Tannin extraction from grape stems through a solid-liquid process: optimizing efficiency by applying the response surface methodology

Extração de tanino do caule da uva por meio de um processo sólido-líquido: otimizando a eficiência aplicando a metodologia de superfície de resposta

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Cristiane Lisboa Giroletti
Mestra em Engenharia Ambiental, Programa de Pós Graduação em Engenharia Ambiental, Universidade Federal de Santa Catarina. Campus Universitário UFSC/CTC, Bairro Trindade - CEP 88040-900. Florianópolis - SC
E-mail: cristiane.lisboa@gmail.com

Jean Carlo Salomé dos Santos Menezes
Doutor em Engenharia, Universidade Federal do Rio Grande do Sul. Pós- doutorando no Programa de Pós- Graduação em Engenharia Civil e Ambiental, Universidade de Passo Fundo. Av. Brasil Leste, 285 - São José, Passo Fundo - RS, 99052-900
E-mail: jeancarlomenezes@gmail.com

Leonardo Dalri-Cecato
Mestre em Engenharia Ambiental, Programa de Pós Graduação em Engenharia Ambiental, Universidade Federal de Santa Catarina. Campus Universitário UFSC/CTC, Bairro Trindade - CEP 88040-900. Florianópolis - SC
E-mail: leonardo.dalri@gmail.com

Beatriz Lima Santos Klienchen Dalari
Mestre em Engenharia Ambiental, Programa de Pós Graduação em Engenharia Ambiental, Universidade Federal de Santa Catarina. Campus Universitário UFSC/CTC, Bairro Trindade - CEP 88040-900. Florianópolis - SC
E-mail: beatriz.lskd@hotmail.com

Maria Eliza Nagel Hassemer
Doutora em Engenharia Ambiental, coordenadora do Programa de Pós Graduação em Engenharia Ambiental, Universidade Federal de Santa Catarina. Campus Universitário UFSC/CTC, Bairro Trindade - CEP 88040-900. Florianópolis - SC
E-mail: maria.eliza@ufsc.br

ABSTRACT
Viticulture is an agro-industrial sector that produces large amounts of waste, with grape seeds, bagasse, and stems standing out among the main types of waste produced. This study evaluated the effects of extraction conditions of total and condensed tannins from grape stems through a solid-liquid process using the response surface methodology. Contact time between matrix and solvent and the concentration of ethanol in the solvent

were monitored as independent variables. Contact time varied between 12 and 24 hours. The extraction solvent was composed of a mixture of ethanol and water in proportions of 0, 50, and 100% (v/v). Total tannins were quantified by the Folin-Ciocalteu method in tannic acid equivalents. Condensed tannins were quantified by the vanillin method in catechin equivalents. Response surface methodology pointed out similar conditions for the extraction of total and condensed tannins. Best yields for total tannins were achieved with a contact time of approximately 21.18 hours and a hydroalcoholic solvent composed of 51.65% ethanol. Regarding condensed tannins, the contact time was slightly inferior, with the best yields obtained in 19.31 hours with a hydroalcoholic solvent composed of 49.19% ethanol. The tannic extract obtained under optimized conditions according to the results of the response surface methodology was characterized by Fourier-transform infrared spectroscopy, allowing the identification of functional groups present in the extract.

Keywords: Tannins, grape stems, response surface methodology, vitis-labrusca, solid-liquid extraction.

RESUMO
A vitivinicultura é um setor agroindustrial que produz grandes quantidades de resíduos e dentre os principais destacam-se as sementes de uva, os bagaços e os engaços. O presente estudo avaliou os efeitos das condições de extração de taninos totais e taninos condensados presentes em engaços de uva, via processo sólido líquido, através da metodologia da superfície de resposta (RSM). As variáveis independentes monitoradas foram o tempo de contato entre a matriz e o solvente e a concentração de etanol no solvente. O tempo de contato variou entre 12 e 24 horas e o solvente de extração foi composto por uma mistura de etanol e água na proporção de 0, 50 e 100%. Os taninos totais foram quantificados pelo método Folin-Ciocalteu equivalente à ácido tânico e os taninos condensados pelo método de vanilina equivalente à catequina. A RSM apontou condições semelhantes para extração de taninos totais e condensados, os melhores rendimentos para taninos totais foram alcançados com tempo de contato de aproximadamente 21,18 horas, com solvente hidroalcoólico composto de 51,65% de etanol. Para taninos condensados, o tempo de contato foi um pouco inferior, sendo os melhores rendimentos obtidos em 19,31 horas, com solvente hidroalcoólico composto de 49,19% de etanol. O extrato tânico obtido nas condições otimizadas conforme os resultados da RSM foi caracterizado pela espectroscopia de Infravermelho com Transformada de Fourier, a qual permitiu identificar seus grupos funcionais.

Palavras-chave: Taninos, engaços de uva, metodologia de superfície de resposta, vitis-labrusca, extração sólido-líquido.

1 INTRODUCTION

Viticulture is an agro-industrial sector with strong economic and social influence in several countries. This sector constantly evolved in the last decades, especially regarding the development of new grape products and culture management techniques. However, this industry still faces problems concerning the solid waste generated during the processing of the fruit, with approximately 30 to 40% of the entire mass of grapes harvested being discarded as waste. This amount is made up of skins, seeds, and stems, and has a negative environmental impact on the site [1,2,3].

Stems, also known as peduncles or stems, are formed by short branches responsible for transporting nutrients and supporting the grape grains. They are seasonally generated residues that represent approximately 25% of the by-products originating in viticulture. Although recent reports point to grape stems as a valuable material rich in phenolic compounds, monomeric and oligomeric flavonols, stilbenes, and tannins, the material has received relatively little attention, being among the least characterized and valued winemaking residues [3,4,5,6,7, 8].

Tannins are phenolic or polyphenolic compounds found in the bark, leaves, fruits, roots, and seeds of various plant species. These substances play an important role in the interactions between plants and the ecosystem, exercising metabolic activity, and protecting species. Formed by monomeric flavonoid units polymerized in various degrees of concentration, with molecular weights between 100 and 30,000 Dalton, tannins are naturally soluble in water, alcohol, and acetone, and insoluble in pure ether, chloroform, and benzene, presenting the ability to precipitate alkaloids, proteins and heavy metal salts [9,10,11,12].

Tannins are chemically reactive and allow the formation of intra and intermolecular hydrogen bonds. These properties encourage such compounds (especially condensate tannins) to turn into relevant subjects of studies regarding the most diverse applications, including leather tanning, manufacture of adhesives (particularly wood adhesives), ore flotation reagents, cement superplasticizers, corrosion inhibitors, polyurethane coatings, epoxy adhesives, binders for polytetrafluoroethylene (PTFE) coatings, several medical and pharmaceutical applications, and in the production of coagulating agents used in water and wastewater treatment [10,13,14,15,16]. In this context, vegetable matrices such as winemaking residues, especially grape stems, may present an interesting alternative for sustainable tannin production [16].

The main industrial techniques for extracting tannins from plant matrices apply simple procedures using water at temperatures between 70 and 100 °C as the main extraction solvent [12]. Other solvents widely used include acetone, ethanol, methanol, and aqueous mixtures, which have been evaluated for the extraction of phenolic compounds [17, 18]. Factors such as particle size of plant material, temperature, solvent concentration, and contact time also present significant effects on the extraction of tannins from plant matrices [19,20,21,22].

Statistical methods can assist in the improvement of the extraction process, with the response surface methodology (RSM), initially described by Box and Behnken (1960) [23], being an important tool that allows the reduction of the number of tests needed to evaluate multiple parameters and interactions involved in a single process. RSM aims to optimize processes while providing as much information about the process as possible, focusing on improving the accuracy of results given by the empirical model. These advantages make RSM a powerful tool applied in the planning, development, and formulation of new products, as well as improving existing projects and products [12, 24].

In this context, this study applied a Central Composite Design (CCD) – one of various RSM methodologies – to optimize the extraction process of tannins from grape stems.

2 MATERIALS AND METHODS

2.1 RAW MATERIAL SAMPLING AND PROCESSING

Grape stems of Vitis labrusca cultivar (Figure 1) were collected in a rural property located in the inland municipality of Videira, state of Santa Catarina, Brazil. The stems were washed with distilled water and dried in an oven at 50 ºC for 12 hours, time necessary to stabilize the humidity of the samples. After drying, the stems were macerated manually and crushed in a mill, following the recommendations of ASTM D-6405 (American Society for Testing and Materials, 2014) [25].

Figure 1- Fresh grape stems (a) and dry macerated grape stems (b).

Grape stems were characterized in terms of moisture, ash, and organic matter content. Moisture content was determined according to the methodology described in ASTM D-6403 [26].

2.2 TANNINS EXTRACTION SYSTEM

Tannins were extracted from grape stems in a continuous solid-liquid process using a Soxhlet extractor mounted under a heating battery at a constant temperature of 90º C, corresponding to the average temperature of the solvent's boiling point. Portions of 25 grams of stems were inserted into Whatman filter-paper cartridges sealed with a thin layer of cotton. The cartridges were then placed inside Soxhlet extraction tubes. A water inlet mechanism was connected to the condensers providing a continuous flow of water to the system. The heating of the battery placed over the extraction system caused the boiling of the solvents present in the balloons, generating steam, which condensed in the extraction tube and dripped into the cartridges containing the stalk samples. When the volume of condensed steam reached the maximum height of the extraction tube's siphon, the liquid overflowed, carrying the substances soluble in the solvent.

The extraction solvent used consisted of 400 mL of a hydroalcoholic solution composed of a mixture of ethanol and water (0.50 and 100%). This type of solvent is safer for humans and the environment since it is not toxic [(27)]. The extraction time varied between 12 and 24 hours, as defined in the central composite design (CCD).

2.3 CENTRAL COMPOSITE DESIGN (CCD)

The extraction of tannin present in the grape stems was optimized by applying the response surface methodology (RSM). The experimental design consisted of three levels and two independent variables: extraction time (x_1, hours) and solvent composition (x_2, v/v, ethanol/water). Total and condensed tannins concentrations in each test were evaluated as dependent variables. Table 1 presents the independent variables, their coded and actual levels considered in the RSM.

Table 1- Coded and actual levels of independent variables.

Independent variable	Factors	Levels		
		-1	0	1
Time (h)	x_1	12	18	24
Solvent (ethanol concentration 1%)	x_2	0	50	100

This study included 12 tests conducted randomly, with 4 factorial points (+1 and -1), 4 axial points (+1.41 and -1.41), and 4 central points (0), as shown in Table 2.

Table 2- Experimental design matrix.

Tests	X_1	Extraction time	X_2	Solvent concentration
1	-1	12	-1	0
2	-1	12	1	100
3	1	24	-1	0
4	1	24	1	100
5	-1.41	9.5	0	50
6	1.41	26.5	0	50
7	0	18	-1.41	-20
8	0	18	1.41	120
9	0	18	0	50
10	0	18	0	50
11	0	18	0	50
12	0	18	0	50

Treatment of the results was performed using STATISTIC v. 8. Statistical analysis was performed considering the significance level of $p < 0.05$.

2.4 DETERMINATION OF TOTAL TANNINS CONTENT: TANNIC ACID EQUIVALENTS

Total tannin content was expressed in tannic acid equivalents in $mg.L^{-1}$, calculated from a linear regression equation obtained from a calibration curve constructed with tannic acid concentrations ranging from 25 to 150 $mg.L^{-1}$ using the Folin-Ciocalteu adapted method with adaptations [(28)]. The reference curve was prepared by adding 2.0 mL of a standard solution of tannic acid, 2 ml of the Folin-Ciocalteu reagent, and 2 ml of a solution of sodium carbonate (Na2CO3) at 8% into test tubes. For the blank test, the 2.0 mL of the standard tannic acid solution were replaced by 2.0 mL of distilled water. The tubes were kept at rest for 30 minutes, then centrifuged for 5 minutes at 2000 RPM. Readings were performed on a spectrophotometer at a wavelength of 760 nm.

2.5 DETERMINATION OF CONDENSED TANNINS CONTENT: CATECHIN EQUIVALENTS

Condensed tannin content was determined through the Vanillin method using catechin as a reading standard. This method is widely used due to its specificity regarding flavonols quantification, a characteristic that provides an accurate estimate of condensed tannin content in several matrices [(29,30)]. The reference curve was obtained from the reaction of 3 mL of an 8% solution of vanillin in methanol, 0.5 mL of the catechin solution with concentrations ranging from 25 to 150 $mg.L^{-1}$, and 1.5 mL of HCl. Methanol is commonly used in the vanillin method since, in the presence of this solvent, the reaction medium becomes more sensitive to polymeric condensed tannins than to monomeric flavonols [31]. The principle of this method consists in the formation of red-colored complexes, which can be read on a spectrophotometer at 510 nm wavelength. The condensed tannin content was expressed in catechin equivalents in $mg.L^{-1.}$

2.6 CHARACTERIZATION OF TANNIC EXTRACT

Tannin extract, obtained under the optimized conditions established was characterized in terms of its physical-chemical composition, by the parameters of density, pH, total solids, and viscosity. The functional groups of the TBC, as well as the tannin extract, were identified by identified by FT-IR analysis, in potassium bromide pastille, in the scanning range of 400 to 4000 cm^{-1}. The samples were freeze-dried in order to remove the possible interferences caused by the presence of the extraction solvents.

3 RESULTS AND DISCUSSION

From an economic and environmental point of view, stems represent a problem due to the lack of application/use of these residues. It is very likely that the lack of necessary information concerning the phytochemical profile of this residue limits its possibilities of application. This study is inserted in the context that guides the principles of sustainability, being one of the pioneers in the extraction and characterization of tannins from grape stems, as well as in the optimization of process parameters, evaluating the influence of contact time and solvent composition on the extraction process via Soxhlet system. The extraction and characterization of tannins present in grape stems represent a fundamental step for the industrial exploration of this winemaking residue.

3.1 GRAPE STEMS AND TANNIN EXTRACT CHARACTERIZATION

Table 3 presents the physical-chemical characterization of the grape stems used in this study.

Table 3- Physical-chemical characterization of grape stems.

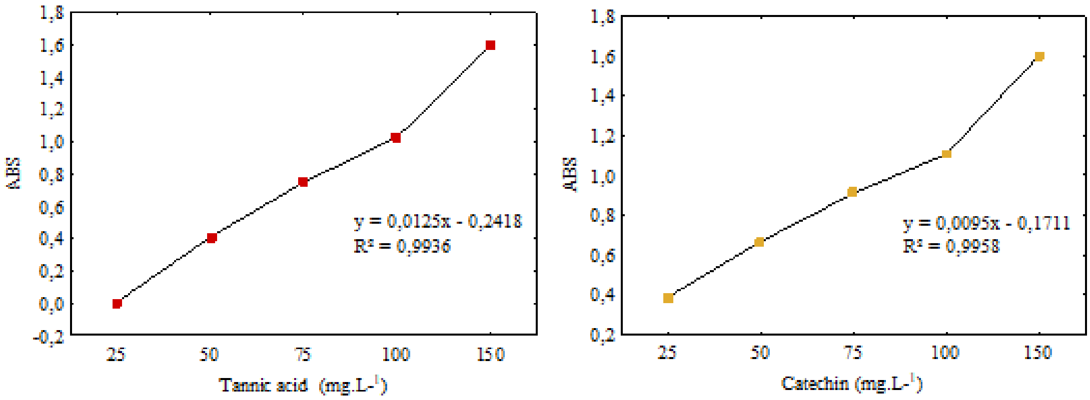
Content	Value (%)
Solid	91,8
Ash	8,2
Moisture	8,68

Grape stems presented a percentage of 91.8% of solids, being the carbonaceous fraction mass of material, and 8.2% of ash, which represents the presence of minerals and inorganic compounds. Due to the need for drying of the stems prior to tannins extraction, the moisture content analysis comprised samples of fresh stems and previously dried stems, thus allowing the determination of moisture loss during sample preparation. The moisture content of fresh stems was 88.67%. Barros et al., (2015) [49], report that moisture content of grape stems can range between 55 to 80%, depending on the species of the cultivar. The moisture content of dried stems was 8.68%. These results show that during the preparation of the stems, preceding maceration and extraction of the tannin, there was a reduction of 79.9% in moisture content.

3.2 TANNINS QUANTIFICATION

Tannins extracted from grape stems were quantified separately, by the Folin-Ciocalteu method for total tannins and the Vanillin method for condensed tannins. Figure 2 present the reference curves constructed with different concentrations of tannic acid and catechin. In both curves, it is possible to observe that the increase in absorbance is directly related to the increase in the concentrations of the reference standards.

Figure 2-Analytical curves with different concentrations of tannic acid and catechin.



Tannic acid was used as a standard in this study as it is a hydrolysable tannin that also allows the characterization of total tannins in a sample. This reagent is very likely to be the best tannin standard commercially available. Catechin, used as a standard for determining condensed tannins by the vanillin method, was selected because it is a precursor to condensed tannins. In addition, the vanillin method is specific for determining flavonoids, so it can be used to selectively determine condensed tannins in the presence of other classes of tannins and other phenolic compounds [31].

3.3 EXPERIMENTAL DESIGN AND CCD RESULTS

In general, the extraction efficiency of a compound is influenced by several parameters such as solvent composition, temperature, contact time, among others. The effects of these variables can be independent, dependent, or interactive [27, 32, 33]. The levels of the independent variables were selected based on the values obtained in preliminary experiments, as the conditions for extracting tannins may not be generalized due to the diversity of plant matrices. Table 4 presents the results of the concentrations of total and condensed tannins obtained in each experiment outlined by the CCD.

Table 4- RSM matrix with decoded levels and tannins concentrations responses in milligrams per stem grams.

Test	Time (h)	Solvent concentration (% ethanol)	Total tannins ($mg.g^{-1}$ stem)	Condensed tannins ($mg.g^{-1}$ stem)
1	12	0	45.23	4.35
2	12	100	39.59	3.39
3	24	0	74.98	5.68
4	24	100	115.1	6.72
5	9.51	50	123.5	9.64
6	26.48	50	244.7	11.31
7	18	50	241.7	13.90
7	18	50	237.3	13.02
9	18	50	225.4	12.67
10	18	50	263.9	14.95

Results show that total tannins concentrations extracted from grape stems ranged between 39.59 and 263.9 $mg.g^{-1}$ of stems. Condensed tannins concentrations ranged from 3.39 to 14.95 $mg.g^{-1}$ of stems. Gonzalez-Centeno et al., (2012) [34] characterized 10 different varieties of Vitis vinifera species, with total tannins concentrations ranging from 47 to 115.25 $mg.g^{-1}$ of grape stems; and condensed tannins values ranging from 0.07 to 2 $mg.g^{-1}$ of grape stems. Poveda et al., (2018) [35], extracted tannins using ultrasound techniques and reported concentrations equal to 46.75 $mg.g^{-1}$ of the plant in grape stems of Vitis vinifera cultivar. In a recent study, Andrade et al., (2021), reported concentrations ranging from 25.32 to 51.5 mg of quercetin ($mg.g-^{1}$ of extract) while determining of total polyphenols in grape marcs were from Bordeaux, Isabel, and Merlot. This results confirm the potential of viticulture residues as a source of sustainable and economical alternative phenolic compounds.

ANOVA allowed evaluating the quality of the adjusted model for total tannins (Table 5) and condensed tannins (Table 6).

Table 5- ANOVA for total tannins concentration at 95% significance level ($p < 0.05$).

Factor	Square sum	df	Mean squares	F	p-value
(1) Time (L)	9458.7	1	9458.68	37.5842	0.008727*
Time (Q)	8999.5	1	8999.51	35.7596	0.009361*
(2) Solvent (L)	50.0	1	50.00	0.1987	0.685993
Solvent (Q)	87971.7	1	87971.66	349.5563	0.000334*
1L by 2L	545.2	1	545.22	2.1664	0.237442
Lack of fit	3147.6	3	1049.19	4.1690	0.135760
Pure error	755.0	3	251.67		
Total	103365.4	11			
R^2	0.9624				
Adj R^2	0.9307				

* Significant at 5% level

Table 6- ANOVA for condensed tannins concentration at 95% significance level ($p < 0.05$).

Factor	Square sum	df	Mean squares	F	p-value
(1) Time (L)	6.1129	1	6.1129	5.8519	0.094247
Time (Q)	25.1854	1	25.1854	24.1100	0.016175*
(2) Solvent (L)	0.6925	1	0.6925	0.6630	0.475136
Solvent (Q)	210.6758	1	210.6758	201.6809	0.000756*
1L by 2L	1.0000	1	1.0000	0.9573	0.400022
Lack of fit	6.6101	3	2.2034	2.1093	0.277789
Pure error	3.1338	3	1.0446		
Total	233.2162	11			
R^2	0.9622				
Adj R^2	0.9233				

* Significant at 5% level

For total tannins, independent variable time showed significant linear and quadratic effects, while the variable solvent showed significant quadratic effect at $p < 0.05$. Interaction between variables and lack of fit were not statistically significant. Regarding condensed tannins, the quadratic variables time and solvent were statistically significant at $p < 0.05$. The linear variables time and solvent, interactions between these variables, and lack of fit were not statistically significant for $p < 0.05$. The second-order polynomial models are presented in equations 1 and 2. The determination coefficients for total tannins and condensed tannins are satisfactory with values of $R^2 = 0.9624$ and 0.9622, respectively.

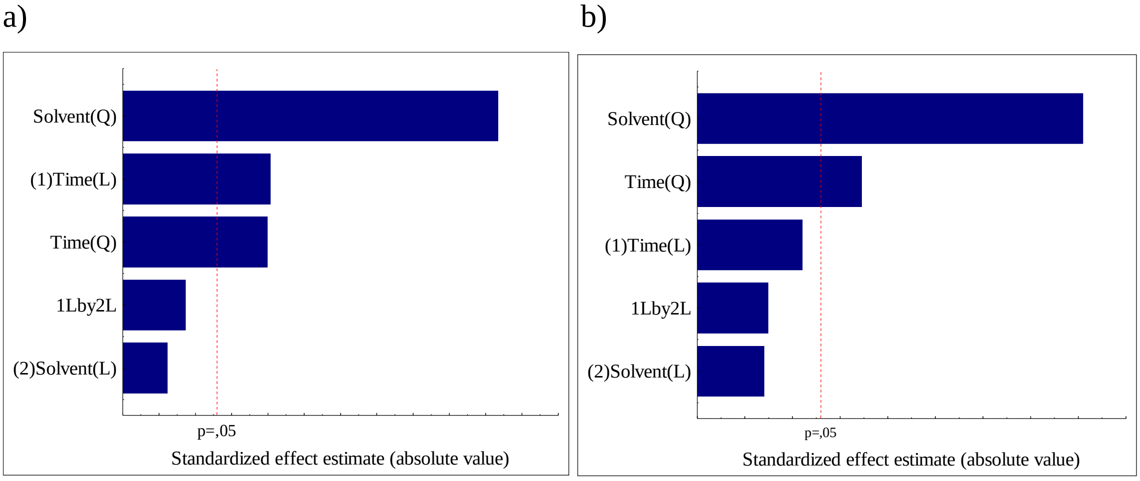
$$\textit{Total tannins} = 241.678 + 68.771_{x1} - 74.926_{x2} + 5.025_{x1}{}^2 - 238.038_{x2}{}^2 \quad (1)$$

$$\textit{Condensed tannins} = -10.849 + 2.044_{x1} - 0.055_{x2} + 0.197_{x1}{}^2 - 0.023_{x2}{}^2 \quad (2)$$

These results indicate that the obtained equations can provide an accurate representation of the relationship between the independent (time and solvent concentration) and dependent (total and condensed tannin) variables evaluated.

The influence and interaction of the monitored factors in the process of extracting tannins from grape stems is shown by the Pareto charts presented in Figures 3 (a) and 3 (b). The significance of the result is represented by the dotted line (p = 0.05) for a 95% confidence interval. The effects of quadratic (Q) and linear (L) variables and their interactions are provided through the horizontal extension of the bars.

Figure 3- Pareto chart for total tannins (a) and condensed tannins (b).



For total tannins, the quadratic terms solvent and time showed statistical significance with a negative effect sign, indicating that longer contact times and ethanol concentrations in the solvent do not promote better yields in the extraction of total tannins present in the grape stems. In contrast to the linear solvent term, time showed a positive sign and significance in relation to p, indicating an increase in total tannins concentrations due to the increase in contact time between the matrix and the solvent. The interaction between the two independent variables and the linear effect of the variable time did not have significant effects on the process. Similar behavior was observed for condensed tannins, except for the linear term for the time variable, which showed a positive sign but no statistical significance.

3.4 RESPONSE SURFACE ANALYSIS BY CCD

CCD allows the reduction in the number of experimental tests needed to model tannin extraction processes while also permitting the definition of the ideal conditions to obtain higher yields using lower solvent ratios and shorter extraction times [24]. The influence of monitored variables on the extraction of tannins from grape stems can be seen in the response surface diagrams shown in Figures 5 and 6. The yield of tannin extraction increased with the addition of ethanol in the hydroalcoholic solvent for both classes of tannins. However, a reduction in the extraction yield was observed when the solvent was composed entirely of ethanol or water. The mixture of both solvents promoted the best extraction yields of the tannins present in the grape stems.

Figure 4- Response surface and contour plots of total tannins extraction optimization.

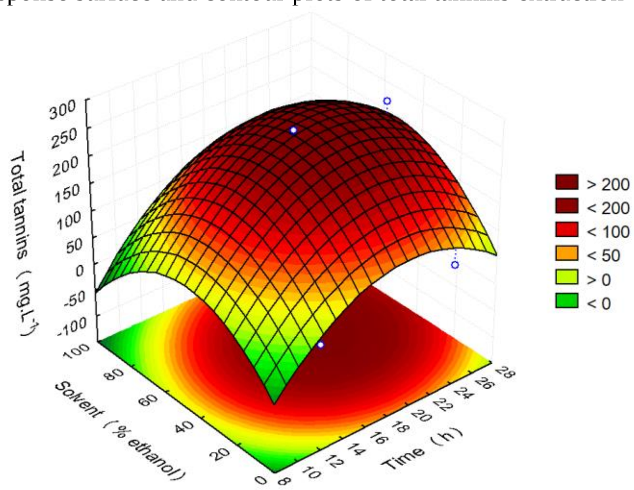
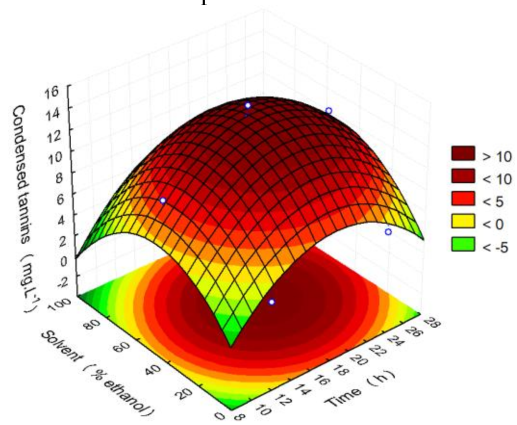


Figure 5- Response surface and contour plots of condensed tannins extraction optimization.



Tables 7 and 8 show optimized results regarding concentration and the contact time obtained by the response surface.

Table 7- Optimized conditions for total tannins extraction.

Optimized conditions		**Total tannins ($mg.g^{-1}$ stems)**	
Ethanol concentration (%)	Contact time (h)	Observed	Predicted
51,65%	21,18	270,04	249,69

Table 8- Optimized conditions for condensed tannins extraction.

Optimized conditions		**Total tannins ($mg.g^{-1}$ stems)**	
Ethanol concentration (%)	Contact time (h)	Observed	Predicted
49.19%	19,31	14.95	13.73

The good correlation between predicted and experimental values demonstrated that RSM is an accurate and reliable tool to determine the best conditions for extracting tannins present in grape stems via Soxhlet process. Ethanol concentrations of 51.65 and 49.19% in the hydroalcoholic solvent agree with the results reported in the literature.

Karvela et al., (2011) [(36)], reported that the best extraction yields of condensed tannins in grape stems were obtained with a hydroalcoholic solvent composed of 44.2 to 53.1% ethanol. Makris et al., (2007) [(37)], evaluated the extraction of polyphenolic compounds in grape skins, seeds, and stems using an aqueous solution of ethanol with concentrations ranging from 28.5 to 85.5% as the extraction solvent. Best yields were observed with a solvent composed of 57% ethanol. Spigno et al., (2007)[(38)], observed an increase in the extraction of total polyphenols in grape marc related to the increase in water percentage in ethanol from 10% to 30%. Over this limit and until increases of 60% (40% ethanol), extraction yields of polyphenols remained unchanged. Downey and Hanlin (2010)[(39)], also reported that the extraction of total tannins in grape skins increased with the addition of ethanol in water; however maximum extraction was reached with an ethanol concentration of 50%. Yields decreased when the solvent was composed of ethanol in concentrations greater than 50%. Similar behavior was observed by Dominguez-Perles et al., (2014)[(8)], when RSM showed that the content of phenolic compounds increased in parallel with the concentration of ethanol up to 40%, with higher or lower concentrations of ethanol in the extraction solvent leading to a decrease in the yield of the phenolic content.

Mildner-Szkudlarz et al., (2010) [(40)], presented a 22% increase in the extraction yield of phenols in grape marc with aqueous solvents composed of 70% ethanol and a reduction in yield when using solvents with 90% ethanol. Bosso et al., (2014)[(41)], report that the best extraction yields of condensed tannins present in grape seeds and skins were obtained with solvents composed of ethanol/water with proportions of 50 and 60%. Rahja et al., (2014) [(42)], observed a trend in the increase of tannins concentrations present in grape by-products related to the increase in ethanol concentrations of up to 67% in the hydroalcoholic solvent at a temperature of 94 ºC. The authors reported that maximum tannin extraction occurs with an extraction solvent composed of 64% ethanol.

This study, in agreement with those mentioned before, verifies that hydroalcoholic solvents can effectively extract tannins present in winemaking residues. However, the composition of the solvent may vary depending on the characteristics of the matrix, mainly the polarity. When compared to the use of aqueous mixtures, the use of pure solvents has shown less efficiency in the extraction of tannins; the same behavior is reported when using only water [43,44,45].

Regarding the contact time between the grape stems and the solvents during the tannin extraction process via Soxhlet extraction, RSM pointed out that the best total tannins yields were obtained with a contact time of 21.7 hours. For condensed tannins, the contact time was slightly shorter, with the best results achieved after 19.47 hours. The authors performed a search for data to be used for comparison. However, there are no records to date of studies that discuss the effect of this variable in the extraction process in relation to the Soxhlet method.

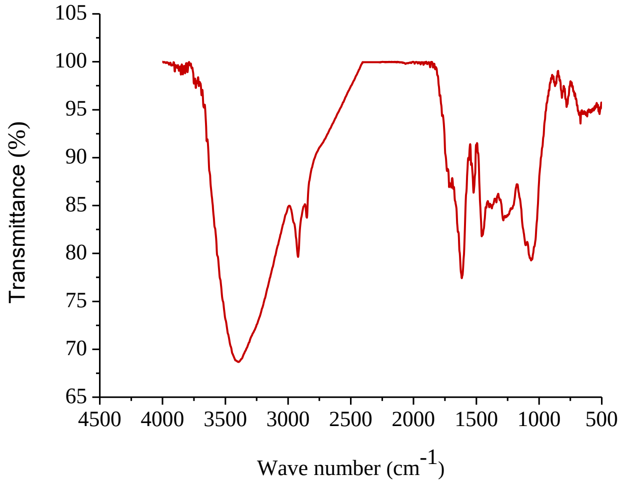
The discoloration of the grape stems, and consequently, the color transfer to the extraction solvents were among the parameters evaluated in the preliminary tests conducted prior to this study, which provided a basis to determine the time variable in the planning of the CCD. In the initial stage of the process, the extraction solvents presented a white color, and the condensed vapor in the tube had a strong brown color. As the contact time increased, an intense coloration was observed in the balloons indicating the transfer of soluble compounds present in the grape stems to the solvents.

In this study, tannin extraction temperature was set at the average boiling point of the solvents, i.e., 90ºC. Previous studies that used RSM to optimize extraction conditions of polyphenolic compounds indicate ideal temperatures ranging from 90 to 95 ºC. Dominguez-Perles et al., (2014) [8],concluded that ideal conditions for the extraction of polyphenols from grape stems occurred at a temperature of 95 ºC, while Rajha et al., (2014) [1], obtained their best results at a temperature of 94 ºC. Connolly (1993) [46], patented the process of extracting tannins from pine bark radiata, reinforcing the preference for high temperatures, between 90 and 100 °C. In general, high temperatures promote greater solubility of phytochemicals present in plant matrices due to the reduction of tension and viscosity in the solvent, triggering a better moistening of the sample [47]. However, high temperatures also tend to negatively influence the extraction yield, since they enable the thermal degradation of phenolic compounds in general [8,48].

3.5 CHARACTERISTIC OF THE TANNIN EXTRACT

The tannin extract obtained from the grape stems showed a density of 0.909 $g.L^{-1}$, kinematic viscosity of 24.88 Cp, pH of 6.7, and a solids concentration of 3%. The functional groups of the extract are as shown in Figure 6.

Figure 6- Spectrum of tannin extract obtained from grape stems.



The tannin extract spectrum showed characteristic bands of phenols from the axial deformation of O-H and C-O, which are sensitive to the formation of hydrogen bonding. Some angular deformations outside the C-H plane were identified in the 740 and 900 cm^{-1} bands. The strong peak at 1052 cm^{-1} is attributed to C-O axial strain vibration. The presence of bands in 1110 cm^{-1}, 1282 cm^{-1}, and 1378 cm^{-1} regions indicate absorption caused by O-H deformation vibrations. In 1450 cm^{-1}, 1515 cm^{-1}, and 1614 cm^{-1} regions, the presence of stretching vibrations of C= C are characteristic of the tannins [(14)]. An axial deformation in the 2919 cm^{-1} region indicates the presence of aromatic compounds. Lastly, in the 3380 cm^{-1} band, a large axial deformation in O-H is observed in intermolecular hydrogen bonding.

4 CONCLUSIONS

Quantification of tannins in grape stems revealed total and condensed tannins concentrations of up to 263.9 $mg.g^{-1}$ and 14.95 $mg.g^{-1}$, respectively, characterizing grape stems as a potential source of tannins for the most diverse applications.

RSM allowed the optimization of tannins extraction conditions from grape stems. Best yields of total tannins were obtained with a contact time of 21,18 hours, and a hydroalcoholic solvent composed of 51.65% ethanol. For condensed tannins, the optimal contact time was slightly shorter, with the best yields achieved in 19,31 hours with the hydroalcoholic solvent composed of 49.19% ethanol.

The high correlation of the model, with R^2 = 0.9624 for total tannins and R^2 = 0.9622 for condensed tannins, showed that second-order polynomial models can be used to maximize extraction of tannins in grape stems.

The characterization of the tannic extract revealed the presence of functional groups characteristic of the tannins, proving once again the presence of these compounds in grape stems.

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