INFLUENCE OF PECTINASE ON THE PRE-TREATMENT OF RESIDUAL GUARANA (*Paullinia cupana*) IN ORDER TO PRODUCE REDUCING SUGARS

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ABSTRACT

Guarana (*Paullinia cupana*) a renowned Amazonian fruit, is highly valued worldwide, notably in Brazil, for its use in soft beverages, syrups, and cosmetics due to its high concentration of bioactive ingredients such as antioxidants and cognitive enhancers. However, the processing of guarana generates enormous waste, particularly wasted seeds, which gives an opportunity for the development of value-added goods. Roasting and hydroalcoholic extraction recover significant bioactives, allowing for enzymatic hydrolysis to extract sugars from the fruit's lignocellulosic part, increasing its versatility as a raw material. Pre-treatment is critical in breaking down the lignocellulosic structure, facilitating penetration and enzymatic activity. Enzymatic hydrolysis, which uses enzymes such as pectinase and cellulase, allows polyphenols to be released without damaging their integrity, in addition to have the potential to produce fermentable sugars from lignocellulosic biomass. With large-scale guarana production, waste management becomes critical, demanding the development of alternative ways to harness waste potential for developing new products and increasing the fruit's value through bioactive recovery. Experimental results show that variations in enzyme load have little influence on glucose levels, with lower loads potentially having a greater impact. However, the regression model utilized for data analysis is insufficiently explanatory, failing to accurately represent experimental results. Notably, enzyme load has a greater effect on enzymatic hydrolysis efficiency than the amount of solids in the substrate.

Keywords, Guarana. Pre-treatment, Enzymatic hydrolysis.

1 INTRODUCTION

Guarana is a fruit native to the Amazon region that, over the years, has been greatly valued in the country, with a high production scale, significantly moving the Brazilian and international markets. It is used in the production of syrups, cosmetics, and, mainly, in the production of soft drinks, as it has beneficial health properties associated with the presence of bioactive compounds and antioxidant, anticarcinogenic, and antitumor effects, as well as improvements in cognitive function. The waste produced, especially exhausted seeds, has great potential for the creation of new products, adding even more value to the fruit, giving a new recipient to what would otherwise be discarded in the environment, and improving the region's socioeconomic status. To exhaust them, the seeds go through the roasting and hydroalcoholic extraction processes to obtain the extract. With these already exhausted, it is possible to recover important bioactives from hydroalcoholic extraction followed by enzymatic hydrolysis with the recovered bioactives, and, thus, taking advantage of the lignocellulosic portion for the production of sugars that will later serve as the basis for obtaining products with greater added value makes guarana a great potential raw material[1,2,3]. To carry out hydrolysis, pre-treatment is extremely important, as it increases the efficiency of hydrolysis of cellulose into glucose due to the presence of lignin and hemicellulose and the crystallinity of cellulose, as it helps to loosen the structure of the lignocellulosic biomass. Carried out under rigorous conditions such as high temperature and pressure, resulting in compounds that prevent the activities of fermentation microorganisms and enzymes that degrade cellulose, such as phenolic compounds and formic and acetic acids[4,5,6]. The main hydrolysis extraction methods are acid, basic, and enzymatic, and each of these has a different way of acting on biomass. Enzymatic hydrolysis, carried out in this study, uses enzymes such as pectinase and cellulase to decompose the cell wall matrix and release the non-extractable polyphenols associated with the polysaccharides in question. This type of hydrolysis does not degrade or break the interflavan bonds of polyphenols, allowing the polymeric structures to be intact, however, it requires the combination of enzymes in an appropriate proportion for the composition of the matrix in question. Some matrices are resistant to enzymatic degradation, impacting extraction yield. The variables considered in this hydrolysis are temperature and pH for the enzymes to function. Enzymatic hydrolysis is considered a promising technique for obtaining fermentable sugars from lignocellulosic biomass due to its specificity and work under mild conditions, despite being expensive for use on an industrial scale[7,8].

2 MATERIAL & METHODS

Samples of roasted *P. cupana* seeds were acquired in the municipality of Urucará, in the Amazonas. For the study, the residual seed (RS) used was obtained through a hydroalcoholic extraction process, similar to the usual method in the beverage industry.[3] RS was subjected to pre-treatment using the enzyme Pectinase from *Aspergillus aculeatus* (P2611, Sigma-Aldrich) in a shaker incubator at different percentages, followed by hydrolysis with Cellulase from *Trichoderma Reesei* (C2730, Sigma-Aldrich). The percentage that presented the best yield of reducing sugars in RS was replicated in the factorial design described in Table 2.

For pre-treatment in a shaker incubator, carried out under shaking at 200 rpm for 24 hours at 45 °C, the RS were weighed and, at 5% solids load, 50 mL of citrate buffer (50 mM, pH 4.8) were added.), subjected to 45 °C, and 0.5 to 2 % (w/v) of the pectinase

enzyme was added, described in Table 1. Then, the enzymatic reaction was stopped with incubation at 100 °C for 5 min, followed by an ice bath. Followed by enzymatic hydrolysis, using 1.22 mL of the Cellulase enzyme, carried out and kept in a shaker for 48 h at 50 °C, shaking at 200 rpm. Upon leaving the shaker, vacuum filtration was performed to separate the solid from the liquid, followed by storage and refrigeration. For second pre-treatment, the RS were weighed and 50 mL of citrate buffer (50 mM, pH 4.8) were added, subjected to 45 °C, and 0.5% (m/v) of the pectinase enzyme was added. Then, the enzymatic reaction was stopped with incubation at 100 °C for 5 min, followed by an ice bath. Followed by enzymatic hydrolysis, using the Cellulase enzyme, through a central composite design of 2^2 rotations with three repetitions at the central point (11 experiments), taking as factors the enzymatic load and solids concentration, described in Table 2. The enzymatic load varied between 15 and 45 FPU/g of solids, and the solid's concentration varied between 3 and 7%. For hydrolysis, the pre-treatment reaction mixture was subjected to 50 °C. Upon reaching the temperature, Cellulose was added and kept in a shaker for 48 h at 50 °C, shaking at 200 rpm. Upon leaving the shaker, vacuum filtration was performed to separate the solid from the liquid, followed by storage and refrigeration.

All analyses were performed in duplicate, and the results were expressed as the mean ± standard deviation. Statistical analyses were performed using Statistica software (Statsoft, version 7.0, USA). The data were subjected to analysis of variance (ANOVA) with a confidence level of 95% ($p < 0.05$). Means were compared using the Tukey test.

3 RESULTS & DISCUSSION

Table 1 shows the yield values after hydrolysis of RS pre-treated in a shaker with Pectinase, together with the results of the Tukey test carried out in each experiment. When observing the results of the Tukey test, there is no significant variation in glucose values between experiments, where the only variation between each situation is the applied enzyme load.

Table 1 Values used in the enzymatic hydrolysis of the residual seed are determined by planning and the yield values that were acquired after hydrolysis of samples that underwent pre-treatment in ultrasound and shaker.

Experiments	Solids (%)	Enzyme load (% w/v)	Performance (%)	Grouping*
A	5	0.5	97.17 ± 6.72	A
B	5	1	86.87 ± 4.49	A
C	5	1.5	91.55 ± 6.17	A
D	5	2	94.80 ± 4.94	A

*Averages followed by the same letter do not differ from each other, using the Tukey test at 5% probability.

Table 2 presents the yield values after the hydrolysis of RS pre-treated in a shaker with 0.5% Pectinase. The highest yield observed was obtained in condition 8, with approximately 81% of reducing sugars. Table 3 presents the analysis of variance (ANOVA) for glucose yield in both treatments.

Table 2 Values used in the enzymatic hydrolysis of the residual seed are determined by planning and the yield values that were acquired after hydrolysis of samples that underwent pre-treatment in ultrasound and shaker.

Experiments	Solids (%)	Enzyme load (FPU/g)	Performance (%)
1	7	15	57.438
2	7,82843	30	65.846
3	3	45	65.751
4	3	15	49.742
5	5	51,2132	69.319
6	7	45	68.533
7	5	8,7868	47.217
8	2,17157	30	80.835
9	5	30	60.531
10	5	30	60.647
11	5	30	61.815

Table 3 Analysis of variance for the variables percentage of solids and enzymatic load (Cellulase) in the yield of reducing sugars obtained from the enzymatic hydrolysis of *P. cupana* processing residue.

Factor	Quadratic sum	Degree of freedom	Quadratic mean	F	P-value
A, Solids	14.3639	1	14.3639	28.4778	0.033367
B, Enzyme load	130.7519	1	130.7519	259.2281	0.003835
AA	425.7500	1	425.7500	844.0901	0.001183
AB	41.9142	1	41.9142	83.0990	0.011821
BB	6.0368	1	6.0368	11.9686	0.074353
Lack of fit	186.7231	3	62.2410	123.3988	0.008049
Pure error	1.0088	2	0.5044		
Total quadratic sum	870.5703	10			

The coefficients of determination R^2 and adjusted R^2 were 0.78436 and 0.56872 respectively, which indicates the lack of adjustment between the regression model and the experimental values. For this model, the R^2 value was less than 80%, that is, it is statistically non-significant, and the lack of adjustment is significant because its value was greater than 0.05. The low R^2 value combined with a significant lack of adjustment leads to the conclusion that the model does not explain the experimental model. In the case of a significant effect on the production of reducing sugars, the efficiency of the different operational factors in the enzymatic hydrolysis of *P. cupana* processing residue occurred in the following order: enzymatic load > percentage of solids, as observed below in Figure 1 .

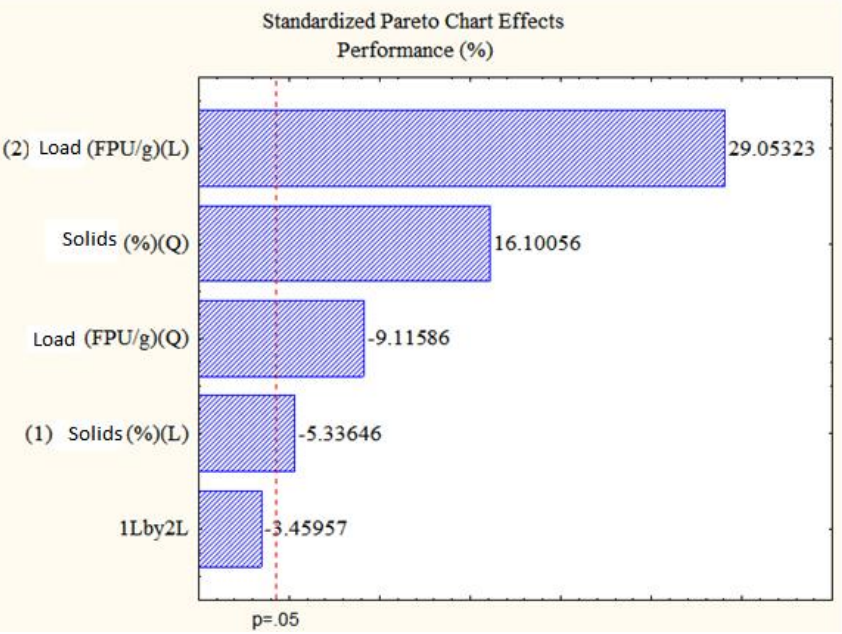

Figure 1 Pareto chart to visualize the effects of the variables' percentage of solids and enzymatic load (cellulase) on the yield of reducing sugars obtained from the enzymatic hydrolysis of *P. cupana* processing residue.

4 CONCLUSION

Due to the high production of *P. cupana* it is necessary to develop alternative solutions for using the waste generated. This is due to the fact that waste has great potential for creating new products and adding even more value to the fruit when its bioactives are recovered. In the work, changes in enzyme load do not have a significant impact on glucose values, but a lower enzyme load may have a more significant impact. However, the regression model used to analyze the data does not have enough explanatory power, meaning it does not describe the results of the experiment effectively. Furthermore, the enzyme load affects the efficiency of enzymatic hydrolysis more than the percentage of solids in the substrate.

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