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FOOD TECHNOLOGY

Enzymatic hydrolysis of lignocellulosic residues and bromatological characterization for animal feed

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ABSTRACT: This study evaluated the action of commercial and non-commercial cellulases and pectinases in the hydrolysis of soybean hulls (SH) and corn stover and cobs (CSC), the effect of temperature and agitation on the lignocellulosic substrate hydrolysis and the bromatological characteristics of hydrolyzed substrates. The effect of pretreatment on the hydrolysis of lignocellulosic residues and bromatological analysis were also evaluated. The highest hydrolytic activity occurred at 300 rpm for SH (47.95 and 51.43% for cellulase and pectinase, respectively) and at 350 rpm for CSC (26.05 and 9.23% for cellulase and pectinase, respectively). Non-commercial enzymes achieved 7.26–30% of the amount of hydrolysis obtained with commercial enzymes, on the same substrates. Pretreatment with 7.5% of NaOH and a particle size of the substrate of 0.5 mm significantly increased the hydrolysis of SH and CSC for both enzymes. The bromatological characteristics showed that soybean hulls hydrolyzed with both commercial cellulase and pectinase have potential for large-scale use in animal feed production.
Key words: waste, corn stover and cobs, digestibility, enzyme, soybean hulls.

Hidrólise enzimática de resíduos lignocelulósicos e caracterização bromatológica para ração animal

RESUMO: Foram avaliadas a ação de celulases e pectinases comerciais e não comerciais na hidrólise de casca de soja (CS) e palha e espigas de milho (PEM), o efeito da temperatura e da agitação na hidrólise do substrato lignocelulósico e as características bromatológicas dos substratos hidrolisados. O efeito do pré-tratamento na hidrólise de resíduos lignocelulósicos e a análise bromatológica também foram avaliados. A maior atividade hidrolítica ocorreu a 300 rpm para CS (47,95 e 51,43% para celulase e pectinase, respectivamente) e a 350 rpm para PEM (26,05 e 9,23% para celulase e pectinase, respectivamente). As enzimas não comerciais atingiram 7,26-30% da quantidade de hidrólise obtida com as enzimas comerciais, nos mesmos substratos. O pré-tratamento com 7,5% de NaOH e um tamanho de partícula do substrato de 0,5 mm aumentou significativamente a hidrólise de CS e PEM para ambas as enzimas. As características bromatológicas mostraram que a casca de soja hidrolisada com celulase e pectinase comercial tem potencial para uso em larga escala na produção de ração animal.
Palavras-chave: resíduos, palha e espigas de milho, digestibilidade, enzima, cascas de soja.

INTRODUCTION

Ruminants are able to take advantage of low-quality substrates due to the synthesis and secretion of cellulolytic and hemicellulolytic enzymes by microorganisms in their rumen (LEO et al., 2019). However, the conversion of lignocellulosic residues by livestock for the production of meat and milk is inefficient, indicating the need for new biotechnological approaches to animal feed in order to maximize the use of nutrients (GRAMINHA et al., 2008). Feeding roughage to pre-ruminant calves has been adopted to promote their development and allow for an earlier diet. Solid fiber-based food results in marked increases in the rumen-reticulum and omasum (CAETANO JUNIOR et al., 2016) and, when previously digested, can improve the energy gain obtained from this feed. Studies indicated that fibrolytic enzymes can act directly on fiber before consumption or increase the degradation of dry matter and fiber in the rumen (YUANGKLANG et al., 2017), increasing milk production or weight gain of cattle after ingestion of lignocellulosic raw materials (GRAMINHA et al., 2008).

The requirement of multiple enzymes to hydrolyze all types of carbohydrates present in the biomass relied on effective biomass pretreatment and optimal mixtures of multiple enzyme activities. Usually, these enzymes are produced from different sources and then blended into cocktails and evaluated

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for hydrolysis effectiveness for particular biomass (LI et al., 2018).

Selection of enzymes/proteins, chemicals for the preparation of cocktail required prior knowledge of the performance of non-catalytic proteins and enzymes or other activators in real-time biomass hydrolysis (ADSUL et al., 2020). Enzyme hydrolysis has been accepted as the most environmentally friendly technology for the conversion of carbohydrate in biomass into monomeric sugars. An enzyme mixture having multiple activities is required to achieve more complete hydrolysis of the complex carbohydrate present in lignocellulosic biomass (RODRIGUES & ODANETH, 2021; LI et al., 2018; SUWANNARANGSEE et al., 2012).

Cellulases are generally used for the enzymatic hydrolysis of lignocellulosic residues and are an enzyme consortium comprising at least three major groups: endoglucanases, exoglucanases and β-glucosidases (MÜLLER et al., 2021; PAYNE et al., 2015). Pectinases are responsible for the degradation of long and complex molecules called pectins, which occur as structural polysaccharides in the middle lamella and primary plants cell walls (KOHLI & GUPTA, 2015), and usually are used in enzyme cocktails along with cellulases.

The release of reducing sugars can be significantly increased by using cellulase in different agro-industrial substrates such as wheat straw (COIMBRA et al., 2016).

SONG et al. (2016) observed better results of corncob, corn stover, and rice straw hydrolysis employing combined cellulase and xylanase compared to their isolated use. The cellulase and pectinase combined use in the soybean hulls hydrolysis has also been reported (ROJAS et al., 2014).

The agricultural biomass is composed of natural fibers referred to as lignocellulosic fibers which mainly consist of cellulose, hemicellulose, and lignin (BAJPAI, 2016). The highly crystalline structure of native cellulose is stabilized by a strong network of hydrogen bonds (AHMADZADEH et al., 2018; JIA et al., 2013). Therefore, and also because of the lignin present in the cell wall, they form a barrier against the attack of enzymes, making it difficult to obtain high yields from the saccharification of lignocellulosic biomass without pre-treatment. Thus, the development of an enzyme cocktail for the hydrolysis of cellulose is one of the main research platforms in biomass conversion (ADSUL et al., 2020). The appropriate combination of these activities is what determines the efficiency of saccharification though it varies with the type of biomass to be pretreated (KHARE & LAROCHE, 2015).

There are few studies in the literature reporting hydrolysis of soybean hulls and, mainly, corn stover and cobs to improve chemical characteristics, comparing the use of commercial and non-commercial enzymes.

Therefore, this study evaluated the commercial and non-commercial cellulases and pectinases action in the hydrolysis of lignocellulosic substrates (SH and CSC) and their effects on the bromatological characteristics of hydrolyzed substrates for use as animal feed.

MATERIALS AND METHODS

The substrates used were soybean hulls (SH) and corn stover and cobs (CSC), both supplied by local farmers from the Rio Grande do Sul, Brazil in the 2018 harvest. The substrates have been stored at room temperature and used without any pre-treatment. The commercial enzymes used were powder cellulase (Sigma-Aldrich® CAS: 9012-54-8) and liquid pectinase (Rohapect DA6L® - AB Enzymes CAS: 9025-98-3) both produced by *Aspergillus niger*.

Non-commercial cellulase was produced using *Trichoderma reesei* NRRL 3652. The culture was performed in a solid-state using soybean hulls as substrate, at pH 4.6, temperature 30 °C, moisture content 70%, and $1x10^7$ spores/g. The cellulase was extracted by adding sodium citrate buffer (0.5 M, pH 5.5) in the ratio 1:15 (substrate:buffer, w:w) and incubated for 30 min at 50 ºC and 100 rpm.

Non-commercial pectinase was obtained by fermentation in a solid-state culture with *Aspergillus niger* ATCC 9642. The culture medium contained orange peel, wheat bran and maize steep-water (8:1:1, w:w:w), and was kept at 30 °C, 65% moisture content, and $5x10^6$ spores/g on a wet basis. The extraction was made with NaCl (0.1 mol/L), with a solvent:substrate ratio of 5:1 (v:w), and incubated for 30 min at 20 °C and 175 rpm.

Determination of the enzymatic activity

All enzyme activity determinations (Exo-PG, PME, PL, FPase, Xylanase, CMCase and Avicelase) were made using the methodologies cited by TEIXEIRA et al. (2019).

Exo-Polygalacturonase (Exo-PG): One exo-PG activity unit was defined as the amount of enzyme that releases 1 mmol of D-galacturonic acid per minute of reaction (U = μmol min^{-1}) from citrus pectin under the test conditions, according to a standard curve (0.1 – 10 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 mL^{-1}).

Pectin Methylesterase (PME): One PME unit was defined as the amount of enzyme able to catalyze pectin demethylation corresponding to 1 µmol NaOH min^{-1} mL^{-1} consumption, under the conditions described on the assay.

Pectin Lyase (PL): 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 (FPase): 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: 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 (CMCase): One CMCase activity unit was defined as the amount of enzyme that releases 1 µmol of reducing sugars equivalent to glucose per minute.

Avicelase: 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 et al., 2014). The reducing sugars released were determined by the dinitrosalicylic acid method cited to TEIXEIRA et al. (2019), 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.

Evaluation of lignocellulosic substrate hydrolysis

Enzymatic substrates saccharification without pretreatment were performed as described by LIU et al. (2011), with some modifications. Assays were performed with 2 g of SH or CSC and autoclaved for 15 min at 121°C. Then, 100 mL of reaction mixture containing 95 mL of 0.05 M sodium citrate buffer at pH 5.0 and 5 mL of commercial cellulolytic (6.35 U g^{-1}) or pectinolytic (290 U mL^{-1}) enzymatic extract, or non-commercial, were added to each substrate. The reactions were carried out in an orbital shaker (Ethiktechnology/Incubator 430) at 150 rpm and 37°C. Commercial cellulase was diluted to 1:100 (w:v) in citrate buffer at pH 5.0, whereas commercial pectinase enzyme and raw extracts (non-commercial cellulase and pectinase) were not diluted. The release of total reducing sugars from the hydrolysis was estimated using the DNS (dinitrosalicylic acid) method. The saccharification percentage was calculated according to equation 1.

$$Saccharification\ (\%) = \frac{RS\ x\ 0.9\ x\ 100}{P} \quad (1)$$

Where: RS = released sugar; 0.9 = correction factor; P = polysaccharides in lignocellulosic substrate (0.0505 for soybean hulls (BRIJWANI et al., 2010) and 0.584 for corn stover and cobs (CRUZ, 1992).

Hydrolysis kinetics of lignocellulosic substrates (SH and CSC) using commercial and non-commercial cellulases and pectinases were measured every 2 hours for 24 h.

To evaluate the enzyme dilution influence on the hydrolysis of lignocellulosic substrates (SH and CSC), assays were performed at different dilutions of commercial cellulase: 1:50; 1:75; 1:100; 1:125; 1:150; 1:175 and 1:200 (g enzyme:mL citrate buffer, pH 5.0), at 150 rpm and 37 °C.

In order to evaluate the temperature and agitation effect on the hydrolysis of lignocellulosic substrates (SH and CSC), a 2^2 factorial design experiment (Central Composite Rotary Design – CCRD) was carried out. The independent variables for cellulase and pectinase were temperature of 29–45 °C (X_1) and agitation of 100–200 rpm (X_2). The variables reaction time and enzymes dilution were kept fixed, as established on previous assays. The dependent variable (response) was the percentage of hydrolysis of lignocellulosic residues.

The effect of increasing the agitation on the hydrolysis of SH and CSC was evaluated by varying the agitation between 100 and 200 rpm, at 45 °C, with all other variables fixed.

To evaluate the interactions between pectinases and cellulases effect in the hydrolysis of SH and CSC, proportions of the different enzymes used in the hydrolysis reaction were combined. The mixtures of cellulases and pectinases used were 100/0, 75/25, 50/50, 25/75 and 0/100 (v/v), respectively. The variables reaction time, dilution, temperature and agitation were fixed as the best results obtained.

The effect of pretreatment on the hydrolysis of lignocellulosic residues

In order to verify whether an increase in the residue surface area influences enzyme activity, SH and CSC were milled into particle sizes of 0.5, 1.0 and 1.5 mm, prior to commercial cellulase enzyme treatment.

The alkaline pretreatment effect of lignocellulosic residues (SH and CSC) on hydrolysis with commercial cellulase was evaluated using commercial sodium hydroxide at 1, 3 and 5% at 100°C for 30, 60 and 90 min. The materials recovered after

boiling were washed with distilled water and dried in an oven at 60 °C to recover the initial moisture content of the residue.

To evaluate the combination of particle size and alkali pretreatment effects, the lignocellulosic substrates (SH and CSC) ground into a particle size of 0.5 mm were treated with sodium hydroxide at 3, 5, 7.5 and 10%, at 100 °C for 90 min, and subjected to hydrolysis with commercial cellulase and pectinase under the optimum conditions of temperature, time and agitation optimized from the previous experiments.

Bromatological analysis

Under the optimal conditions of time, temperature and agitation for lignocellulosic residues hydrolysis with commercial and non-commercial cellulase and pectinase, bromatological analysis was performed of neutral detergent fiber (NDF), acid detergent fiber (ADF), total digestible nutrients (TDN). The tests were conducted using the Reflectance Near-Infrared Spectrophotometry EFQ 49 (FOSS/ Denmark) (700 – 2500 nm) (KONG et al., 2005). The total digestible nutrients (TDN) were calculated by equation 2. 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 control. All the analyzes are described in Brazilian Compendium of Animal Feeding.

$$TDN = 87.84 - (ADF * 70) \quad (2)$$

Where: TDN= total digestible nutrients and ADF= acid detergent fiber.

Statistical analysis

The results of time, enzyme dilution, interactions between pectinases and cellulases and pretreatment on the hydrolysis of lignocellulosic residues (in triplicate) were statistically processed by analysis of variance (ANOVA) and the differences in average were compared by Tukey or Students tests using Statistica software (StatSoft Inc., EUA, version 5.0), at 95% significance level ($P < 0.05$). The results of temperature and agitation effect on the hydrolysis of lignocellulosic substrates obtained in the factorial design were statistically analyzed according to the experimental design methodology, using the same software.

RESULTS AND DISCUSSION

Hydrolysis of lignocellulosic substrates with commercial enzymes

The most effective hydrolysis was observed after 22 hours for SH and 14 hours for the CSC with commercial cellulase, and 22 hours for both substrates with commercial pectinase (Table 1). These reaction times were fixed and used in other experiments. A short reaction time is not enough to degrade both the amorphous fraction and the crystalline cellulose fraction. The hydrolysis time should be sufficient to ensure complete pulp degradation without encountering enzyme deactivation. When evaluating the commercial cellulase dilution effect on the substrates hydrolysis, 23.7 and 13.2% hydrolysis was obtained in SH and CSC, respectively, in higher evaluated concentration (2 g/100mL), corroborating the results obtained by MENEGOL et al. (2014). This concentration was; therefore, used for the other experiments.

There was a linear increase in the hydrolysis of SH and CSC by commercial cellulase with increasing enzyme concentration (SH: $y = 12.35x + 1.906$, $R^2 = 0.93$; CSC: $y = 6.377x + 1.171$, $R^2 = 0.97$). This assessment was not carried out for commercial pectinase due to the lower activity.

The 2^2 factorial design (Table 2) indicated that the highest hydrolysis percentages were obtained when the higher agitation (200 rpm) was used. The effect of agitation on the hydrolysis of SH and CSC with cellulase and pectinase is shown in figure 1.

The interaction effect between commercial pectinases and cellulases in the hydrolysis of lignocellulosic residues (Table 3) demonstrates that the best hydrolysis for SH is obtained with 100% cellulase or pectinase (50.2% of hydrolysis), while for CSC the best condition was with 100% cellulase only (26.2% of hydrolysis). Soybean hull consists mainly of three major plant carbohydrates, i.e., cellulose, hemicellulose and pectin (LI et al., 2017). This could be due the fractions of soluble carbohydrates in neutral detergent: pectin accounts for the largest fraction (62%), while starch (19%) and simple sugars (19%) are present in smaller proportions in the soybean hulls. With 75/25; 50/50 and 25/75 % cellulase/pectinase ratio for SH hydrolysis, the results were similar (around 43%), but significantly lower ($P < 0.05$) than percentages of hydrolysis obtained with 100% cellulase or pectinase.

For the CSC residue, using the same enzymes proportions, a reduction in the hydrolysis with an increase of the pectinase concentration was verified. Among the various factors that affect the enzymatic cellulose hydrolysis are agitation and temperature of reactions. Agitation had a significant positive effect ($P < 0.05$) on the hydrolysis of SH with cellulase (Figure 1a) and pectinase (Figure 1b). For CSC, there was a positive effect of agitation and

Table 1 - Influence of time (h) on the hydrolysis of soybean hull (SH) and corn stover and cobs (CSC) with commercial cellulase and pectinase.

Time (h)	Hydrolysis (%)			
	Commercial Cellulase		Commercial Pectinase	
	SH	CSC	SH	CSC
0.1	$7.67^{k} \pm 0.01$	$2.06^{f} \pm 0.22$	$1.10^{f} \pm 0.01$	$0.88^{g} \pm 0.02$
2	$8.38^{ijk} \pm 0.01$	$4.55^{e} \pm 0.47$	$8.70^{e} \pm 0.70$	$1.38^{f} \pm 0.16$
4	$9.18^{ij} \pm 0.05$	$5.08^{e} \pm 0.01$	$12.70^{d} \pm 0.10$	$1.42^{f} \pm 0.02$
6	$10.94^{fgh} \pm 0.26$	$6.93^{d} \pm 0.02$	$16.20^{c} \pm 1.27$	$1.98^{e} \pm 0.12$
8	$11.57^{ef} \pm 0.21$	$7.19^{cd} \pm 0.40$	$18.97^{b} \pm 0.40$	$3.28^{d} \pm 0.18$
10	$12.12^{de} \pm 0.09$	$7.43^{cd} \pm 0.01$	$20.67^{b} \pm 0.06$	$3.29^{d} \pm 0.07$
12	$12.72^{cd} \pm 0.01$	$7.76^{c} \pm 0.01$	$20.70^{b} \pm 0.81$	$3.05^{d} \pm 0.06$
14	$12.77^{cd} \pm 0.14$	$9.98^{a} \pm 0.10$	$20.30^{b} \pm 0.10$	$3.99^{c} \pm 0.08$
16	$13.22^{c} \pm 0.07$	$8.76^{b} \pm 0.05$	$19.87^{b} \pm 0.15$	$3.63^{cd} \pm 0.10$
18	$15.04^{b} \pm 0.75$	$9.03^{b} \pm 0.12$	$19.67^{b} \pm 0.61$	$3.91^{c} \pm 0.19$
20	$15.48^{b} \pm 0.97$	$9.15^{b} \pm 0.25$	$20.00^{b} \pm 0.60$	$4.72^{b} \pm 0.18$
22	$17.10^{a} \pm 0.57$	$8.75^{b} \pm 0.05$	$26.17^{a} \pm 0.96$	$5.20^{a} \pm 0.08$
24	$15.41^{b} \pm 0.09$	$8.39^{b} \pm 0.35$	$25.27^{a} \pm 0.15$	$5.21^{a} \pm 0.37$

Means ± standard deviation, followed by the same letter in the columns do not differ statistically from each other by the Tukey test at the 95% probability level.

temperature on the action of cellulase (Figure 1c), whereas for pectinase, only agitation was significant (Figure 1d). There was a positive agitation effect on commercial cellulase and pectinase hydrolysis activity, with the highest percentages of hydrolysis at 300 rpm for SH and at 350 rpm for CSC (Figure 2). The hydrolysis of SH by commercial cellulase was higher than that reported by ROJAS et al. (2014) with the same substrate and enzyme.

Different enzymes groups acting synergistically may be more effective in the breakdown of complex polysaccharides (REIS et al., 2014). The simplest way of making a cocktail is to mix the two or three crude enzyme preparations from different sources that vary in the amount and type of cellulolytic enzymes. There are three main reasons for cocktail development: (i) to reduce the amount of enzymes required for biomass hydrolysis, (ii) to convert all carbohydrate into fermentable sugars within a short period and (iii) to work at high substrate loading (ADSUL et al., 2020).

In this study, the highest hydrolysis of CSC was obtained with the highest percentages of cellulase. However, the results are low overall

Table 2 - Factorial design matrix 2^2 (real and coded values) for soybean hulls (SH) and corn stover and cobs (CSC) hydrolysis using commercial cellulase and pectinase, at different temperatures and agitations.

Assays	Independent Variables*		SH Hydrolysis (%)		CSC Hydrolysis (%)	
	Temperature (ºC)	Agitation (rpm)	Cellulase	Pectinase	Cellulase	Pectinase
1	29 (-1)	100 (-1)	14.34	28.34	5.40	3.08
2	29 (-1)	200 (1)	31.83	29.60	8.01	6.04
3	45 (1)	100 (-1)	26.19	26.70	11.20	3.55
4	45 (1)	200 (1)	32.08	29.65	15.62	6.18
5	37 (0)	150 (0)	25.73	31.88	6.64	5.60
6	37 (0)	150 (0)	23.01	31.63	7.48	5.40
7	37 (0)	150 (0)	21.46	32.12	7.68	5.50

*Fixed independent variables: cellulase 1:50 dilution (w:v) and pectinase without dilution; 22 hours of reaction for soybean hulls and 14 hours for corn stover and cobs.

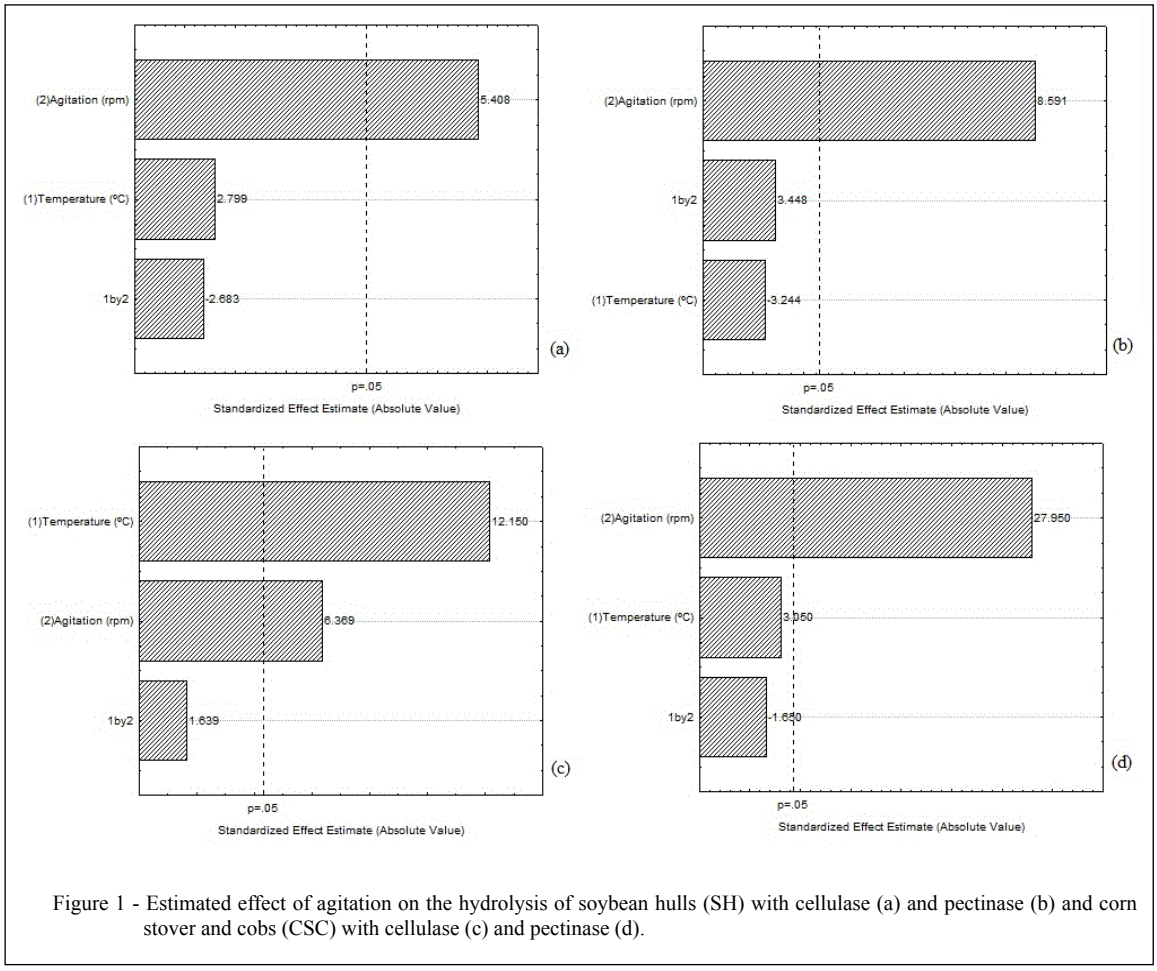

Figure 1 - Estimated effect of agitation on the hydrolysis of soybean hulls (SH) with cellulase (a) and pectinase (b) and corn stover and cobs (CSC) with cellulase (c) and pectinase (d).

when compared to those obtained by CHEN et al.(2007) with cellulase from *Trichoderma reesei* (12 U cellobiase activity) of 79% yield. For SH, higher hydrolysis values were obtained than for CSC, both with pectinase and cellulase. A reduction of approximately 13% hydrolysis was observed in different mixing ratios evaluated. This result may be due to a large pectin quantity in soybean hulls, which also contain cellulose in significant quantities (39.7%) (CASSALES et al., 2011). Soybean hulls pectin corresponds to 62.4% of non-fiber carbohydrates, which is equivalent to 8.8% of dry matter. Similar results were obtained by ROJAS et al. (2014), whereby 55% hydrolysis of SH was reached under the conditions of 50°C, pH 4.8, 200 rpm and 20 U cellulase/g. When the authors supplemented the medium with beta-glucosidase at 120 U/g and pectinase 1% w/w in order to increase the percentage of hydrolysis, the conversion remained similar (49–55%) (ROJAS et al., 2014).

Table 3 - Effect of the interaction between commercial cellulase and pectinase on the hydrolysis of lignocellulosic substrates (soybean hull and corn stover and cobs).

Residue	----------Cellulase/Pectinase Mixture (%)----------				
	100/0	75/25	50/50	25/75	0/100
SH	$50.2^{aA} \pm 4.8$	$44.3^{bA} \pm 4.0$	$43.0^{bA} \pm 3.9$	$43.7^{bB} \pm 4.0$	$50.2^{aA} \pm 4.6$
CSC	$26.2^{aC} \pm 2.4$	$24.4^{bC} \pm 2.2$	$17.7^{cB} \pm 1.6$	$9.9^{dC} \pm 8.12$	$2.5^{eD} \pm 0.2$

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

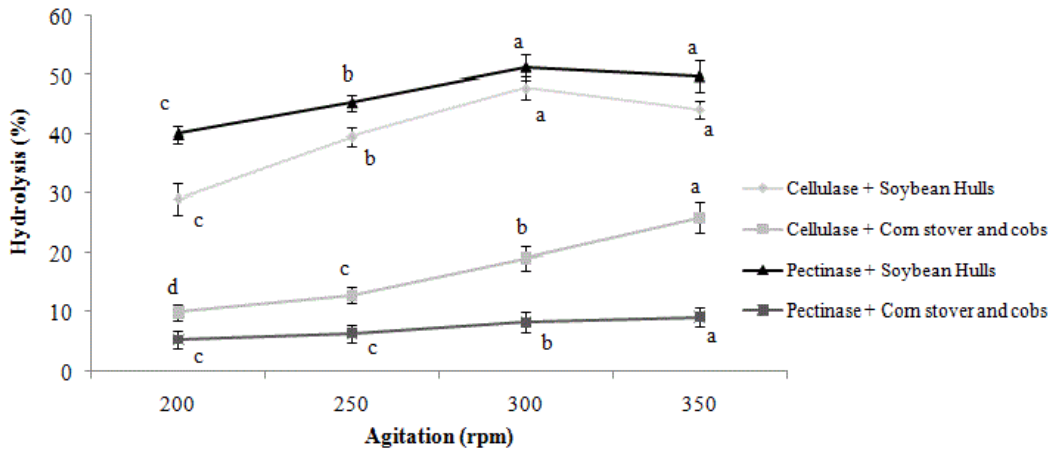

Figure 2 - Influence of agitation on hydrolysis of soybean hulls or corn stover and cobs with commercial cellulase or pectinase. [Different letters indicate significant differences according to a Tukey test ($P < 0.05$) for each treatment.

Hydrolysis of lignocellulosic substrates using non-commercial enzymes

Hydrolysis of CSC with non-commercial enzymes was only 17.16 and 7.26% of the total obtained with the commercial cellulase and pectinase, and for SH the hydrolysis was 20.71 and 29.7%, respectively (Table 4). In the crude extract of non-commercial cellulase, activity of FPase, xylanase, CMCase and avicelase was determined, with values of 6.02, 972.74, 7.76 and 2.02 U/g, respectively. In the non-commercial pectinase crude extract, activity of polygalacturonase (PG), pectin methylesterase (PME) and pectin lyase (PL) was determined, with values of 1.83, 3.80 and 29.00 U/g, respectively. Hydrolysis of SH and CSC by non-commercial cellulase and pectinase is low in comparison to commercial enzymes. However, these results may still be relevant, since the cost of obtaining non-commercial enzymes is much lower than that of commercial enzymes.

However, the results are promising when compared to those obtained by YANG et al. (2018), that showed P33 enzyme cocktail acted synergistically with a commercial cellulase to promote the hydrolysis of delignified corn stover, resulting in significant increases in cellulose and hemicellulose conversion without increasing overall cellulase loading.

The effect of pretreatment on the hydrolysis of lignocellulosic residues

The hydrolysis of substrates with a grain size of 5 mm to 0.5 mm using commercial cellulase ranged from 47.95 to 49.92% and 25.86 to 26.87% with increments of 4.1 and 0.8% compared to the unmilled substrate for soybean hulls and corn stover

Table 4 - Comparison of hydrolysis of lignocellulosic substrates (SH and CSC) using commercial and non-commercial cellulase and pectinase.

Residue	Commercial	Non-commercial	P*	% Hydrolysis**
----------Cellulase----------				
SH	47.95 ± 0.15	9.93 ± 0.62	< 0.01	20.71
CSC	26.05 ± 0.14	4.47 ± 0.22	< 0.01	17.16
----------Pectinase----------				
SH	51.4 ± 1.75	15.27 ± 0.96	< 0.01	29.7
CSC	9.23 ± 0.75	0.67 ± 0.05	< 0.01	7.26

*Student t test. **Percentage of non-commercial/commercial hydrolysis.

and cobs, respectively, without statistical difference (P > 0.05) between treatments.

The isolated treatment effect with 1–5% NaOH and duration of 30–90 min produced 48.8–49.3% hydrolysis for soybean hulls and 26.8–27.4% hydrolysis for corn stover and cobs, without statistical difference (P > 0.05) between treatments. The combined treatment of 0.5 mm particle size and 7.5% NaOH produced hydrolysis yields with cellulase and pectinase of 73.08 and 59.52% for soybean hulls and 34.4 and 17.7% for corn stover and cobs (Table 5).

In the pretreatments that reduce the cellulose DP, e.g., dilute acid hydrolysis, chains with different sizes are formed, including soluble and insoluble cellulosic polymers and oligomers AHUJA et al., 2018; KARIMI & TAHERZADEH, 2016). MENEGOL et al. (2014) indicated that sodium hydroxide pretreatment was more effective at lignin removal and the release of reducing sugars and glucose from elephant grass biomass.

Bromatological characterization of lignocellulosic residues

Neutral detergent fiber (NDF) estimates the content in cellulose, hemicellulose, lignin, cutin and insoluble minerals in the cell wall, and is determined as being the residue remaining after extraction with the neutral detergent solution (made up of sodium lauryl sulphate and EDTA). Acid detergent fiber (ADF) is an estimator of the content in cellulose, lignin, cutin and insoluble minerals in the cell wall and it is determined as the residue remaining after the digestion of the sample with an acid detergent solution.

The difference between NDF and ADF is the fraction of hemicellulose. With the ADF method the hemicellulose is hydrolyzed so that the determination of ADF is more closely associated with degradability and digestibility, whereas the NDF content is only related to ingestion or to a fraction of fiber still highly usable by the organism. There is the negative correlation existing between the content of NDF and ADF with the digestibility of vegetable products (OBREGÓN-CANO et al., 2019).

Results from the fiber measurements, neutral detergent fiber (NDF), acid detergent fiber (ADF) total digestible nutrients (TDN) and nitrogen free extract (NFE) are shown in table 6. The treatments reduced the NDF and FDA percentage in CSC and increased the percentage of total digestible nutrients (TDN) and nitrogen free extract (NFE) in the evaluated residues, especially when commercial cellulase and pectinase were used.

This result is positive, because the lower the NDF percentage, which is the nutritional fraction of hemicellulose, cellulose and lignin, the better the nutritional value of animal feed. For SH, both the NDF and ADF decreased with enzymatic treatment, and the best results were produced by commercial and non-commercial pectinase. According to IPHARRAGUERRE & CLARK (2003), good degradation of NDF occurs in diets containing soybean hulls due to its chemical composition, which is high in cellulose and hemicellulose and low in lignin. Analyses for CSC demonstrated that the enzymes used reduced the percentage of NDF and ADF to values of 20% and 17% respectively, which were lower than the untreated residue. For CSC, the NDF and ADF reduction percentages were lower when treated with non-commercial cellulase and pectinase. These results are important because neutral detergent fiber is a good indicator of potential food consumption in ruminant animals, where an increase in consumption corresponds to the lowest percentage of NDF.

Treatment with cellulase and pectinase improved the percentage of total digestible nutrients (TDN) and nitrogen free extract (NFE) in

Table 5 - Hydrolysis of lignocellulosic substrates (CS and SPM) without pretreatment and ground in 0.5 mm granulometry, treated with NaOH (3, 5, 7.5 and 10%) at a temperature of 100ºC for 90 min, applying the enzymes cellulase and pectinase commercial.

Pretreatment	----------Soybean Hulls Hydrolysis (%)----------		----------Corn stover and cobs Hydrolysis (%)---------	
	Cellulase	Pectinase	Cellulase	Pectinase
Without pretreatment	$49.92^{cA} \pm 3.87$	$51.40^{bA} \pm 3.28$	$26.07^{cB} \pm 2.16$	$9.2^{dC} \pm 0.56$
3% NaOH	$50.06^{cA} \pm 2.16$	$52.55^{bA} \pm 2.85$	$30.20^{bB} \pm 2.32$	$11.6^{cC} \pm 0.95$
5% NaOH	$55.55^{bA} \pm 2.56$	$52.2^{bA} \pm 2.80$	$30.30^{bB} \pm 2.52$	$15.0^{bC} \pm 0.98$
7.5% NaOH	$73.08^{aA} \pm 4.58$	$59.52^{aB} \pm 3.02$	$34.40^{aC} \pm 2.68$	$17.7^{aD} \pm 1.10$
10% NaOH	$72.8^{aA} \pm 4.52$	$58.71^{aB} \pm 4.06$	$34.00^{aC} \pm 2.49$	$17.9^{aD} \pm 1.25$

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.

Table 6 - Bromatological analysis of lignocellulosic residues with commercial and non-commercial cellulase and pectinase.

Treatment	NDF (g/100 g)	%	ADF (g/100 g)	%	TDN (g/100 g)	%	NFE (g/100g)	%
----------Soybean Hulls----------								
Without treatment	76.28	0	53.75	0	29.54	0	43.38	0
Commercial Celulase	60.36	-20.9	46.16	-14.1	35.67	20.8	46.61	7.4
Non-commercial Celulase	61.54	-19.3	48.43	-9.9	34.52	16.9	44.09	1.6
Commercial Pectinase	59.26	-22.3	41.47	-22.8	37.1	26.3	47.54	9.6
Non-commercial Pectinase	67.01	-12.2	43.35	-19.4	33.18	12.3	43.98	1.4
----------Corn stover and cobs----------								
Without treatment	81.36	0	49.60	0	53.12	0	46.93	0
Commercial Celulase	64.37	-20.9	41.18	-16.9	73.01	37.4	71.33	51.9
Non-commercial Celulase	67.87	-16.6	42.33	-14.7	61.01	14.8	53.28	13.5
Commercial Pectinase	64.55	-20.7	40.87	-17.6	63.58	19.7	68.93	46.9
Non-commercial Pectinase	69.52	-14.6	43.34	-12.6	61.00	14.8	52.98	12.9

the evaluated residues, especially when using the commercial enzymes. The results of TDN and NFE obtained using commercial cellulase in corn stover and cobs showed the highest increase in percentage compared to the untreated residue (37.44% and 51.99%, respectively). TEIXEIRA et al. (2019) evaluated the action of cellulase and commercial and non-commercial pectinase in the hydrolysis of rice husk and Tifton 85 hay. The bromatological analysis showed that the use of these enzymes improved the percentage of total digestible nutrients (NDT) and non-nitrogenous extracts (NNE). Commercial cellulase showed the best results for rice husks in relation to NDT (61.94) and NNE (69.57). TDN is one of the most widely used methods of evaluating the food energy content for ruminants. Many chemicals are related to the concentration of energy available, and the commonly evaluated constituents are digestible crude protein, digestible ether extract, digestible neutral detergent fiber (corrected for ash and protein) and digestible non-fibrous carbohydrates (ROCHA et al., 2003).

CONCLUSION

Hydrolysis was highest under the highest agitation, revealing the importance of this parameter for the hydrolysis efficiency of SH and CSC. Both commercial cellulase and pectinase showed good hydrolytic efficiency in soybean hulls due to the large quantity of pectin and cellulose in its composition, and may improve the quality of SH in animal feed by reducing the NDF and ADF. Hydrolysis with non-commercial cellulase and pectinase was lower than with commercial enzymes, but more effective in SH than in CSC. Smaller particle size and pretreatment with NaOH caused a significant increase in hydrolysis of both substrates with both enzymes. The best hydrolysis results were obtained with pretreatment with 7.5% NaOH and 0.5 mm particle size independent of the substrate using commercial enzyme. The best digestibility results were obtained using commercial pectinase for SH and commercial cellulase for CSC.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

ADSUL, M., et al. Designing a cellulolytic enzyme cocktail for the efficient and economical conversion of lignocellulosic

biomass to biofuels. **Enzyme and Microbial Technology**, v.133, p.109442, 2020. Available from: <https://doi.org/10.1016/j.enzmictec.2019.109442>. Accessed: Jun. 07, 2021.

AHMADZADEH, S., et al. Effect of electrohydrodynamic technique as a complementary process for cellulose extraction from bagasse: Crystalline to amorphous transition. **Carbohydrate Polymers**, v.188, p.188-196, 2018. Available from: <https://doi.org/10.1016/j.carbpol.2018.01.109>. Accessed: Jun. 07, 2021.

AHUJA, D., et al. Simultaneous extraction of lignin and cellulose nanofibrils from waste jute bags using one pot pre-treatment. **International Journal of Biological Macromolecules**, v.107 (Part A), p.1294-1301, 2018. Available from: <https://doi.org/10.1016/j.ijbiomac.2017.09.107>. Accessed: Jun. 07, 2021.

BAJPAI, P. Structure of Lignocellulosic Biomass. In:_____ **Pretreatment of Lignocellulosic Biomass for Biofuel Production, Springer Briefs in Molecular Science**. Springer, Singapore, 2016, p.7-12.

BRIJWANI, K., et al. Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. **Process Biochemistry**, v.45, n.1, p.120-128, 2010. Available from: <https://doi.org/10.1016/j.procbio.2009.08.015>. Accessed: Jun. 07, 2021.

CAETANO JUNIOR, M. et al. A influência da dieta no desenvolvimento ruminal de bezerros. **Nutritime**, v.13, n.6, p.4902-4918, 2016. Accessed: Jun. 20, 2022.

CASSALES, A., et al. Optimization of soybean hull acid hydrolysis and its characterization as a potential substrate for bioprocessing. **Biomass & Bioenergy**, v.35, n.11, p.4675-4683, 2011. Available from: <https://doi.org/10.1016/j.biombioe.2011.09.021>. Accessed: Jun. 07, 2021.

CHEN, M., et al. Enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. **International Biodeterioration and Biodegradation**, v.59, n.2, p.85-89, 2007. Available from: <https://doi.org/10.1016/j.ibiod.2006.07.011>. Accessed: Jun. 07, 2021.

COIMBRA, M. C., et al. Sugar production from wheat straw biomass by alkaline extrusion and enzymatic hydrolysis. **Renewable Energy**, v.86, p.1060-1068, 2016. Available from: <https://doi.org/10.1016/j.renene.2015.09.026>. Accessed: Jun. 07, 2021.

CRUZ, G. M. Utilização dos restos de culturas e palhas na alimentação de ruminantes. In:____**Utilização de Subprodutos Agroindustriais e Resíduos de Colheita na Alimentação de Ruminantes**. Embrapa, São Carlos, 1992, p.99-121.

GRAMINHA, E. B. N., et al. Enzyme production by solid-state fermentation: Application to animal nutrition. **Animal Feed Science and Technology**, v.144, p.1-22, 2008. Available from: <https://doi.org/10.1016/j.anifeedsci.2007.09.029>. Accessed: Jun. 07, 2021.

IPHARRAGUERRE, I. R.; CLARK, J. H., Soyhulls as an alternative feed for lactating dairy cows: A review. **Journal of Dairy Science**, v.86, n.4, p.1052-1073, 2003. Available from: <https://doi.org/10.3168/jds.S0022-0302(03)73689-3>. Accessed: Jun. 07, 2021.

JIA, X., et al. Preparation and Characterization of Cellulose Regenerated from Phosphoric Acid. **Journal of Agricultural and Food Chemistry**, v.61, n.50, p.12405-12414, 2013. Available from: <https://doi.org/10.1021/jf4042358>. Accessed: Jun. 07, 2021.

KARIMI, K.; TAHERZADEH, M. J. A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. **Bioresource Technology**, v.203, p.348–356, 2016. Available from: <https://doi.org/10.1016/j.biortech.2015.12.035>. Accessed: Jun. 07, 2021.

KHARE, S. K. A.; LARROCHE, C. Current perspectives in enzymatic saccharification of lignocellulosic biomass. **Biochemical Engineering Journal**, v.102, p.8-44, 2015. Available from: <https://doi.org/10.1016/j.bej.2015.02.033>. Accessed: Jun. 07, 2021.

KOHLI, P.; GUPTA, R. Alkaline pectinases: A review. **Biocatalysis and Agricultural Biotechnology**, v.4, n.3, p.279-285, 2015. Available from: <https://doi.org/10.1016/j.bcab.2015.07.001>. Accessed: Jun. 07, 2021.

KONG, X., et al. Rapid prediction of acid detergent fiber, neutral detergent fiber, and acid detergent lignin of rice materials by near-infrared spectroscopy. **Journal of Agricultural and Food Chemistry**, v.53, p.2843-2848, 2005. Available from: <https://doi.org/10.1021/jf047924g>. Accessed: Jun. 07, 2021.

LEO, V. V., et al. Chapter 4 - Microorganisms as an Efficient Tool for Cellulase Production: Availability, Diversity, and Efficiency. In: SRIVASTAVA, N., et al. **New and Future Developments in Microbial Biotechnology and Bioengineering**. Elsevier, 2019, p.45-61. Available from: <http://doi.org/10.1016/B978-0-444-64223-3.00004-7>. Accessed: Jun. 07, 2021.

LI, Q., et al. Leveraging pH profiles to direct enzyme production (cellulase, xylanase, polygalacturonase, pectinase, A-galactosidase, and invertase) by *Aspergillus foetidus*. **Biochemical Engineering Journal**, v.137, p.247-254, 2018. Available from: <https://doi.org/10.1016/j.bej.2018.06.008>. Accessed: Sept. 13, 2021.

LI, Q., et al. Soybean hull induced production of carbohydrases and protease among *Aspergillus* and their effectiveness in soy flour carbohydrate and protein separation. **Journal of Biotechnology**, v.248, p.35-42, 2017. Available from: <https://doi.org/10.1016/j.jbiotec.2017.03.013>. Accessed: Sept. 13, 2021.

LIU, J., et al. Enzymatic hydrolysis of cellulose in a membrane bioreactor: Assessment of operating conditions. **Bioprocess and Biosystems Engineering**, v.34, n.5, p.525-532, 2011. Available from: <https://doi.org/10.1007/s00449-010-0501-z>. Accessed: Sept. 13, 2021.

MENEGOL, D., et al. Potential of a Penicillium echinulatum enzymatic complex produced in either submerged or solid-state cultures for enzymatic hydrolysis of elephant grass. **Fuel**, v.13, p.232-240, 2014. Available from: <https://doi.org/10.1016/j.fuel.2014.05.003>. Accessed: Sept. 13, 2021.

MÜLLER, A., et al. Applications of Fungal Cellulases. In: ____ **Reference Module in Life Sciences**. Elsevier, 2021. Available from: <https://doi.org/10.1016/B978-0-12-819990-9.00044-5>. Accessed: Sept. 13, 2021.

OBREGÓN-CANO, S., et al. Analysis of the Acid Detergent Fibre Content in Turnip Greens and Turnip Tops (*Brassica rapa* L. Subsp. *rapa*) by Means of Near-Infrared Reflectance. **Foods**, v.8,

n.9, p.364-379, 2019. Available from: <https://doi.org/10.3390/foods8090364>. Accessed: Sept. 13, 2021.

PAYNE, C. M., et al. Fungal cellulases. **Chemical Reviews**, v.115, p.1308-1448, 2015. Available from: <https://doi.org/10.1021/cr500351c>. Accessed: Sept. 13, 2021.

REIS, L., et al. Cellulase and Xylanase Expression in Response to Different pH Levels of *Penicillium echinulatum* S1M29 Medium. **BioEnergy Research**, v.7, p.60-67, 2014. Available from: <https://doi.org/10.1007/s12155-013-9345-0>. Accessed: Sept. 13, 2021.

ROCHA, V. R., et al. Estimativa do Valor Energético dos Alimentos e Validaçã o das Equações Propostas pelo NRC (2001). **Revista Brasileira de Zootecnia**, v.32, n.2, p.480–490, 2003. Available from: <https://doi.org/10.1590/S1516-35982003000200029>. Accessed: Sept. 13, 2021.

RODRIGUES, V. J.; ODANETH, A. A. Industrial application of cellulases. In: TULI, D.K.; KUILA, A. Current Status and Future Scope of Microbial Cellulases. **Elsevier**, 2021, p.189-209. Available from: <https://doi.org/10.1016/C2019-0-04287-2>. Accessed: Sept. 13, 2021.

ROJAS, M. J., et al. Sequential proteolysis and cellulolytic hydrolysis of soybean hulls for oligopeptides and ethanol production. **Industrial Crops and Products**, v.61, p.202-210, 2014. Available from: <https://doi.org/10.1016/j.indcrop.2014.07.002>. Accessed: Sept. 13, 2021.

SONG, H.T., et al. Synergistic effect of cellulase and xylanase during hydrolysis of natural lignocellulosic substrates. **Bioresource Technology**, v.219, p.710-715, 2016. Available from: <https://doi.org/10.1016/j.biortech.2016.08.035>. Accessed: Sept. 13, 2021.

SUWANNARANGSEE, S, et al. Optimisation of synergistic biomass-degrading enzyme systems for efficient rice straw hydrolysis using an experimental mixture design. **Bioresource Technology**, v.119, p.252-261, 2012. Available from: <https://doi.org/10.1016/j.biortech.2012.05.098>. Accessed: Sept. 13, 2021.

TEIXEIRA, A.J., et al. Commercial and non-commercial pectinase and cellulase on the enzymatic hydrolysis efficacy of rice husk and Tifton 85 hay. **Acta Scientiarum. Animal Sciences**, v.41, n.1, p.e45100, 2019. Available from: <https://doi.org/10.4025/actascianimsci.v41i1.45100>. Accessed: Sept. 13, 2021.

YANG, Y., et al. The composition of accessory enzymes of *Penicillium chrysogenum* P33 revealed by secretome and synergistic effects with commercial cellulase on lignocellulose hydrolysis. **Bioresource Technology**, v.257, p.54-61, 2018. Available from: <https://doi.org/10.1016/j.biortech.2018.02.028>. Accessed: Sept. 13, 2021.

YUANGKLANG, C., et al. Growth performance and macronutrient digestion in goats fed a rice straw based ration supplemented with fibrolytic enzymes. **Small Ruminant Research**, v.154, p.20-22, 2017. Available from: <https://doi.org/10.1016/j.smallrumres.2017.06.009>. Accessed: Sept. 13, 2021.