



Acta Scientiarum. Animal Sciences

ISSN: 1807-8672

Editora da Universidade Estadual de Maringá - EDUEM

Teixeira, Amito José; Menoncin Weschenfelder, Leonardo; Antunes, Angela; Zeni, Jamile; Toniazzo Backes, Geciane; Cansian, Rogério Luis

Commercial and non-commercial pectinase and cellulase on the enzymatic hydrolysis efficacy of rice husk and Tifton 85 hay

Acta Scientiarum. Animal Sciences, vol. 41, e45100, 2019

Editora da Universidade Estadual de Maringá - EDUEM

DOI: <https://doi.org/10.4025/actascianimsci.v41i1.45100>

Available in: <https://www.redalyc.org/articulo.oa?id=303160553013>

- How to cite
- Complete issue
- More information about this article
- Journal's webpage in redalyc.org

redalyc.org

Scientific Information System Redalyc

Network of Scientific Journals from Latin America and the Caribbean, Spain and Portugal

Project academic non-profit, developed under the open access initiative

Commercial and non-commercial pectinase and cellulase on the enzymatic hydrolysis efficacy of rice husk and Tifton 85 hay

Amito José Teixeira, Leonardo Menoncin Weschenfelder, Angela Antunes, Jamile Zeni^{*}, Geciane Toniazco Backes and Rogério Luis Cansian

Departamento de Engenharia de Alimentos, Universidade Regional Integrada do Alto Uruguai e das Missões, Avenida 7 de Setembro, 1621, 99709-910, Erechim, Rio Grande do Sul, Brazil. *Autor correspondente. E-mail: jamilenzi@uricer.edu.br

ABSTRACT. The aim of this study was to evaluate the action of commercial and non-commercial cellulase and pectinase on rice husk and Tifton 85 hay hydrolyses. The hydrolysis kinetics of the substrates with commercial cellulase and pectinase were evaluated and the hydrolysis at different temperature and agitation conditions was maximized using experimental design. The combined use of commercial and non-commercial enzymes under optimized conditions was evaluated. The pre-treatment of the residues was also investigated by milling and different concentrations of NaOH. Finally, the effect of the hydrolysis on the bromatological composition of the residues was evaluated. The best hydrolysis times of rice husk and Tifton 85 hay were 10 and 12h for commercial cellulase, 12 and 14h for non-commercial cellulase, 10 and 14h for commercial pectinase and 16 and 20h for non-commercial pectinase, respectively. The highest hydrolysis values were obtained using commercial cellulase with 1:50 (w:v enzyme:water) dilution rate, at 45°C and 300 rpm agitation for both substrates, reaching 20.6% maximum percentage for Tifton 85 hay and 11.6% for rice husk. The combined use of commercial enzymes did not increase hydrolysis percentage. The pre-treatment using 7.5% NaOH and 0.5 mm grain size significantly increased the rice husk and Tifton 85 hay hydrolyses (60-80%), either using commercial cellulase or pectinase enzymes. The use of non-commercial enzymes provided 18-30% hydrolysis obtained from commercial ones. Bromatological analyzes indicated a reduction in neutral detergent fiber and acid detergent fiber content for rice husk and Tifton 85 hay when using pectinases and commercial cellulases.

Keywords: Enzyme; fiber; forage; residues.

Received on October 26, 2018.
 Accepted on December 18, 2018.

Introduction

Cellulose in lignified plant cell walls can be an energy source in applications such as animal nutrition (Van Kuijk et al., 2017). However, the use of cellulose in lignocellulosic biomass cannot be directly used for such purposes due to the presence of lignin. Lignin cannot be degraded through anaerobic fermentation in the rumen and has been negatively related to digestibility in ruminant animals (Azizi-Shotorkhoft, Mohammadabadi, Motamedi, Chaji, & Fazaeli, 2016). Lignocellulose is composed of cellulose (38-50%) and hemicellulose (23-32%) bound together by an impermeable lignin layer (15-25%) (Han, Guo, Liu, Xia, & Wang, 2019; Sindhu, Binod, & Pandey, 2016).

The use of cellulolytic enzyme cocktails is among the most promising ways to convert cellulose and hemicellulose into reducing sugars such as glucose and xylose (Xu, Lin, Zang, & Shi, 2018; Yadav, 2017). Pectinase, another enzyme group, is responsible for the degradation of long and complex molecules called pectin, which occurs as structural polysaccharides in the middle lamella and primary cell walls of plants (Martos, Zubreski, Combina, Garro, & Hours, 2013). Enzymes such as pectinase and xylanase may break hemicellulose heterogeneous polymers that interconnect with cellulose microfibrils. Therefore, supplementation with cellulase and pectinase enzymes can benefit cellulose hydrolysis (Gupta et al., 2016), although it has been identified as the most expensive step in the hydrolytic process (Castro & Pereira Júnior, 2010).

Cellulolytic enzymes behavior on substrates may differ due to the substrate type (amorphous and crystalline cellulose). This is explained by many factors divided into two groups: those related to

lignocelluloses structural features and those related to mechanism and interactions of the cellulolytic enzyme. The latter includes (a) lignin presence, that although to a small extent, acts as a physical barrier, restricting cellulase access to cellulose and is able to adsorb cellulase enzymes and (b) hemicellulose and pectin effect, which are barriers limiting enzyme action or reducing local water activity (Eibinger, Bubner, Ganner, Plank, & Nidetzky, 2014).

Factors affecting enzymatic hydrolysis include substrate concentration, cellulase and auxiliary enzymes activities (or amounts) and operational conditions such as agitation or mixing, pH, temperature, and particle diameter (Sun, Sun, Cao, & Sun, 2016; Takahashi, Sato, Ito, & Mori, 2014).

The aim of this study was to evaluate commercial and non-commercial cellulase and pectinase enzymes action on lignocellulosic substrates hydrolysis (rice husk and Tifton 85 hay), by analyzing contact time, enzyme dilution, temperature, agitation, alkaline treatment and grain size, aiming at improving substrates' nutritional quality for animal feed.

Material and methods

Substrates and enzymes used on hydrolysis

The substrates used on enzymatic hydrolysis were rice husk (RH), a rice-processing byproduct (Josapar, Pelotas-RS, Brazil) and Tifton 85 hay (TH), both supplied by local farmers from the Alto Uruguay Region. The substrates have been stored at room temperature and used without any pre-treatment. The commercial enzymes used were cellulase (Sigma-Aldrich®) and pectinase (Rohapect DA6L® - AB Enzymes) produced from *Aspergillus niger*.

Non-commercial cellulase production was using *Trichoderma reesei* NRRL 3652. Solid state culture was carried out using soybean hull as substrate at pH 4.6, 30°C, 70% humidity, and 1.0×10^7 spores $\text{g}_{\text{wm}}^{-1}$. Extraction was performed by adding sodium citrate buffer solution (0.5 M, pH 5.5) at 1:15 (substrate:buffer, w:w) ratio, incubated for 30 min at 50°C, and 100 rpm.

Non-commercial pectinase enzyme was obtained according to methodology described by Borszcz et al. (2017) by solid-state culture (SSC) using *Aspergillus niger* ATCC 9642 in a 500 mL polypropylene beaker, with 9 g orange peel, 4 g corn steep liquor, and 7 g wheat bran concentrations, at 30°C, and 5×10^6 spores $\text{g}_{\text{wm}}^{-1}$. Extraction was done with NaCl (0.1 mol L^{-1}), 5:1 (v:w) solvent: substrate ratio, for 30 min, at 20°C, and 180 rpm.

Lignocellulosic substrates hydrolysis evaluation using commercial and non-commercial enzymes

Enzymatic saccharification for each non-previously-treated substrate was carried out as described by Liu, Lu, and Cui (2011). The assays were done in erlenmeyer with the addition of 2 g lignocellulosic residue (RH and TH) and autoclaved for 15 min at 121°C. Subsequently, 100 mL of a reactional mixture containing 95 mL sodium citrate buffer 0.05 M, pH 5.0 and 5 mL commercial enzymatic extract (cellulase or pectinase, respectively with 6.35 U g^{-1} and 290 U mL^{-1} activities) or 5 mL crude enzymatic extract from non-commercial cellulase or pectinase enzymes.

The kinetic behavior of lignocellulosic substrates hydrolysis process (RH and TH), using both commercial and non-commercial cellulase and pectinase enzymes, was monitored with samples removal from the reactional mixture at various time intervals. Hydrolysis reactions were carried out using an orbital shaker at 150 rpm and 37°C. Reactions with non-commercial enzymes were achieved by adding 5 mL crude enzymatic extract, whereas for commercial ones it was added 5 mL pectinase extract and 5 mL 1:50 cellulase dilution. Total reducing sugars (TRS) release on hydrolysis was estimated using DNS methodology (dinitrosalicylic acid) (Miller, 1959).

In order to evaluate the influence of enzyme dilution on lignocellulosic substrates (RH and TH) hydrolysis, studies at different commercial cellulase concentrations (1:50, 1:75, 1:100, 1:125, 1:150, 1:175 and 1:200, w:v) diluted in a citrate buffer pH 5.0 were carried out. No evaluation was performed for commercial pectinase enzyme as they show the lowest lignocellulosic residues hydrolysis activity.

In order to evaluate temperature and agitation effects on lignocellulosic substrates (RH and TH) hydrolysis, a 2^2 factorial design experiment (Central Composite Rotary Design – CCRD) (Table 1) was carried out. The independent variables were temperature (°C, X_1) and agitation (rpm, X_2). The variables reaction time and enzyme dilution were kept fixed, as established on previous assays.

Different ratios of cellulase and pectinase commercial enzyme were combined in order to evaluate their interaction effects on RH and TH hydrolyses. The cellulase/pectinase mixtures (%) were 100/0, 75/25, 50/50, 25/75, and 0/100.

Pre-treatments effect on the hydrolysis of lignocellulosic residues

Substrates were ground to 0.5, 1.0, and 1.5 mm particle sizes prior to treatment with commercial cellulase enzyme in order to verify if an increase of superficial area in the residues would influence on enzymatic activity.

The effect of alkaline treatment on RH and TH residues on the hydrolysis using commercial cellulase was evaluated using 1, 3, and 5% commercial sodium hydroxide, at 100° C for 30, 60, and 90 min. The materials recovered after boiling were washed using running distilled water and then stove-dried at 60°C until the residue reached its natural humidity.

In order to evaluate the effects of grain size and alkaline pre-treatment combination, RH and TH substrates ground at 0.5 mm granulometry underwent treatment with 3% commercial sodium hydroxide, at 100°C for 90 min. Afterwards, hydrolysis using commercial cellulase at 1:50 dilution rate was done under optimal temperature (45°C), time (10 h for rice husk and 14 h for Tifton 85 hay) and agitation (300 rpm for rice husk and 150 rpm for Tifton 85 hay) conditions reached on previous stages. Subsequently, the 0.5 mm ground grain size substrates underwent commercial sodium hydroxide (NaOH) treatment at different (3, 5, 7.5 and 10%) concentrations, also under optimal temperature, time, and agitation conditions reached on earlier stages.

Bromatological analysis

Bromatological analyzes of neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN) and nitrogen-free extract (NFE) were carried out with lignocellulosic residues hydrolysed with commercial and non-commercial cellulase and pectinase by Research in Food (CEPA) of the University of Passo Fundo, Brazil. The assays were conducted using the Reflectance Near-Infrared Spectrophotometry EFQ 49 (FOSS/Denmark) (700 – 2500 nm). The total digestible nutrients (TDN) were calculated by Equation 1. The amount of nitrogen-free extract (NFE) was calculated by subtracting the sum of NDF, Crude protein (CP), ether extract (EE), and crude ash (Ash) from 100. Unhydrolyzed residues were analyzed as a control. All the analyzes calculations are described in Brazilian Compendium of Animal Feeding.

$$\text{TDN} = 87.84 - (\text{ADF} * 70) \quad (1)$$

where TDN= total digestible nutrients and ADF = acid detergent fiber.

Analytical determinations

Total Reducing sugars: The amount of total reducing sugars was estimated using the 3.5 dinitrosalicylic acid method with glucose as standard (Miller, 1959). The saccharification percentage was calculated according to Equation 2 (Association Official Analytical Chemist [AOAC], 2005).

$$\text{Saccharification (\%)} = \frac{\text{RS} \times 0.9 \times 100}{\text{P}} \quad (2)$$

where RS = released sugar; 0.9 = correction factor; P = polysaccharides in lignocellulosic substrate (0.0505 for soybean hulls (Brijwani, Oberoi, & Vadlani, 2010) and 0.584 for corn stover and cobs).

Exo-Polygalacturonase (Exo-PG) activity: It was determined using methodology by Miller (1959). One exo-PG activity unit was defined as the amount of enzyme that releases 1 mmol D-galacturonic acid per minute of reaction ($U = \mu\text{mol min}^{-1}$) from citrus pectin under the test conditions, according to a standard curve ($0.1 - 10 \text{ mg mL}^{-1}$) established with α -D-galacturonic acid (Sigma-Aldrich, São Paulo-SP, Brazil) as the reducing sugar. The exo-PG activity was expressed in activity units per milliliter ($U \text{ mL}^{-1}$).

Pectin Methylesterase (PME) activity: PME activity was determined following methodology described by Hultin, Sun, and Bulger (1966). One PME unit was defined as the amount of enzyme able to catalyze pectin demethylation corresponding to $1 \mu\text{mol NaOH min}^{-1} \text{ mL}^{-1}$ consumption, under the conditions described on the assay.

Pectin Lyase (PL) activity: It was determined using methodology described by Pitt (1988). One enzyme activity unit was defined as the amount of enzyme that resulted in a 0.01 change in absorbance at 550 nm.

Total cellulase activity (FPase): It was measured according to Ghose (1987). The mixtures Optical Density was recorded at 540 nm and compared with the standard glucose curve to determine the amount of reducing sugar (mg mL^{-1}) produced during cellulose hydrolysis.

Xylanase activity: It was measured according to Bailey, Biely, and Poutanen (1992). One xylanase activity unit was defined as the amount of enzyme that releases 1 μmol of reducing sugars equivalent to xylose per minute.

Carboxymethyl cellulase activity (CMCase): It was measured according to Ghose (1987). One CMCase activity unit was defined as the amount of enzyme that releases 1 μmol of reducing sugars equivalent to glucose per minute.

Avicelase activity: It consisted of adding 1 mL crude enzyme extract in 1 mL of 1% microcrystalline cellulose (Avicel) solution in 0.05 M acetate buffer, pH 5.0 and incubated at 50° C for 30 min, under constant agitation (Menegol, Scholl, Fontana, Dillon, & Camassola, 2014). The reducing sugars released were determined by the dinitrosalicylic acid method according to Miller (1959) and one activity unit (U) was defined as 1 μmol of glucose equivalent released per minute under the conditions described above, using a glucose standard curve.

Statistical analysis

The results were statistically processed by analysis of variance (ANOVA) and the differences in average were compared by Tukey test using Statistica software (Statsoft Inc, 2008), at 95 % significance level ($p < 0.05$).

Results and discussion

Lignocellulosic substrates hydrolysis using commercial and non-commercial enzymes

The evaluation of the kinetic behavior for the hydrolysis process of rice hull substrates and Tifton 85 hay using commercial and non-commercial enzymes of cellulase and pectinase presented different times for each enzyme, being 10 and 12h for commercial cellulase, 12 and 14h for non-commercial cellulase, 10 and 14h for commercial pectinase, and 16 and 20h for non-commercial pectinase, respectively (data not shown). Menegol et al. (2014) obtained high reducing sugar concentrations after 8-12h elephant-grass hydrolysis, which corroborated with the results from this study.

When evaluating the influence of commercial cellulase dilution on rice husk and Tifton 85 hay hydrolysis, it was noticed that the highest hydrolysis values were obtained at a 1:50 (w:v enzyme:water) dilution ratio for both substrates, differing statistically ($p > 0.05$) from other assays. Based on these results 1:50 dilution rate was used for the experiments that followed. Different dilutions were not evaluated for commercial pectinase enzyme as it presented the lowest hydrolysis activity.

Table 1 shows the results 2^2 factorial design obtained through temperature and agitation for rice husk and Tifton 85 hay hydrolysis using commercial cellulase and pectinase enzymes. The best hydrolysis results using commercial cellulase were obtained at 29 and 37°C and 200 and 150 rpm for RH and TH, respectively. Whereas, using commercial pectinase, assay 4 presented the highest hydrolyses for rice husk and Tifton 85 hay with 1.34 and 9.01%, respectively, at 45°C and 200 rpm.

Table 1 results were statistically treated and Figure 1 shows the Pareto charts, which validated the estimated effects of variables studied for rice husk and Tifton 85 hay hydrolysis, with commercial cellulase and pectinase enzymes, respectively. Agitation and temperature presented a positive effect ($p < 0.05$) on the hydrolysis of substrates, either with cellulase or pectinase, indicating that an increase in agitation and temperature will increase their hydrolysis. Hu, Jing, Zhang, Guo, and Lee (2017), when evaluating enzyme hydrolysis of maize straw, obtained the highest sugar concentrations at 50°C and hydrolysis reduction at higher temperatures.

Taking into account that temperatures above 45°C could limit enzymes, tests in triplicate were carried out, varying only in agitation (200 to 350 rpm) at a constant 45°C temperature (Figure 2). Agitation had a significant positive effect for all substrates hydrolyses, with cellulase as much as with pectinase, showing the highest hydrolysis levels at 30 rpm, expect for Tifton 85 hay treated with pectinase that had the highest percentage of hydrolysis at 350 rpm. It was also noted higher percentages of Tifton 85 hay hydrolysis compared to rice husk, independently of the enzyme used and with cellulase in comparison to pectinase, independently of the substrate.

For the interaction between commercial cellulases and pectinases on substrates hydrolysis, the highest hydrolysis for RH (12.1%) and TH (20.6%) was obtained with the highest amounts of cellulase (100%). In that case, considering that the major composition of these residues is cellulose, higher hydrolysis was obtained in assays where cellulase enzyme prevailed.

Table 1. 2² Factorial design matrix (real and coded values) for rice husk (RH) and Tifton 85 hay (TH) hydrolysis using commercial cellulase and pectinase enzymes

Assays	Independent variables*		Cellulase		Pectinase	
	Temperature (°C)	Agitation (rpm)	RH Hydrolysis (%)	TH Hydrolysis (%)	RH Hydrolysis (%)	TH Hydrolysis (%)
1	29 (-1)	100 (-1)	6.35	11.32	0.22	3.54
2	29 (-1)	200 (1)	10.87	15.86	0.58	7.54
3	45 (1)	100 (-1)	9.96	17.83	0.84	4.05
4	45 (1)	200 (1)	10.67	18.84	1.34	9.01
5	37 (0)	150 (0)	6.90	18.70	0.07	6.28
6	37 (0)	150 (0)	6.94	19.24	0.07	6.42
7	37 (0)	150 (0)	6.27	19.76	0.03	6.59

*Fixed independent variables: amount of enzyme: 5 mL (1:50 dilution only for cellulase); reaction time 10h for rice hulls with pectinase and cellulase and for Tifton 85 hay, 12h with cellulase and 14h with pectinase.

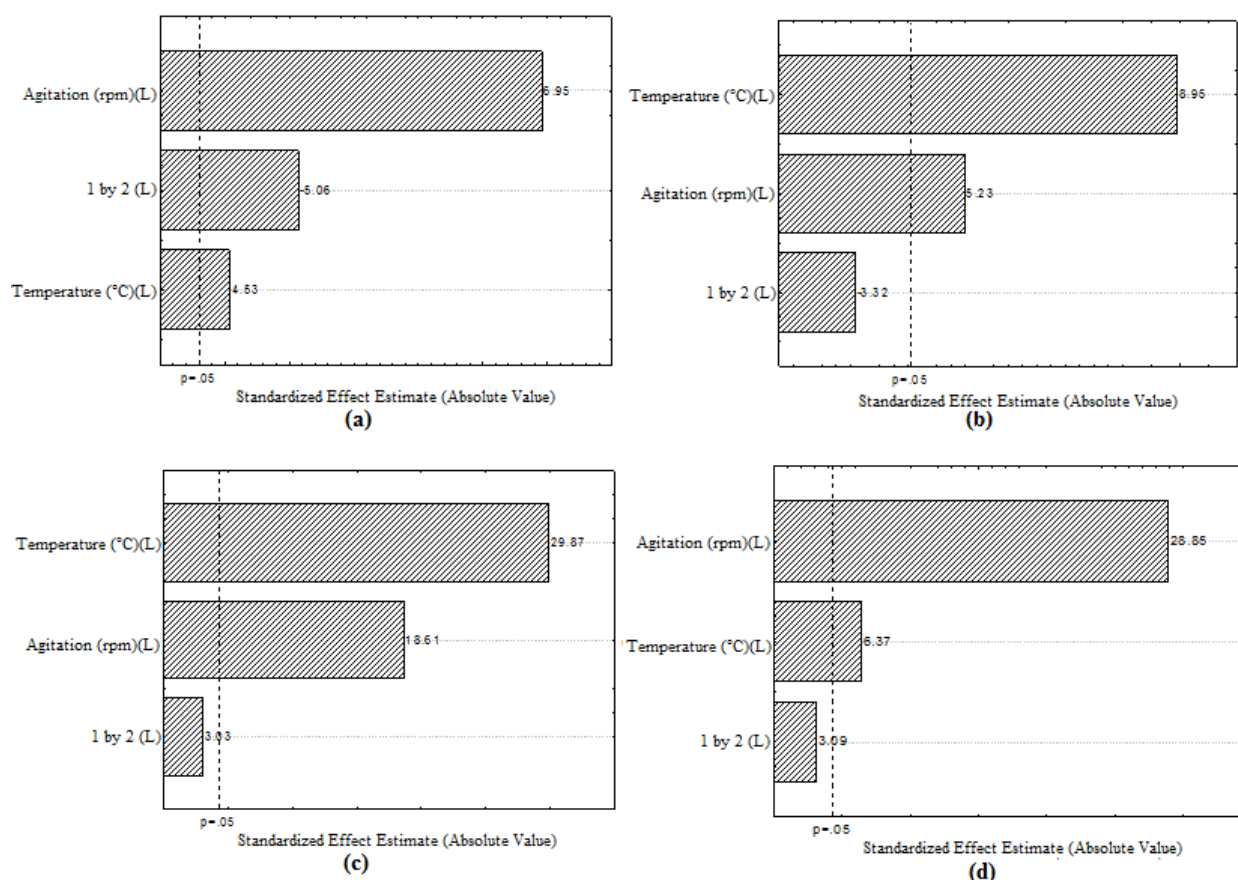
**Figure 1.** Pareto chart with estimated effect (absolute value) for variables tested of a full 2² factorial design for rice husk (a) and (b) and Tifton 85 hay (c) and (d) hydrolysis with commercial cellulase and pectinase enzymes, respectively.

Table 2 shows the results of substrates hydrolysis using commercial and non-commercial pectinase and cellulase enzymes. It was observed that the percentage of hydrolysis with non-commercial cellulase was lower than the one with commercial cellulase. The best percentage for hydrolysis using non-commercial enzyme was obtained with Tifton 85 hay, at 23.65% of total hydrolysis obtained using commercial cellulase. Rice husk hydrolysis was just 18.09% of total reached with the commercial enzyme. Substrates hydrolysis with non-commercial pectinase enzymes (Table 2) reached only 30% of the total compared to commercial pectinase enzyme. The best hydrolysis percentage for non-commercial pectinase was 30.07% obtained for Tifton 85 hay. Despite being relatively low, such results could be satisfactory, since the costs for obtaining non-commercial pectinase enzyme is lower than the commercial one.

The non-commercial enzymes used in this study are a crude extract composite of a set of enzymes. Thus, FPase, Xylanase CMCase and Avicelase activities were determined in the cellulase crude extract, showing 6.02, 972.74, 7.76, and 2.02 U g⁻¹ values, respectively. PG, PME and PMGL enzymes activity in the pectinase crude extract were also determined presenting 1.83, 3.80, and 29.0 U g⁻¹ values, respectively.

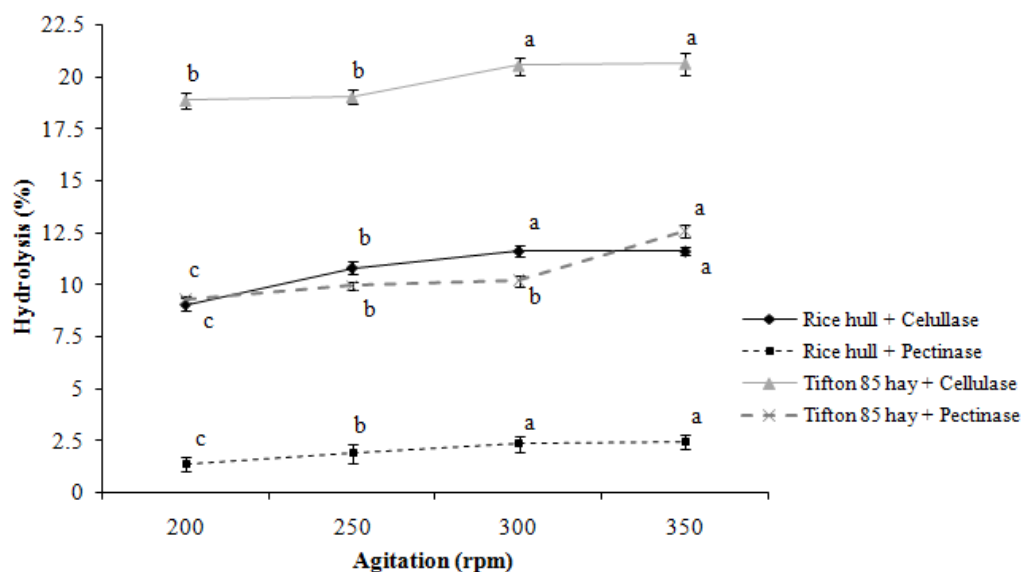


Figure 2. Agitation influence on rice husk hydrolysis with commercial cellulase and pectinase on Tifton 85 hay.

Pre-treatment effects on hydrolysis of lignocellulosic residues

Despite presenting a slight percentage improvement on hydrolysis at most grinding levels and substrates, physical treatment alone did not have a positive effect on hydrolysis of lignocellulosic residues. Hydrolysis for RH and TH substrates with 5.0 to 0.5 mm grain size using commercial cellulase varied from 11.54 to 12.69% and from 20.0 to 21.30% with 9.96 and 6.5% increments in relation to rice husk and Tifton 85 hay non-ground substrate, respectively, without statistical difference ($p > 0.05$) among treatments. Figure 3 shows results of rice husk and Tifton 85 hay hydrolysis treated with NaOH and using commercial cellulase and pectinase enzymes. The highest percentages for rice husk hydrolysis (20.43% with commercial cellulase and 22.9% with commercial pectinase enzyme) and for Tifton 85 hay (35.63% with commercial cellulase and 22.9% with commercial pectinase enzyme) occurred at 7.5% NaOH concentration, not differing much from the 10% concentration. Menegol et al. (2014) obtained high reducing sugars and glucose yields for elephant-grass samples pre-treated with 6% NaOH, caused by lignin reduction. The lignocellulosic complex consists of a cellulose and lignin matrix bound by hemicellulose chains (Isikgor & Becer, 2015). Pre-treatment is used to break such matrix in order to reduce the cellulose crystallinity degree and increase amorphous cellulose fraction, which is more appropriated for the enzymatic attack (Kumar & Sharma, 2017).

Lignocellulosic residues characterization before and after enzymatic hydrolysis

The results of neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN), and non-nitrogen extractives (NNE) from substrates treated with commercial and non-commercial cellulase and pectinase enzymes are shown in Table 3.

The use of enzymes on rice husk reduced NDF and ADF, highlighting the results achieved with commercial cellulase. Tifton 85 hay showed a reduction of approximately 20% for NDF using commercial cellulase and pectinase. Whereas, for ADF, Tifton 85 hay presented a 14% reduction with the same enzymes. Tifton 85 hay had its ADF percentage reduced by only 8% using non-commercial enzymes.

Bromatological analyses indicated that in both substrates evaluated, neutral detergent fiber (NDF) and acid detergent fiber (ADF) substrates were reduced with the use of all commercial and non-commercial enzymes. That is considered a positive result, since the lower the ADF percentage, which the nutritional fraction is corresponding to hemicellulose, cellulose, and lignin sum, the better the food nutritional value (Sousa et al., 2018).

Table 2. Effect of the interaction between commercial cellulase and pectinase on the hydrolysis of lignocellulosic substrates (RH and TH).

Byproduct	Cellulase/Pectinase Blends (%)				
	100/0	75/25	50/50	25/75	0/100
RH	12.07 ^{aE} ± 1.10	10.27 ^{bE} ± 1.02	8.17 ^{cD} ± 0.65	2.74 ^{dF} ± 2.03	0.41 ^{eE} ± 0.03
TH	20.63 ^{aD} ± 1.90	18.06 ^{bD} ± 1.77	12.50 ^{cC} ± 1.23	6.23 ^{dD} ± 5.83	2.13 ^{eD} ± 0.19

Means ± standard deviation followed by the same lowercase letters in the columns and capitals in the rows do not differ significantly by the Tukey test with 95% confidence.

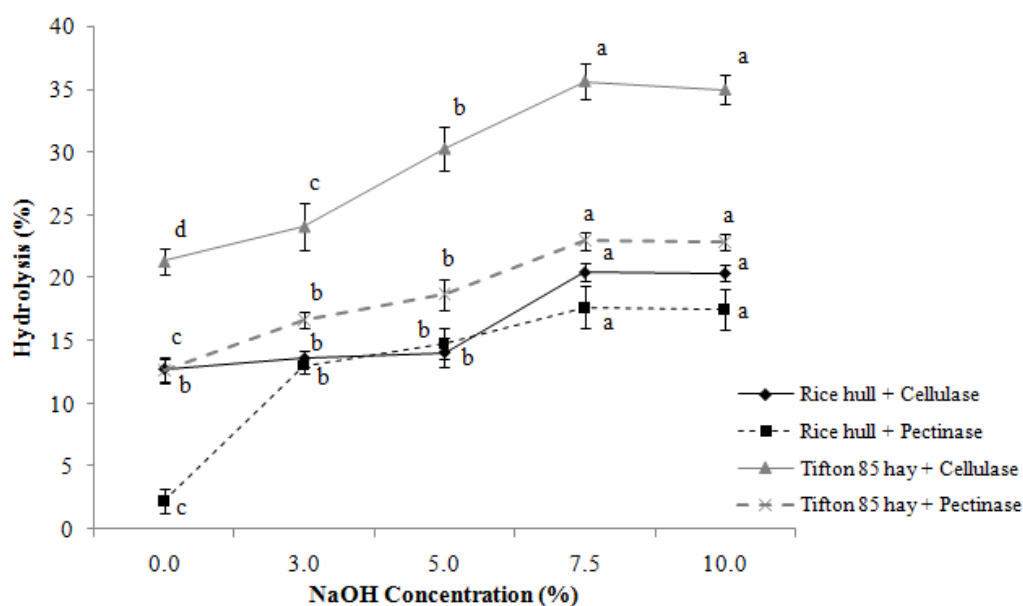


Figure 3. Rice husk hydrolysis with cellulase (a) and pectinase (b) and Tifton 85 hay with cellulase (c) and pectinase (d), ground at 0.5 mm grain size, without alkaline pre-treatment and NaOH treated (3.5, 7.5, and 10%) at 100°C for 90 min.

Table 3. Lignocellulosic residues centesimal composition before and after using commercial and non-commercial cellulase and pectinase enzymes.

Treatments	NDF (g 100 g ⁻¹)	%	ADF (g 100 g ⁻¹)	%	TDN (g 100 g ⁻¹)	%	NFE (g 100g ⁻¹)	%
Rice husk								
Without treatment	92.62		70.48		19.21		28.13	
Commercial Cellulase	65.14	29.67	38.64	45.18	31.11	61.94	47.70	69.57
Non-commercial Cellulase	79.85	13.79	61.08	13.34	21.12	9.94	32.44	15.32
Commercial Pectinase	75.05	18.97	59.65	15.37	24.35	26.75	35.92	27.69
Non-commercial Pectinase	78.75	14.98	62.49	11.34	20.58	7.13	31.24	11.05
Tifton 85 hay								
Without treatment	68.13		33.71		64.24		55.68	
Commercial Cellulase	53.98	20.77	29.06	13.79	67.70	5.38	62.97	13.10
Non-commercial Cellulase	57.84	15.10	30.98	8.09	65.53	2.01	58.89	5.77
Commercial Pectinase	54.79	19.58	28.72	14.80	69.78	8.62	60.12	7.97
Non-commercial Pectinase	60.56	11.11	31.02	7.98	65.13	1.39	57.27	2.85

* NDF stands for neutral detergent fiber, ADF - acid detergent fiber; TDN - total digestible nutrients and NNE non-nitrogen extractives.

The bromatological analysis results (Table 3) showed that the use of cellulase and pectinase enzymes improved the total digestible nutrients (TDN) and non-nitrogen extractives (NNE) percentages in the evaluated residues, mainly when using commercial cellulase and pectinase enzymes. TDN and NNE results using commercial cellulase enzyme for rice husk are highlighted as they showed better percentages (61.94 and 69.57), respectively.

Conclusion

Commercial and non-commercial pectinases and cellulases were effective in the enzymatic hydrolysis of Tifton 85 hay and rice husk.

Acknowledgements

This study was financed in part by the Coordination for the Improvement of Higher Education Personnel (CAPES – Finance code 001), National Council for Scientific and Technological Development (CNPq), Research Support Foundation of the State of Rio Grande do Sul (FAPERGS) and URI Erechim.

References

Association Official Analytical Chemist [AOAC]. (2005). *Official Methods of Analysis* (18th ed.). Gaithersburg, MD: AOAC International.

- Azizi-Shotorkhoft, A., Mohammadabadi, T., Motamedi, H., Chaji, M., & Fazaeli, H. (2016). Isolation and identification of termite gut symbiotic bacteria with lignocellulose-degrading potential, and their effects on the nutritive value for ruminants of some by-products. *Animal Feed Science and Technology*, 221, 234-242. doi: 10.1016/j.anifeedsci.2016.04.016.
- Bailey, M. J., Biely, P., & Poutanen, K. (1992). Interlaboratory testing of methods for assay of xylanase activity. *Journal of Biotechnology*, 23(3), 257-270. doi: 10.1016/0168-1656(92)90074-J.
- Borszcz, V., Boscato, T. R. P., Antunes, A., Zeni, J., Backes, G. T., & Valduga, E. (2017). Recovery of Pectinase Obtained by Solid-State Cultivation of Agro-Industrial Residues. *Industrial Biotechnology*, 13(3), 141-148. doi: 10.1089/ind.2017.0005.
- Brijwani, K., Oberoi, H. S., & Vadlani, P. V. (2010). Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochemistry*, 45(1), 120-128. doi: 10.1016/j.procbio.2009.08.015.
- Castro, A. M., & Pereira Júnior, N. (2010). Produção, propriedades e aplicação de celulases na hidrólise de resíduos agroindustriais. *Química Nova*, 33(1), 181-188. doi: 10.1590/S0100-40422010000100031.
- Eibinger, M., Bubner, P., Ganner, T., Plank, H., & Nidetzky, B. (2014). Surface structural dynamics of enzymatic cellulose degradation, revealed by combined kinetic and atomic force microscopy studies. *Federation of European Biochemical Societies Journal*, 281(1), 275-290. doi: 10.1111/febs.12594.
- Ghose, T. K. (1987). Measurement of cellulase activities. *Pure and Applied Chemistry*, 59(2), 257-268. doi: 10.1007/s12010-007-9064-0.
- Gupta, V. K., Kubicek, C. P., Berrin, J.-G., Wilson, D. W., Couturier, M., Berlin, A., ... Ezeji, T. (2016). Fungal enzymes for bio-products from sustainable and waste biomass. *Trends in Biochemical Sciences*, 41(7), 633-645. doi: 10.1016/j.tibs.2016.
- Han, X., Guo, Y., Liu, X., Xia, Q., & Wang, Y. (2019). Catalytic conversion of lignocellulosic biomass into hydrocarbons: a mini review. *Catalysis Today*, 319, 2-13. doi: 10.1016/j.cattod.2018.05.013.
- Hu, J., Jing, Y., Zhang, Q., Guo, J., & Lee, D.-J. (2017). Enzyme hydrolysis kinetics of micro-grinded maize straws. *Bioresource Technology*, 240, 177-180. doi: 10.1016/j.biortech.2017.02.078.
- Hultin, H. O., Sun, B., & Bulger, J. (1966). Pectin methyl esterases of the banana. Purification and properties. *Journal of Food Science*, 31(3), 320-327. doi: 10.1111/j.1365-2621.1966.tb00500.x.
- Isikgor, F. H., & Becer, C. R. (2015). Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. *Polymer Chemistry*, 6(25), 4497-4559. doi: 10.1039/C5PY00263J.
- Kumar, A. K., & Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresources and Bioprocessing*, 4(7), 1-19. doi: 10.1186/s40643-017-0137-9.
- Liu, J., Lu, J., & Cui, Z. (2011). Enzymatic hydrolysis of cellulose in a membrane bioreactor: assessment of operating conditions. *Bioprocess and Biosystems Engineering*, 34(5), 525-532. doi: 10.1007/s00449-010-0501-z.
- Martos, M. A., Zubreski, E. R., Combina, M., Garro, O. A., & Hours, R. A. (2013). Isolation of a yeast strain able to produce a polygalacturonase with maceration activity of cassava roots. *Food Science and Technology*, 33(2), 332-338. doi: 10.1590/S0101-20612013005000047.
- Menegol, D., Scholl, A. L., Fontana, R. C., Dillon, A. J. P., & Camassola, M. (2014). Potential of a *Penicillium echinulatum* enzymatic complex produced in either submerged or solid-state cultures for enzymatic hydrolysis of elephant grass. *Fuel*, 133, 232-240.
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426-428.
- Pitt, D. (1988). Pectin lyase from *Phoma medicaginis* var. pinodella. *Methods in Enzymology*, 161, 350-354.
- Sindhu, R., Binod, P., & Pandey, A. (2016). A novel sono-assisted acid pretreatment of chili post harvest residue for bioethanol production. *Bioresource Technology*, 213, 58-63.
- Sousa, N. M., Oliveira, J. S., Silva, D. S., Santos, E. M., Medeiros, A. N., Ramos, J. P. F., & Brito, E. A. (2018). Levels of neutral detergent fiber in diets with forage palm for dairy goats. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 70(5), 1595-1604.
- Statsoft Inc. (2008). *Statistica for Windows (data analysis software system), version 8.0*. Tulsa, OK: Statsoft, Inc.

- Sun, S., Sun, S., Cao, X., & Sun, R. (2016). The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresource Technology*, 199, 49-58.
- Takahashi, T., Sato, Y., Ito, K., & Mori, H. (2014). Effect of agitation speed on enzymatic saccharification of dry-pulverized lignocellulosic biomass. *Renewable Energy*, 62, 754-760.
- Van Kuijk, S. J. A., Sonnenberg, A. S. M., Baars, J. J. P., Hendriks, W. H., José, C., Rencoret, J., ... Cone, J. W. (2017). Chemical changes and increased degradability of wheat straw and oak wood chips treated with the white rot fungi *Ceriporiopsis subvermispota* and *Lentinula edodes*. *Biomass and Bioenergy*, 105, 381-391.
- Xu, X., Lin, M., Zang, Q., & Shi, S. (2018). Solid state bioconversion of lignocellulosic residues by *Inonotus obliquus* for production of cellulolytic enzymes and saccharification. *Bioresource Technology*, 247, 88-95.
- Yadav, S. K. (2017). Technological advances and applications of hydrolytic enzymes for valorization of lignocellulosic biomass. *Bioresource Technology*, 245, 1727-1739.