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Evaluation of acid hydrolysis parameters for the efficient solubilization of the xylose content of bagasse and sugarcane straw

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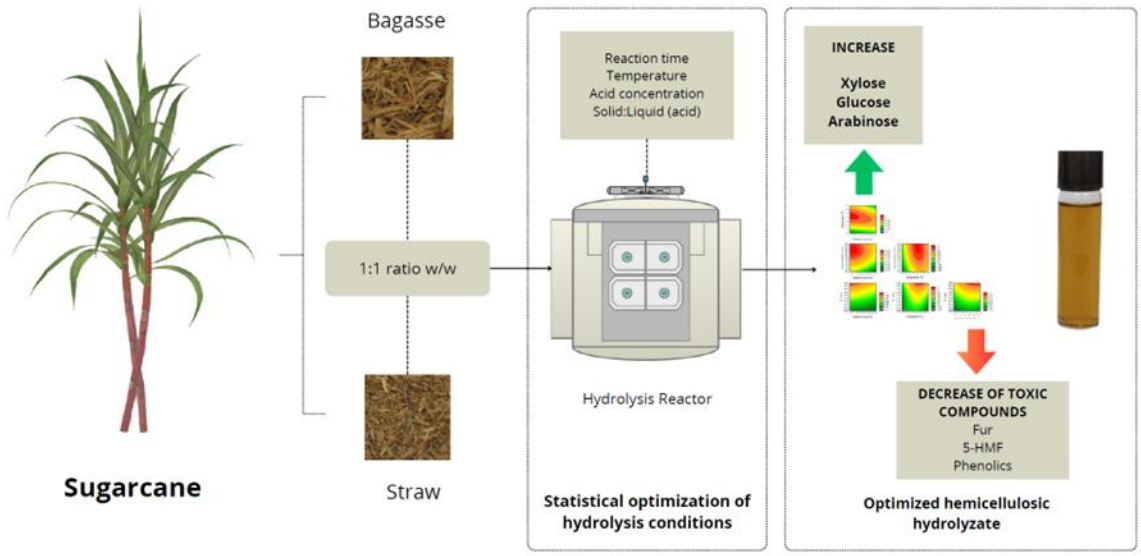
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Graphical abstract



Abstract

Much of our current energy and material production relies on non-renewable fossil resources. As a result, there's a growing exploration of alternative sources, such as plant biomass in a biorefinery context. Brazil, a country with an extensive experience in utilizing sugarcane for sugar, bioethanol, and electricity, is also investigating the potential of producing high value-added biomolecules from sugarcane bagasse and straw, by-products of the sugar-alcohol sector. These by-products contain a hemicellulosic fraction primarily composed of xylose. To obtain a xylose-rich solution, breaking down lignocellulosic biomass is essential, with dilute acid hydrolysis being a common technique. However, this process also releases inhibitors like furans, acetic acid, and phenolic compounds. This study aimed to establish dilute acid hydrolysis conditions to optimize the xylose yield. Dilute acid hydrolysis experiments were conducted in a 1L capacity reactor using a Central Composite Rotatable Design (CCRD), with temperature, reaction time, sulfuric acid concentration, and solid:liquid ratio as variables. Optimal conditions yielding the highest sugar concentrations (approximately 60 g/L xylose) were identified: 135 ºC temperature, 7 minutes reaction time, 2.25% sulfuric acid concentration, and 0.25 (w/v) solid-liquid ratio. However, these conditions also resulted in the presence of toxic compounds like acetic acid, furfural, 5-HMF, and total phenolics. In this case, detoxification could be an alternative to avoid the inhibitory effect of these compounds. Statistical modeling highlighted temperature as the main factor influencing sugar and toxic compound concentrations, indicating that increasing hydrolysis temperature could enhance xylose yield in the process and reduce energy-intensive steps like vacuum concentration.

Keywords: Hemicellulosic hydrolysate; dilute acid hydrolysis; toxic compounds; sugarcane by-products; xylitol.

1. Introduction

The abundance of lignocellulosic biomass worldwide makes it a rich source of carbohydrates and an essential raw material for the development of new technologies involving bioprocesses (Mujtaba et al., 2023). This is especially true for those technologies that contribute to utilizing all the constituent fractions of the plant cell wall, particularly focusing on the hemicellulosic fraction, which is usually underutilized (Sun et al,. 2021).

Scientific investigations about the use of these materials have grown over the years, particularly in the case of Brazil, the world's leading sugarcane producer. Despite their primary use in the sugar energy sector, there is an abundance of sugarcane bagasse and straw (Bordonal et al., 2018; Cherubin et al., 2021). However, the use of lignocellulosic materials in industrial sectors must consider their particular composition, which includes three main fractions: cellulose, a homopolymer of glucose, hemicellulose, an heteropolymer with predominance of xylose units and, lignin, a polyphenolic macromolecule can represent a crucial step for the efficacy of an bioprocess. This structure is resistant to chemical and enzymatic agents, impairing the utilization of its monomeric constituents of carbohydrate fractions in biotechnological processes (Chandra, Yadav, Kumar, 2015; Ballesteros et al., 2018).

The use of these biomasses in bioprocesses requires the deconstruction of the plant cell wall (lignocellulose), which can be performed by different methods, such as chemical, physical or a combination of both, to obtain hydrolysates rich in carbohydrates. Regarding the recovery of xylose from hemicellulose, the most common hydrolysis method includes dilute acid and hydrothermal (Wolf et al, 2023). Regarding the dilute acid hydrolysis, several acids such as sulfuric, hydrochloric, or phosphoric are employed to solubilize the hemicellulosic fraction into its monomeric sugars. Important parameters control the biomass hydrolysis: acid concentration (0.5% to 2%), residence time (up to 40 min), temperature (100°C to 200°C), and solid-to-liquid ratio (Pasquier, Paës, Perré, 2023). The main advantages include its efficacy in converting hemicellulose to xylose, process control, and adaptability to several types of plant biomass, including agro-industrial byproducts. The resulting hemicellulosic

hydrolysate serves as a feedstock for subsequent fermentation processes, enabling the production of biofuels, biochemicals, and other value-added products.

During the deconstruction of the polymeric structure of the plant cell wall, there is also the liberation and/or formation of toxic compounds for microorganisms, such as acetic acid, phenolic compounds, furfural, and 5-hydroxymethylfurfural (HMF) (Pereira, Mussatto, Roberto, 2011). These compounds inhibit the microbial metabolism, depending on their concentration and synergistic action, varying according to the microbial species and culture conditions (Nichols et al., 2008; Chandel, Silva, Singh, 2013; Adeboye, Bettiga, Olsson, 2014). Thus, different strategies have been employed to minimize these compounds toxicity and to enhance the efficiency of bioprocesses. The alternatives include the utilization of microorganisms capable of tolerating certain concentrations of toxic compounds (Adeboye, Bettiga, Olsson, 2014), techniques to increase the robustness of microorganisms such as metabolic engineering (Chandel, Silva, Singh, (2013), and evolutionary adaptation (Bianchini et al., 2022). Additionally, detoxification of the hydrolysate before its utilization in media formulation is carried out through pH neutralization, ion exchange resins, adsorption on activated charcoal, or their combination with ion exchange resins, as well as flocculation with plant-based polymers (Carvalho et al., 2006; Chandel, Silva, Singh, 2013; Silva-Fernandes et al., 2017).

Aiming the enhance of xylose bioconversion into several biomolecules such as xylitol, arabitol, lactic acid, ethanol, isobutanol, carotenoid and organic acids, it is usually necessary an additional step to increase the initial xylose concentration in hemicellulosic hydrolysates, such as vacuum concentration (Jagtap and Rao, 2018; Demesa et al., 2020). The increase in xylose concentration in the medium leads to an increase in non-volatile toxic compounds in the hydrolysate, such as phenolics (Rodrigues et al., 2001), negatively impacting fermentation performance, thus resulting in a consequent loss of economical viability (Pereira, Mussatto, Roberto, 2011). In this context, studies aimed at optimizing process parameters by establishing hydrolysis conditions to obtain a xylose-rich hydrolysate with low levels of toxic compounds, such as acetic acid, phenolics, furfural, and hydroxymethylfurfural, are crucial (Roberto, Mussatto, Rodrigues, 2003; Pienkos, Zhang, 2009).

In this research, different conditions of dilute acid hydrolysis of sugarcane bagasse and straw were evaluated to obtain a xylose-rich hemicellulosic hydrolysate. A central composite rotatable design was employed considering the following variables: sulfuric acid concentration, solid:liquid ratio, reaction time, and temperature.

2. Materials and Methods

2.1 Raw material

The sugarcane by-products, bagasse and straw, were generously provided by Usina Pederneiras, located in São Paulo, Brazil. Both biomasses were air-dried and kept at 30°C until their moisture content reached approximately 10%. The straw was macerated in a mill and subsequently a mixture of bagasse and straw in a 1:1 (w/w) ratio was prepared (Hernández-Pérez et al., 2020).

2.2 Chemical characterization of sugarcane bagasse and straw

The chemical composition of sugarcane bagasse and straw (SBS) including polysaccharides (xylan, arabinan and glucan), lignin, extractives and ash content, was determined according to Ferraz et al. (2000). Briefly, for polysaccharides and lignin content, the raw materials were submitted to acid hydrolysis using sulfuric acid (72%) (v/v) for 1 h at 30°C. The hydrolysed content was diluted with distilled water and subjected to autoclaving at 120°C for 1 h. Insoluble lignin was separated by filtration from the hydrolysate and the mass of the dried sample was measured. Additionally, ash content was determined using a muffle furnace at 575°C for 24 h. Extractives were determined with 95% ethanol.

2.3 Optimization of acid hydrolysis conditions

The study of the acid hydrolysis of the sugarcane bagasse and straw mixture was conducted through a CCRD (central composite rotatable design), and the independent variables investigated are detailed in Table 1. The response variables were the concentration of sugars (xylose, glucose, and arabinose), total

phenolics, as well as furfural and 5-hydroxymethylfurfural (HMF), resulting in different acid hydrolysis conditions.

Table 1. CCRD to evaluate the acid hydrolysis of sugarcane bagasse and straw under different conditions (independent variables).

Independent variables	**Symbol**	**Levels**				
		-α	**-1**	**0**	**+1**	**+α**
Time (min)	X_1	5	10	15	20	25
Temperature (°C)	X_2	105	120	135	150	165
H_2SO_4 (%)	X_3	1.0	1.5	2.0	2.5	3.0
Solid:liquid ratio (w/v)	X_4	0.09	0.13	0.17	0.21	0.25

The acid hydrolysis experiments were carried out using an autoclave system (Regmed AU/E-20, Brazil), with four compartments with a maximum volume of 1L each. At the end of the hydrolysis process, the solid fraction (cellulignin) was separated from the liquid fraction (hemicellulosic hydrolysate) and stored at 4°C for chemical analysis.

2.4 Analytical techniques

Acid hemicellulosic hydrolysate sugar concentrations were determined through HPLC employing an Aminex HPX-87H column and eluting with H_2SO_4 0.01 N at 45°C, with a flow rate of 0.06 mL/min (Agilent 1200, Kyoto Japan). To characterize the cellulignin, the soluble lignin content was assessed using UV spectroscopy at 205 nm (utilizing the Beckman Coulter DU 800, USA). The total phenolic content was measured through spectroscopy at 280 nm, with the sample pH adjusted to 12 using NaOH (6 mol/L) (Arruda et al., 2017).

2.5 Statistical analysis

The statistical analysis of the results presented in the experiments was performed using STATISTICA 10.0 software. A significance level of 90% ($p < 0.1$) was considered.

3. Results and Discussion

3.1 Sugarcane bagasse and straw chemical composition

The concentration of sugars and other compounds from the mixture (1:1 w/w) of sugarcane bagasse and straw (SBS) was determined through the chemical characterization analysis. Considering the sugars, the SBS mixture exhibited 34.4% cellulose and 22.9% hemicellulose, while other fractions corresponded to 22.0% total lignin, 3.5% ashes and 9.3% extractives.

In terms of the hemicellulose content in the sugarcane bagasse and straw mixture, it is observed that this value is close to the range reported in the literature (between 20% - 30%) for this type of vegetal biomass. Regarding the cellulosic fraction, it constituted 60% of the carbohydrate content in the SBS, similar as described by Hernández-Pérez (2019) for sugarcane bagasse and straw mixture.

The total lignin content of SBS *in natura* determined in this study (22%) are similar with the literature for sugarcane bagasse and straw mixture. Despite both being byproducts of the same plant biomass, sugarcane bagasse and straw exhibit distinctive characteristics. Del Rìo et al. (2015) reported that the lignin in the straw has a higher concentration of syringyl groups, whereas in the bagasse, guaiacyl groups predominate. The variability in chemical composition of carbohydrate and non-carbohydrate content of the same type of material depends on several factors, such as plant genetics, growth environment and processing conditions, as well as the methods used to analyze the chemical composition (Canilha et al. al., 2012). These authors also state that in sugarcane straw, this variation may be linked to factors such as the harvesting system, height, sugarcane variety, harvest age, climate, and soil.

3.2 Evaluation of different parameters in sugarcane bagasse and straw acid hydrolysis

The results regarding the chemical composition of the hemicellulosic hydrolysate from the mixture of bagasse and sugarcane straw (1:1, w/w) subjected to dilute acid hydrolysis, according to the Central Composite Rotatable Design (CCRD), are detailed in Table 2. Notably, the highest values for xylose (64.20 g/L), glucose (9.61 g/L), arabinose (9.95 g/L), and acetic acid (9.38 g/L) were achieved under the conditions where the time was set at 15 min, temperature at 135 ºC, sulfuric acid concentration at 2%, and solid:liquid ratio of 0.25 (w/v) (Run 24).

Table 2. CCRD design responses for optimization of dilute acid hydrolysis conditions of the sugarcane bagasse and straw mixture.

Run	Actual variables				Responses (g/L)							
	RT (min)	T (°C)	AC % w/v	S:L ratio	Xyl	Glu	Ara	AA	Fur	5 - HMF	Phe	pH
1	10.0	120	1.50	0.13	10.760	1.590	2.480	1.950	0.002	0.018	3.500	0.940
2	10.0	120	1.50	0.21	17.070	3.110	4.920	3.610	0.0010	0.023	4.130	0.920
3	10.0	120	2.50	0.13	22.060	2.910	3.390	3.770	0.008	0.022	7.290	0.540
4	10.0	120	2.50	0.21	39.520	4.330	5.770	6.360	0.007	0.041	7.740	0.630
5	10.0	150	1.50	0.13	14.550	4.270	3.870	4.150	0.079	0.048	26.340	0.940
6	10.0	150	1.50	0.21	32.85	6.97	6.93	6.56	0.072	0.055	24.55	0.92
7	10.0	150	2.50	0.13	20.230	5.080	3.920	4.990	0.142	0.045	35.860	0.560
8	10.0	150	2.50	0.21	48.320	9.600	7.950	7.990	0.061	0.081	16.140	0.630
9	20.0	120	1.50	0.13	10.670	2.270	3.170	2.280	0.001	0.014	6.200	0.610
10	20.0	120	1.50	0.21	28.770	3.210	4.760	4.720	0.004	0.043	6.080	0.580
11	20.0	120	2.50	0.13	24.680	4.280	4.850	4.730	0.009	0.045	8.430	0.380
12	20.0	120	2.50	0.21	41.230	4.560	5.930	6.750	0.007	0.045	7.580	0.380
13	20.0	150	1.50	0.13	14.330	4.160	2.830	4.010	0.093	0.048	33.890	0.870
14	20.0	150	1.50	0.21	25.580	5.190	5.230	5.230	0.020	0.074	14.970	1.030
15	20.0	150	2.50	0.13	12.190	4.730	3.070	5.230	0.085	0.030	46.270	0.670
16	20.0	150	2.50	0.21	32.060	7.390	5.870	7.660	0.265	0.091	47.170	0.680
17	5.0	135	2.00	0.17	30.280	4.180	5.040	4.500	0.006	0.000	3.100	0.300
18	25.0	135	2.00	0.17	42.180	7.680	7.040	7.340	0.039	0.045	11.170	0.390
19	15.0	105	2.00	0.17	2.020	2.170	2.950	2.080	0.002	0.033	2.190	0.210
20	15.0	165	2.00	0.17	5.360	4.440	1.510	8.320	0.521	0.213	65.280	0.780

21	15.0	135	1.00	0.17	33.130	2.940	3.520	3.150	0.014	0.050	6.510	1.110
22	15.0	135	3.00	0.17	41.720	6.680	5.320	6.460	0.047	0.057	13.470	0.660
23	15.0	135	2.00	0.09	18.430	3.200	2.730	2.770	0.018	0.026	10.340	0.740
24	15.0	135	2.00	0.25	64.200	9.610	9.950	9.380	0.022	0.027	11.570	0.330
25	15.0	135	2.00	0.17	43.660	7.800	6.690	6.820	0.010	0.037	11.400	0.220
26	15.0	135	2.00	0.17	33.550	5.650	4.920	5.960	0.024	0.050	10.520	0.820
27	15.0	135	2.00	0.17	38.780	3.850	4.200	4.430	0.022	0.052	11.530	0.850

RT: Reaction time; T: Temperature; AC: Acid concentration; S:L : Solid material: Acid liquid solution ratio; Xyl: xylose; Glu: Glucose; Ara: Arabinose; AA: Acetic acid; Fur: Furfural; 5-HMF: 5-Hydroxymethylfurfural; Phe: Phenolic compounds; pH: final pH.

The minimum values found for xylose (2.02 g/L) (Run 19), glucose (1.59 g/L) (Run 1), arabinose (1.51 g/L) (Run 20) and acetic acid (1.95 g/L) (Run 1), were obtained in tests in which the time, temperature, acid concentration and solid:liquid ratio varied from 10 to 15 min, 105 to 165 ºC, 1.5 at 2% and 0.13 to 0.17 (w/v) respectively.

Regarding the concentration of furfural compounds, 5-HMF and total phenolics, the highest values found were 0.52 g/L, 0.21 g/L and 65.28 g/L, respectively, in Run 20, in which the temperature was 165 ºC. In Run 19, the conditions used remained the same as in Run 17, except for the time being 15 minutes and the temperature 105 ºC, thus suggesting that the increase in temperature and the time that the temperature remains influence the concentration of these compounds in the hemicellulosic hydrolysate.

Run 24 presented a higher xylose concentration (64.20 g/L) compared to Hernádez-Pérez (2020) (47.63 g/L). This was achieved by the implementation of elevated temperature conditions (135 ºC), higher sulfuric acid concentration (2%), and solid-to-liquid ratio of 0.25 (w/v). These findings suggest the potential elimination of the vacuum concentration step in the biotechnological production process of xylitol from sugarcane bagasse and straw mixture. Moreover, the results indicate advancements in dilute acid hydrolysis conditions, due to the increased xylose concentration in the hydrolysate when compared with the prior study. The obtained results were submitted to statistical analyses to evaluate these observations.

3.3 Statistical analysis of concentration of monosaccharides and toxic compounds in hemicellulosic hydrolysate

3.3.1 Monosaccharides

The concentrations of xylose, glucose, arabinose and final pH obtained under different acid hydrolysis conditions (Table 2) for the mixture of sugarcane bagasse and straw (1:1, w/w) were analyzed using analysis of variance (ANOVA) (Table 3). This analysis was conducted to develop a quadratic model that incorporates the variables of time, temperature, acid concentration, and solid-to-liquid ratio. Response surface methodology (RSM), was employed to optimize experimental conditions by analyzing the relationships between input factors and outputs within a limited number of runs.

Table 3. Analysis of variance (ANOVA) and coefficients of determination for the fitted models of xylose, glucose, arabinose, and final pH values.

	Xylose					Glucose					Arabinose					Final pH				
Source	Sum squares	df	Mean squares	F value	P value	Sum squares	df	Mean squares	F value	P value	Sum squares	df	Mean squares	F value	P value	Sum of squares	df	Mean squares	F value	P value
Model	4919.96	6	819.99	23.81	<0.001[a]	86.84	4	21.71	14.21	<0.001[a]	77.56	7	11.08	23.81	<0.001[a]	1.7	7	0.12	0.11	<0.001[a]
Residual	6688.92	20	34.45			33.61	22	1.53			14.97	19	0.79			0.19	19	0.79		
Lack of fit	637.79	18	35.43	1.39	0.5004[b]	25.79	20	1.29	0.33	0.9223[b]	11.69	17	0.69	0.42	0.8782[b]	0.17	17	0.03	0.2	0.97[b]
Pure error	51.13	2	25.56			7.82	2	3.91			3.28	2	1.64			0.02	2	0.13		
Total	5608.88	26				120.45	26				92.53	26				1.96	26			

R^2	0.8772	0.7209	0.8382	0 5

df: degrees of freedom; R^2: Coefficient of determination; Significance at 90% confidence level
[a] Model is significant
[b] Lack of fit is not significant

Based on our results, mathematical models were obtained that accurately demonstrate the behavior of the variables under study as a function of the concentration of sugars, such as xylose (Eq. 1), glucose (Eq. 2) and arabinose (Eq. 3). These mathematical models were used to plot response surfaces and contour plots (Figure 1), which facilitated the understanding of significant variables at a 90% confidence level. In addition, the analysis of the effects of the independent variables on the yield of sugars generated during acid treatment is presented in Table 4.

Xylose yield (g/L) = $35.73 + 0.33A + 0.50B + 4.29C + 9.48 / D - 2.98 AB - 9.02 (B^2)$

(Eq. 1)

Glucose yield (g/L) = $5.45 + 1.07B + 0.82 C + 1.16 D - 0.64 (B^2)$

(Eq. 2)

Arabinose yield (g/L) = $5.85 + 0.020A + 0.063B + 0.42C + 1.43D - 0.49AB - 0.88 (B^2) - 0.33 (C^2)$

(Eq. 3)

Where,

A - Time (min)

B - Temperature (ºC)

C - Sulfuric acid concentration (%)

D - Solid:liquid ratio (w/v)

For xylose concentration, it was observed that the variables acid concentration and solid-liquid ratio significantly affect its concentration in the hemicellulosic hydrolysate, with a confidence level of 90%. The condition resulting in the highest concentration (approximately 70 g/L) corresponds to intermediate temperature values (135 ºC). In a study conducted by Dionísio et al. (2021), dilute acid hydrolysis of sugarcane bagasse was performed at 140 ºC in a stainless-steel reactor with a capacity of 350L, solid ratio of 9% (w/w), and acid concentration of 0.5% (v/v) for 15 min, yielding 25.14 g/L of xylose. However, a decrease in xylose concentration was observed in the present study during acid hydrolysis experiments performed with temperatures above 150 ºC (Figure 1a). This effect can be attributed to the significant influence of high temperatures on the degradation of xylose into furfural

(Redding et al., 2011). According to Zhang et al. (2022), elevated temperatures accelerate both the hydrolysis rate of cellulose/hemicellulose into glucose/xylose and the degradation rate of glucose/xylose into HMF/furfural.

Table 4. Estimates of the effects of the studied variables, standard error e values of "t" and "p" for xylose, glucose, arabinose yields and final pH values.

	Xylose				Glucose				Arabinose				final pH			
	Effect	Std. Err.	t	p	Effect	Std. Err.	t	p	Effect	Std. Err.	t	p	Effect	Std. Err.	t	p
Mean	38.66	3.51	11.01	0.00[a]	5.77	0.77	7.44	0.00[a]	5.27	0.54	9.82	0.00[a]	0.63	0.12	5.32	0.00[a]
X_1	0.66	2.48	0.27	0.79	0.41	0.55	0.75	0.47	0.04	0.38	0.11	0.92	-0.06	0.08	-0.70	0.50
X_1^2	-2.88	2.63	-1.09	0.30	-0.09	0.58	-0.15	0.88	0.36	0.40	0.90	0.39	-0.10	0.09	-1.08	0.30
X_2	1.00	2.48	0.40	0.69	2.14	0.55	3.91	0.00[a]	0.13	0.38	0.33	0.74	0.21	0.08	2.45	0.03[a]
X_2^2	-19.15	2.63	-7.27	0.00[a]	-1.40	0.58	-2.41	0.03[a]	-1.54	0.40	-3.84	0.00[a]	-0.02	0.09	-0.23	0.82
X_3	8.57	2.48	3.45	0.00[a]	1.63	0.55	2.98	0.01[a]	0.85	0.38	2.23	0.05[a]	-0.27	0.08	-3.23	0.01[a]
X_3^2	-2.28	2.63	-0.87	0.40	-0.65	0.58	-1.12	0.29	-0.45	0.40	-1.12	0.29	0.17	0.09	1.96	0.07[a]
X_4	18.96	2.48	7.64	0.00[a]	2.32	0.55	4.24	0.00[a]	2.85	0.38	7.51	0.00[a]	-0.05	0.08	-0.56	0.59
X_4^2	-0.34	2.63	-0.13	0.90	0.15	0.58	0.26	0.80	0.51	0.40	1.27	0.23	0.00	0.09	-0.01	0.99
$X_1 X_2$	-5.97	3.04	-1.96	0.07[a]	-0.85	0.67	-1.27	0.23	-0.98	0.46	-2.10	0.06[a]	0.16	0.10	1.56	0.14

X_1: Coded levels of reaction time; X_2: Coded levels of the temperature; X_3: Coded levels of the acid concentration; X_4: Coded levels of S:L ratio
[a] Significant at 90% confidence level

For glucose yield, the proposed model for dilute acid hydrolysis was also significant ($p<0.01$), with a non-significant lack of fit ($p>0.10$). The R^2 value of 0.7209 indicates that the model explains 72.09% of the variability in the response. Temperature was a significant factor that influences the glucose concentration, with values higher than 9 g/L observed at temperatures above 120 ºC. Regarding acid concentration and the solid-to-liquid ratio, a linear relation was observed. Increasing both factors also increased the glucose concentration, starting from a temperature of 115 ºC (Figure 1b). Particularly, acid concentration resulted in a higher release of xylose than glucose, attributed to the susceptibility of the hemicellulose fraction to sulfuric acid attack (Ogata, 2013).

The behavior of arabinose concentration in the hydrolysate under different hydrolysis conditions exhibited similarities with xylose and glucose. Temperature was the factor that most influenced the yield, with a confidence level of 90%. On the other hand, time presented minimal influence on yield. Specifically, at elevated temperatures (above 145 ºC) and extended reaction time, the degradation of

arabinose was observed (Fig. 1c). In a study performed by Bravo et al. (2017), acid hydrolysis of *Pinus* sp. sawdust was optimized to selectively recover arabinose from the hemicellulose fraction via an environmentally friendly process utilizing dilute HCl as a catalyst under mild conditions and atmospheric pressure. Investigation of temperature, HCl concentration, and reaction time revealed that the most favorable conditions for arabinose extraction were achieved at 65°C for 18 hours with a 3% HCl concentration, resulting in over 3.8 g/L of arabinose in the liquid phase.

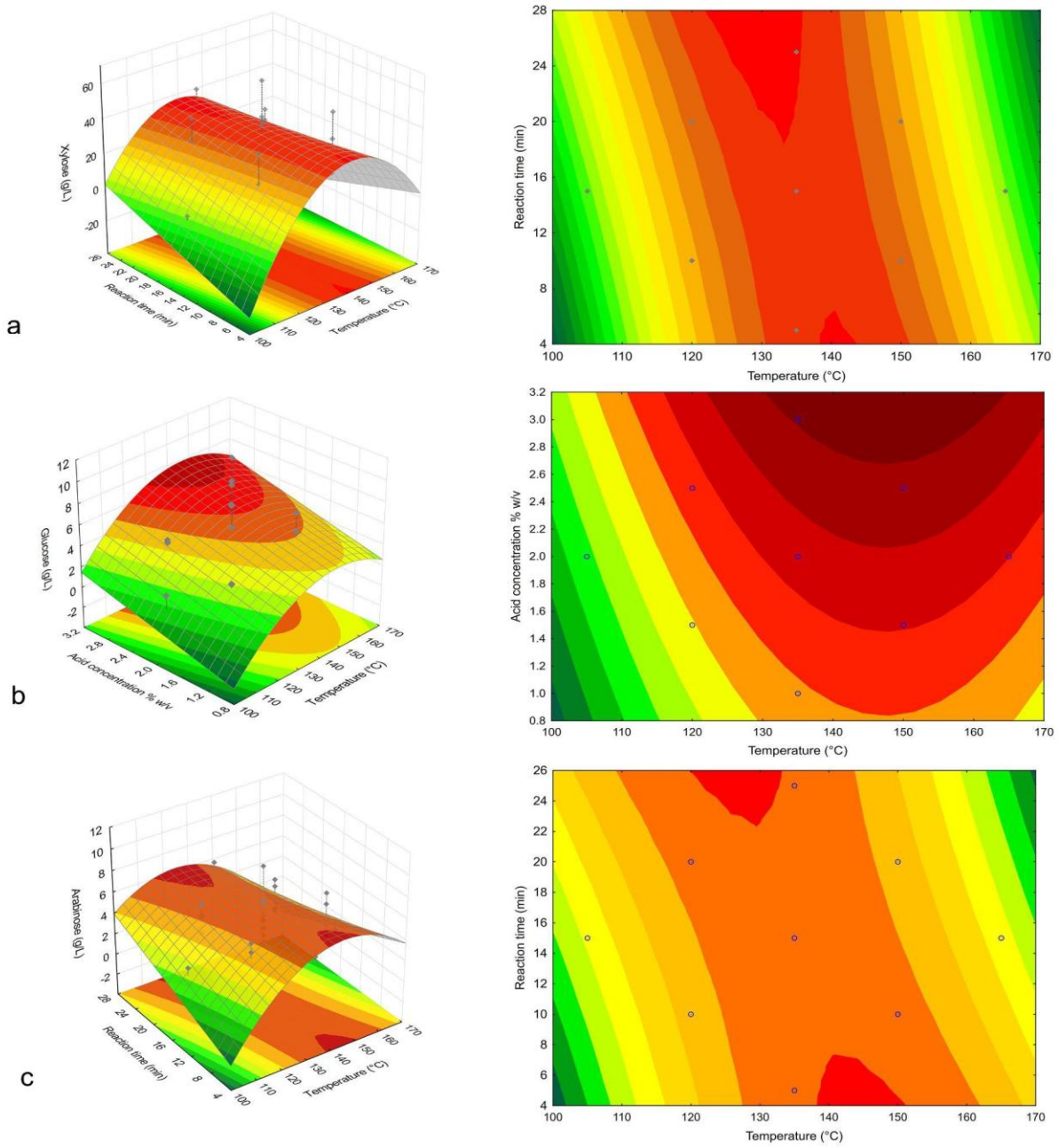


Fig. 1. Response surface plot and contour plot of (a) xylose yield (g/L) as a function of the temperature (°C) and the reaction time (min); (b) glucose yield (g/L) as a function of the acid concentration % (°C) and the temperature (°C) and (c) arabinose yield (g/L) as a function of the temperature (°C) and the reaction time (min).

3.3.2 Toxic compounds

In addition to evaluating sugar yields from dilute acid hydrolysis experiments, the relationships between hydrolysis parameters and concentrations of toxic compounds (acetic acid, total phenolic compounds and furans) were also statistically evaluated. The analysis of variance for these compounds (Table 5) showed that the selected models were significant (p<0.1), while the lack of fit of the models is not significant for the same p-value, being able to conclude that the models correctly express the release of toxic compounds in the acid hydrolysis process.

Table 5. Analysis of variance (ANOVA) and coefficient of determination for the fitted models of acetic acid, furfural, 5-HMF, and phenolic compounds values.

	Acetic acid					Furfural					5 - HMF					Phenolic Compounds				
Source	Sum of squares	*df*	Mean squares	*F* value	*P* value	Sum of squares	*df*	Mean squares	*F* value	*P* value	Sum of squares	*df*	Mean squares	*F* value	*P* value	Sum of squares	*df*	Mean squares	*F* value	*P* value
Model	75.04	14	5.36	3.34	0.02[a]	0.25	14	0.02	3.48	0.02[a]	0.03	14	0.00	3.35	0.02	6056.82	14	432.63	10.34	<0.001[a]
Residual	19.26	12	1.70			0.06	12	0.01			0.01	12	0.00			502.07	12	41.84		

Lack of fit	16.33	10	1.63	1.11	0.56	0.06	10	0.01	105.6	0.1	0.01	10	0.00	11.58	0.18	50 1.46	10	50.15	166.10	0.11
Pure error	2.93	2	1.47			0.00	2	0.00			0.00	2	0.00			0.60	2	0.30		
Total	94.31	26				0.31	26				0.04	26				65 58.89	26			
R^2	0.80					0.80					0.80					0.92				

df: degrees of freedom; R^2: Coefficient of determination; Significant at 90% confidence level

[a] Model is significant

The estimated effects (Table 6) and the Pareto plots (see supplementary material, Fig. S1) were used to establish a relationship between the concentration of toxic compounds generated and the response variables. Acetic acid release in our analyses was statistically dependent (p<0.1) on temperature (Linear), acid concentration % w/v (Linear) and S:L ratio (Linear and quadratic).

In a study carried out by Hernández-Perez et al. (2016) for sugarcane straw subjected to acid hydrolysis diluted with just 1.0% (w/v) of H_2SO_4 at 121 °C for 20 min at a solid/liquid ratio of 1:10, obtained a value for acetic acid of 2.23 g/L. Aguilar et al. (2002) also for diluted acid hydrolysis, but from sugarcane bagasse, maintaining the same solid liquid ratio 1:10, however increasing the acid concentration to 6% (w/v) of H_2SO_4, temperature to 122 ºC during the fermentation time. reaction of 40 minutes, obtained an even higher acetic acid concentration of 5.1 g/L, thus showing that with the increase in these variables there is an increase in the concentration of acetic acid.

Table 6. Estimates of the effects of the studied variables, standard error e values of "t" and "p" for acetic acid, furfural, 5-HMF, and phenolic compounds values.

	Acetic acid				Furfural				5- HMF				Phenolic Compounds			
	Effect	Std. Err.	t	p	Effect	Std. Err.	t	p	Effect	Std. Err.	t	p	Effect	Std. Err.	t	p
Mean	5.74	0.73	7.84	0.00	0.02	0.04	0.45	0.66	0.05	0.01	3.14	0.01[a]	11.15	3.73	2.99	0.01[a]
X_1	0.58	0.52	1.11	0.29	0.01	0.03	0.51	0.62	0.01	0.01	1.17	0.26	5.10	2.64	1.93	0.08[a]
X_1^2	0.23	0.55	0.42	0.68	-0.01	0.03	-0.26	0.80	-0.02	0.01	-1.41	0.18	-0.99	2.80	-0.35	0.73
X_2	2.01	0.52	3.89	0.00[a]	0.15	0.03	5.21	0.00[a]	0.05	0.01	4.65	0.00[a]	26.70	2.64	10.11	0.00[a]
X_2^2	-0.13	0.55	-0.24	0.82	0.11	0.03	3.62	0.00[a]	0.03	0.01	3.13	0.01[a]	12.31	2.80	4.40	0.00[a]
X_3	1.80	0.52	3.48	0.00[a]	0.03	0.03	1.08	0.30	0.01	0.01	0.73	0.48	5.90	2.64	2.23	0.05[a]
X_3^2	-0.33	0.55	-0.60	0.56	0.00	0.03	-0.13	0.90	0.00	0.01	-0.01	0.99	0.44	2.80	0.16	0.88
X_4	1.37	0.52	2.64	0.02[a]	0.00	0.03	0.07	0.94	0.02	0.01	1.48	0.16	-3.08	2.64	-1.17	0.27
X_4^2	-1.52	0.55	-2.77	0.02[a]	-0.01	0.03	-0.30	0.77	-0.01	0.01	-1.24	0.24	0.92	2.80	0.33	0.75
X_1 X_2	-0.54	0.63	-0.86	0.41	0.01	0.04	0.37	0.72	0.00	0.01	-0.28	0.78	4.22	3.23	1.31	0.22

X_1: Coded levels of reaction time; X_2: Coded levels of the temperature; X_3: Coded levels of the acid concentration; X_4: Coded levels of S:L ratio

[a] Significant at 90% confidence level

3.4 Statistical desirability and model validation

After optimization through MSR, it is crucial to understand which values of the variables under study represent a maximization considering multiple responses, i.e., which condition maximizes a desirable set of variables. To achieve this goal, we employ desirability analysis, a widely used statistical tool for optimizing processes with multiple responses (Yupanqui-Mendoza et al., 2024).

In our analysis, we have not only focused on maximizing the concentration of sugars, but also on reducing the presence of compounds such as furfural, 5-HMF and acetic acid. Previous studies have indicated that these three components most significantly impact downstream microbial metabolism (Bedzo et al., 2024; Bhavana et al., 2024). The choice of an optimal condition to achieve a higher sugar yield with minimal inhibitor generation is crucial in the production of a viable hydrolysate that preserves microbial metabolism during fermentation. In this sense, we selected these three components as variables for minimization, as opposed to the sugars that we needed to maximize, in order to more accurately represent the behavior of the hydrolysis process and its impact on the response variables.

Using the desirability function, the optimal hydrolysis conditions to optimize xylose, glucose and arabinose concentration were shown to be 7 min, 135 °C, 2.25% v/v acid concentration and an S:L ratio of 0.25 (see Figure 2). According to this function, the response variables for sugars, as a function of xylose, glucose and arabinose concentration, were predicted to be 58.42, 9.48 and 10.54 g/L, respectively. Likewise, for components such as furfural, 5-HMF and acetic acid, the predicted concentrations were 0.05, 0.00045 and 4.69 g/L, respectively. The value of D was equal to 0.893, indicating that these predicted conditions are representative and ideal.

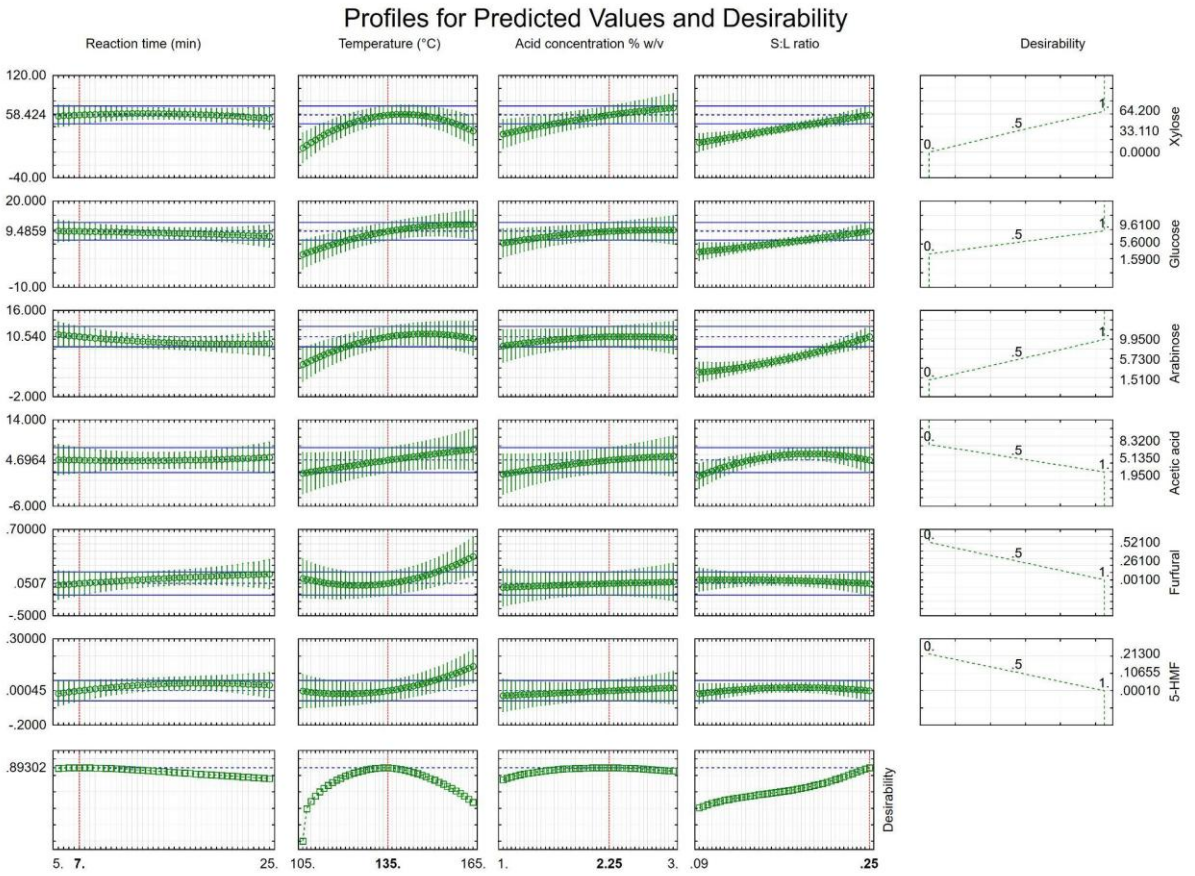


Fig. 2 Desirability plot for xylose, arabinose and toxic compounds of the hemicellulosic hydrolysate of bagasse and rice straw as a function of reaction time, temperature, acid concentration and Solid:Liquid ratio

The contour curves (Figure 3) show the hydrolysis conditions that maximize the production of sugars from the hemicellulosic hydrolysate. It can be deduced that the desirability function was influenced by the four independent variables. Thus, the highest concentrations of sugars were related to values of D close to 1. To validate the prediction efficiency of our model, an experimental adjustment was performed for the concentration of these three sugars, obtaining concentration values of 57.23 g/L for xylose, 10.48 g/L for glucose and 9.16 g/L for arabinose, while maintaining a minimum concentration of furfural, 5-HMF and acetic acid of 0.057, 0.048 and, 2.88 g/L. The results predicted by the desirability function and observed by the validation tests showed that the regression models were satisfactory for predicting the production of sugars obtained from the hemicellulosic hydrolysate of bagasse and straw.

From a general framework, the optimized condition obtained (135 °C, 7 min, 2.25% H_2SO_4 and S:L ratio of 0.25) could be attractive for larger scale processes based on xylose release at low concentrations of furfural, HMF and acetic acid, due to the reduction of the treatment time. This temperature-associated factor has a significant influence on the occurrence of decomposition reactions leading to the degradation of pentoses into furfural and hexoses into HMF and formation of acetic acid (Delfín-Ruíz et al., 2020). Maintaining HMF and furfural concentrations at minimum levels is a crucial factor since higher concentrations can negatively affect xylose consumption in fermentation (Bhavana et al., 2024). In addition, the release of acetic acid at concentrations higher than 3 g/L can affect cell growth, because the dissociated form of acetic acid can penetrate the cell membrane causing a decrease in intracellular pH, leading to a decrease in ATP production resulting in cell death (Mussatto, 2016). According to previous studies, we can find several statistically optimized conditions as a function of temperature, acid concentration and S:L ratio, all of them related to different substrates such as, wheat straw (Beckendorff et al., 2023), sugarcane bagasse (Varilla-Mazaba et al., 2024) and Agave bagasse (Delfín-Ruíz et al., 2020), however, as an important point, our approach result to be positioned in a shorter residence time compared to these studies. The production of a hemicellulosic hydrolysate release inhibitory compounds at reduced residence times and makes it an ideal candidate for conversion processes by microorganisms into compounds of industrial interest, such as xylitol, relevant in the food and pharmaceutical industry. In addition, other products derived from this process may include valuable compounds such as ethanol, lactic acid, and short-chain fatty acids, all of which have significant applications in industry.

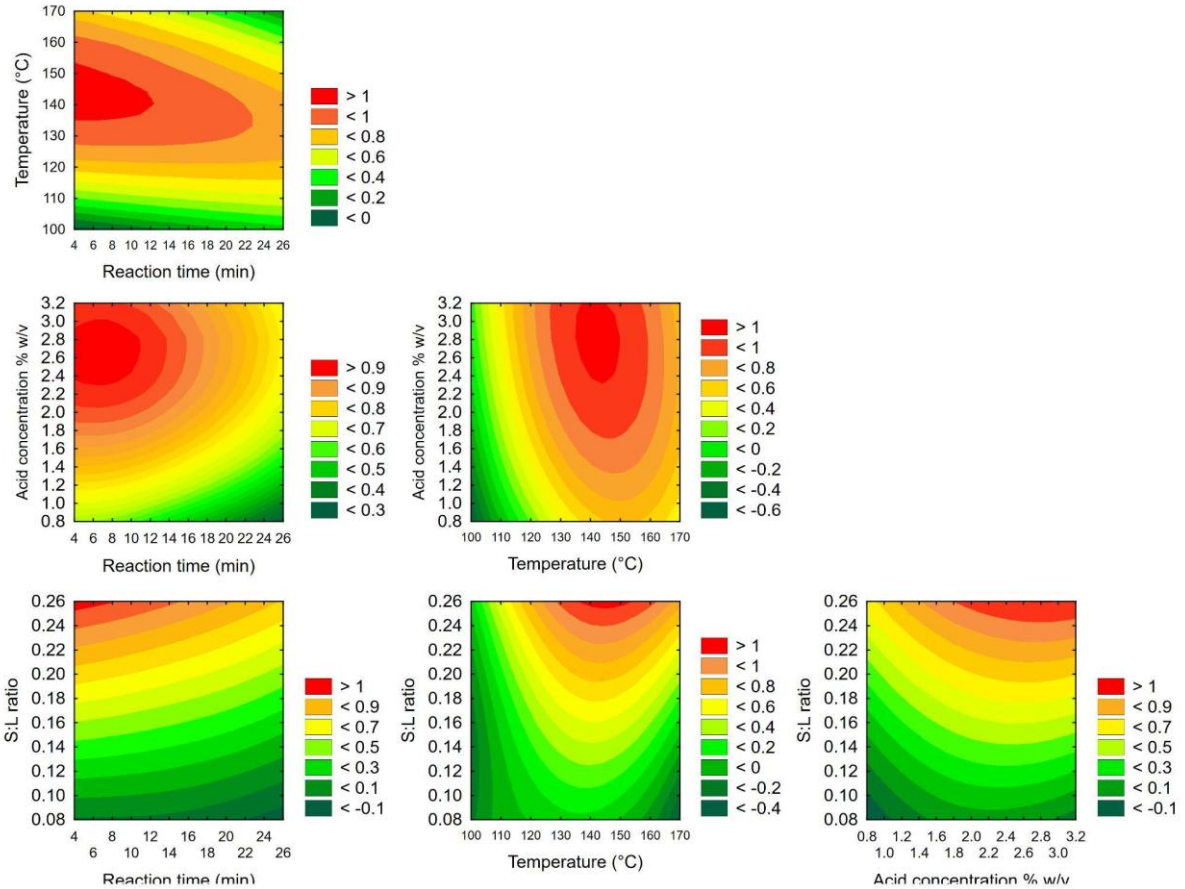


Fig. 3 Contour curves generated by the desirability function, in relation to the influence of the independent variables on the sugar concentrations of the hemicellulosic hydrolysate.

4. Conclusions

The optimization of dilute acid hydrolysis of sugarcane bagasse and straw mixture was carried out to increase the efficiency of xylose solubilization. Using a central composite rotatable design, different acid hydrolysis parameters were evaluated, such as temperature, reaction time, sulfuric acid concentration and solid:liquid ratio. Among the several dilute acid hydrolysis conditions tested, the highest concentrations of xylose (64.20 g/L), glucose (9.61 g/L), and arabinose (9.95 g/L) was achieved. The sugar concentration within the hemicellulose hydrolysate was mainly affected by temperature, with intermediate temperatures (135ºC) proving to be the optimal choice. Lower temperatures (120ºC) and higher temperatures (150ºC) yielded suboptimal sugar yields due to incomplete solubilization or

degradation into inhibitory compounds and by-products. These findings lead to the determination of optimal hydrolysis conditions for maximizing xylose yield, particularly relevant for xylitol production.

5. Statements and Declarations

Competing Interests and Funding

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper and no funding was received.

Author Contributions

Fanny Machado Jofre: Methodology, Investigation, Data Curation, Writing - Original Draft. Tatiane da Silva Boaes: Methodology, Investigation. Priscila Vaz De Arruda: Writing – Original Draft, Review and Editing. Sergio Luis Yupanqui-Mendoza: Formal Analysis, Writing – Original Draft, Review and Editing. Júlio César Dos Santos: Conceptualization, Formal Analysis, Investigation, Writing – Review & Editing. Maria Das Graças De Almeida Felipe: Conceptualization, Investigation, Supervision.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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