/ mL, who used *Aspergillus foetidus* MTCC 4898 instead of *A. niger* and applied additional steps for enzyme purification, including ammonium sulfate precipitation and dialysis, achieving a pure enzyme with high xylanase activity but also with additional costs of production [16].

3.3.2. Enzymatic extraction

Enzymatic extraction was performed using the produced xylanase from *A. niger* 3T5B8 and Viscozyme®, with enzyme load of 10 and 100 IU/g. Extraction times were 1, 2, 4, and 6 hours. Results of total extraction of XOS and xylose are presented in Figure 4.

Extraction of XOS using the mixture of enzymes produced by *A. niger* 3T5B8 with activity of 10 IU/g for 1 and 4 hours, allowed to extract from 22.20 ± 0.87 and 51.52 ± 0.70 % of XOS, respectively. After 6 hours of extraction, the amount of xylose extracted (45.39 ± 6.25%) was much higher than the amount of XOS (14.09 ± 3.17%). The same mixture of enzymes with final concentration of 100 IU/g allowed to produce the maximum amount of XOS (88.68 ± 0.13%) after 4 hours of extraction, without producing xylose monomers. The other extraction times allowed to produce from 13.00 ± 0.38 to 74.83 ± 3.89% of XOS. While the extraction of 4 and 6 hours produced no xylose monomers, the other extraction times produced higher amount of xylose, not being suitable for XOS extraction.

The use of 10 IU/g of Viscozyme® had a slight produce of XOS within 1 and 2 hours. Extraction at 4 hours allowed to produce 36.26 ± 0.13% of total XOS with minimal production of xylose. Extraction for 6 hours had superior extraction of XOS but also a superior production of xylose: 20.88% of sugar monomers. At last, the use of 100 IU/g of Viscozyme® allowed to recover from 25.60 to 84.09 ± 2.40% of total XOS, without production of any xylose monomers. This data is in accordance with the results obtained by Akpinar and co-workers, who used a commercial xylanase (Veron 191 from *A. niger*, AB Enzymes, Germany) to extract XOS from cotton stalks, and produced ca. 53% of total XOS [39]. However, these authors produced a majority of xylohexaose and xylopentaose instead of the xylotetraose recovered in this work (Table 5).

Although extractions with Viscozyme® produced more quantity of XOS ($p =$ 0.050), the use of our enzyme (condition of 100 IU/g for 4 hours) allowed to extract XOS with a minimum amount of xylose monomers. This capacity could be due to the substrate composition or due to other enzymatic activities associate with our enzymatic cocktail that were not analyzed (hemicellulolytic activity, for instance). Extractions performed with 100 IU/g of enzyme allowed to produce more quantity of XOS than extractions with 10 IU/g ($p < 0.05$), in accordance with the results obtained by Akpinar *et al.,* who concluded that xylan hydrolysis yield increases with enzyme concentration as production of XOS using lower enzyme concentrations is too slow [39].

The use of both enzymes for enzymatic treatment of grape pomace allowed to produce high amounts of xylotetraose but also some xylopentaose, depending on the quantity of enzyme used and time of incubation. Detailed data is show in table 5.

In conclusion, enzymatic extractions proved to be as efficient as acid or alkali, without the need of using environmental unfriendly solvents, and should be used in the future for studies on bioactivities

4. Conclusion

The present study demonstrated different feasible methods to produce high added value molecules, the xylooligosaccharides, from *Syrah* grape pomace flour, as an alternative to valorize this major by-product.

Enzymatic XOS production is affected by the enzyme type and enzyme loading: xylanases mixture produced by *A. niger* 3T5B8 at 100UI/ g was found to be the most suitable condition. The use of enzymatic cocktails demonstrated to be an alternative to the conventional methods, as they allowed to obtain similar yields of XOS, but within an eco-friendly and sustainable grape pomace extract. Also, in both chemical and

enzymatic extracts, produced XOS were mainly composed by xylotetraose and xylopentaose. The biological activities of XOS, particularly their described biological activities with impact on gastrointestinal health, allow to conclude that these enzymatic grape pomace extracts can be a potential candidate to be used in the development of a new functional ingredient. The development of such product requires further studies on biological activities, specifically the demonstration of prebiotic activity and absence of toxicity.

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Table 1 – Coded variables studied in the XOS produced by acid hydrolysis.

Coded variables	-1.41	-1.0	0	+1.0	+1.41
S : L ratio	1 : 8	1 : 10	1 : 14	1 : 18	1 : 20
H_2SO_4 concentration (%)	0.1	0.8	2.5	4.2	4.9

Table 2 – Coded variables studied in the XOS produced by alkaline hydrolysis.

Coded variables	-1.41	-1.0	0	+1.0	+1.41
S : L ratio	1 : 8	1 : 10	1 : 14	1 : 18	1 : 20
NaOH concentration (%)	0.2	1.6	5.0	8.4	9.8

Table 3. XOS produced (%) in the acid and alkaline extractions

Acid hydrolysis				Alkaline hydrolysis			
Trial	S : L	H_2SO_4 conc. (%)	XOS produced (%)	Trial	S : L	NaOH conc. (%)	XOS produced (%)
1	1 : 10	0.8	49.49	1	1 : 10	1.6	21,12
2	1 : 18	0.8	74.58	2	1: 18	1.6	89.95
3	1 : 10	4.2	40.92	3	1 : 10	8.4	33.94
4	1 : 18	4.2	60.10	4	1 : 18	8.4	96.28
5	1 : 8	2.5	21.83	5	1 : 8	5.0	10.57
6	1 : 20	2.5	48.49	6	1 : 20	5.0	58.60
7	1 : 14	0.1	73.97	7	1 : 14	0.2	5.17
8	1 : 14	4.9	65.14	8	1 : 14	9.8	73.76
9	1 : 14	2.5	67.27	9	1 : 14	5.0	76.47
10	1 : 14	2.5	66.73	10	1 : 14	5.0	78.22
11	1 : 14	2.5	65.95	11	1 : 14	5.0	75.48

Table 4. Coded second-order regression coefficients for encapsulation efficiency and bulk density.

Coefficient	% XOS produced by acid hydrolysis	% XOS produced by alkaline hydrolysis
β_0	66.63	72.47
β_1	-4.45	13.36
β_2	10.26	24.18
β_{11}	2.47	--
β_{22}	-14.82	--
β_{12}	-1.48	-10.23
R^2	0.980	0.966
F	48.76	28.67

Table 5. Content (g/ kg of grape pomace) of each xylooligosaccharide present in the enzymatic extracts of grape pomace

	3T5B8		Viscozyme	
	10 UI/ g	100 UI/ g	10 UI/ g	100 UI/ g
1 h	$X_4 = 31.8$	$X_4 = 22.2$ $X_5 = 28.9$	-	$X_4 = 49.2$
2 h	$X_4 = 21.3$	$X_4 = 10.6$ $X_5 = 18.5$	-	$X_4 = 80.8$
4 h	$X_4 = 49.5$	$X_4 = 85.2$	$X_4 = 34.8$ $X_5 = 0.1$	$X_4 = 71.9$
6 h	$X_4 = 13.5$	$X_4 = 71.9$	$X_4 = 55.9$ $X_5 = 0.7$	$X_4 = 55.3$
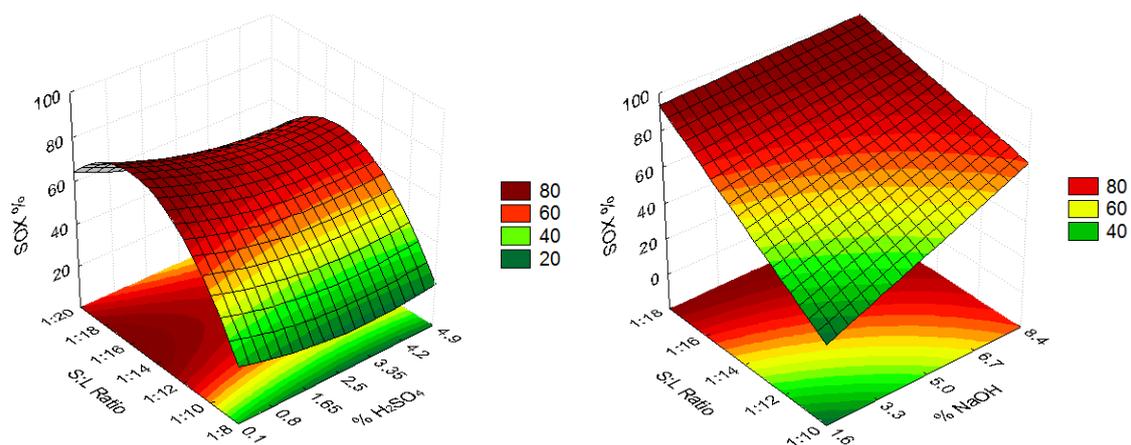
Figure 1 – Surface response for extraction through conventional methods: A) acid and

B) alkaline.
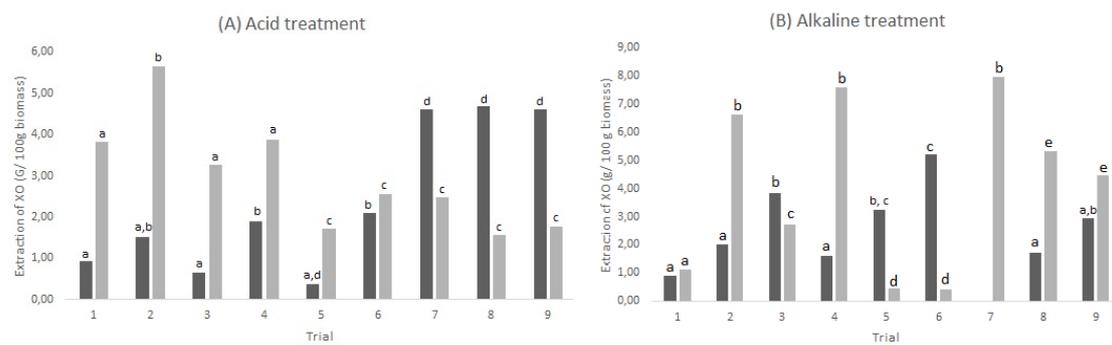
Figure 2 – Total extraction of each xylooligosaccharide (xylotetraose) (■) and xylopentaose (□) for both conventional treatments: A) acid extraction and B) alkaline extraction
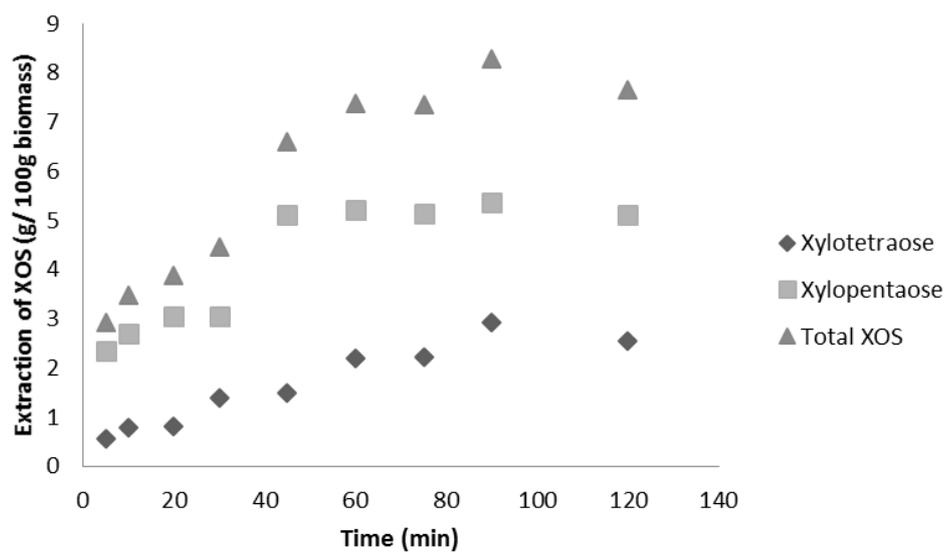
Figure 3 – Kinetics of conventional extraction of XOS using NaOH.
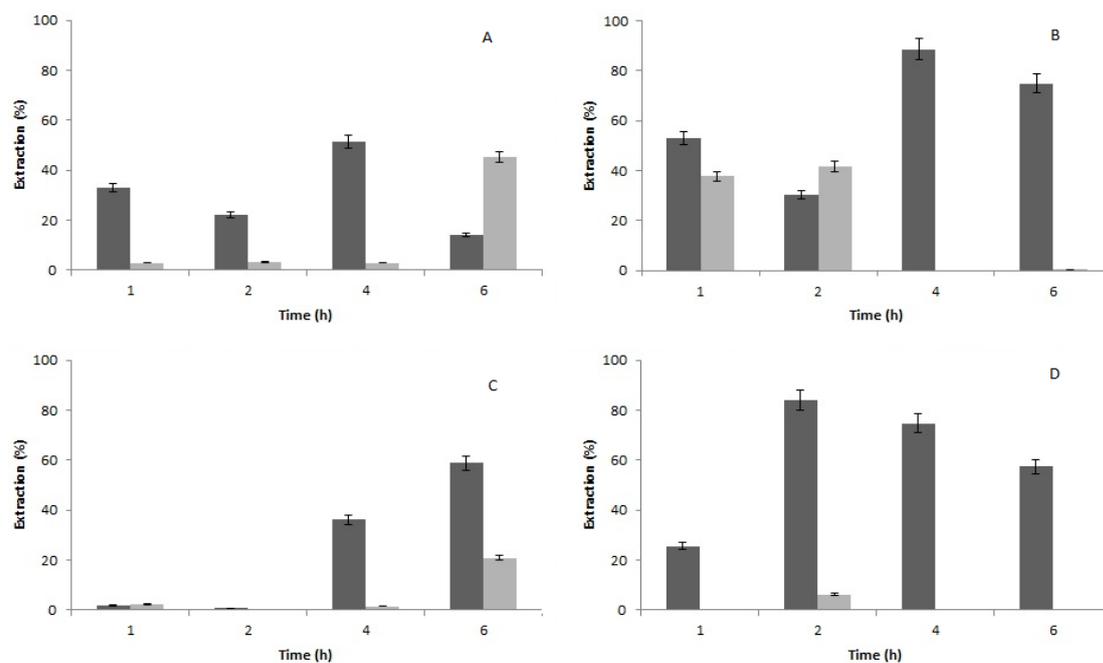
Figure 4 – Enzymatic extraction of XOS (■) and xylose (□) from grape pomace, using a mixture of enzymes from *A. niger* with final activity of (A) 10 IU/g and (B) 100 IU/g, and commercial Viscozyme with final activity of (C) 10 IU/g and (D) 100 IU/g.