

## NR 06. SUGAR PRODUCTION FROM RICE STRAW

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### Resumen

#### Producción de azúcar de la paja de arroz

Se investigó un proceso de presurización y despresurización amoniacal (PDA) para incrementar la susceptibilidad de la paja de arroz a la hidrólisis enzimática. Se determinaron las fracciones de carbohidratos y las muestras se sometieron a hidrólisis por enzimas celulolíticas comerciales en condiciones estándar. El tratamiento PDA aumentó significativamente el contenido de sustancias solubles y disminuyó el contenido de hemicelulosa ( $P < .001$ ) de la paja de arroz. El contenido de celulosa disminuyó en muestras con alta humedad tratadas por 6 y 29 min ( $P < .05$ ). La lignina permaneció invariable. El tratamiento PDA aumentó la producción de azúcar de la paja no tratada de 10 % hasta 67 % de la conversión teórica en el mejor tratamiento ( $P < .001$ ). La producción de azúcar fue significativamente mayor ( $P < .05$ ) a mayor contenido de humedad y a mayor tiempo de tratamiento. Mientras que la paja de arroz es un material resistente a la hidrólisis enzimática, el tratamiento PDA produce un material inmediatamente disponible para la acción de enzimas hidrolíticas. La paja de arroz procesada se puede utilizar como una materia prima para producir azúcares para alimentación animal.

**Palabras claves:** Paja de arroz, azúcares, hidrólisis, amoniaco.

**Key words:** Rice straw, sugars, hydrolysis, ammonia

### Introduction

There is a great deficit of energetic feedstuffs for ruminant as well as non ruminant animals such as poultry and swine in Venezuela. Traditional feeds supplying energy are cereals. However, 80 % of the cereals used for feeding are imported costing \$ 1.5 million annually (M.A.C., 1994) and compete with humans for corn. Lignocellulosic wastes such as rice straw have a high polysaccharide content, mainly cellulose and hemicellulose, which have energy levels similar to corn (Ferrer *et al.*, 1994), but these compounds are not digested by poultry and only partly digested by swine. Novel ammonia reactor processes have recently been developed (AFEX, TAME, PDA) which greatly increase the ruminal digestibility of forages and cereals such as coastal bermudagrass (De La Rosa *et al.*, 1994), sorghum and corn (Turner *et al.*, 1995) and dwarf elephantgrass (Ferrer *et al.*, 1997). It has also been possible to enzymatically hydrolyze ammonia-processed Coastal bermudagrass (De La Rosa *et al.*, 1994) and corn fiber (Moniruzzaman *et al.*, 1996) up to 90 % of theoretical yields. As a result, a mixture of glucose and pentoses have been obtained. Glucose is readily used by animals, but pentoses such as xylose and arabinose are digested with lower efficiency. Poultry should not have more than 15 % of pentoses in the diet whereas swine withstand higher concentrations due to their greater ability to adapt. Rice straw has approximately 45 % cellulose and 28 % hemicellulose. If only 50 % of the fibers were converted into sugars, 360 mg sugar/g straw could be obtained. Since annual rice straw availability in Venezuela reaches about 700 000 MT of dry matter, 252 000 MT of sugar could be produced at 50 % enzymatic hydrolysis, which could replace 230 000 MT of corn/year, valued at \$ 42.5 million/year. Sugars produced this way could also economically be used to feed ruminants, which readily digest these sugars, as a replacement for molasses, and to produce ethanol and organic acids by fermentation processes. The objective of this work was to assess the utility of the Ammonia Pressurization and Depressurization (PDA) process to enhance the enzymatic production of reducing sugars from rice straw.

### Materials and methods

A laboratory-scale ammonia reactor unit consisting of a 4-L reactor with appropriate support equipment was used for the treatment of rice straw. Rice straw obtained from California was used in these studies. Liquid anhydrous ammonia was added to 80 g-samples and temperature was rapidly elevated to the desired temperature. After the selected treatment time, pressure was suddenly released and samples allowed to air-dry overnight. An ammonia loading of 1.5 g ammonia/g dry matter, delivered in 4 min and an initial biomass temperature of 90 °C

were used in all the treatments. Two experiments were carried out in duplicate. The first experiment involved a 20 min-treatment time and three moisture levels (15, 35 and 60 %). In the second experiment, three treatment times (0, 6 and 20 min) were tested with 35 % moisture-samples. NDF, ADF and ADL were determined in quadruplicate in the samples to estimate solubles, cellulose, hemicellulose and lignin (Goering and Van Soest, 1970). Samples were subjected to enzymatic hydrolysis by cellulase (Spezyme CP, GENENCOR, Inc., San Francisco, CA), cellobiase (Novozym 188, Novo Laboratories, Wilton, CT) and hemicellulase (Bio-Feed Plus, Novo Laboratories). Cellulase, cellobiase and hemicellulase loadings were kept at 5 IU/g DM, 28.4 CBU/g and 2.5 mg/g, respectively. Hydrolysis was carried out at a solids loading of 5 % w/v in 100 mL of 0.05 M citrate buffer at pH 4.8. Sodium azide was added (0.15 %) for preservation. Duplicate 500 mL erlenmeyer flasks containing the samples were placed in air-incubators at 50 °C at 100 rpm. Prior to analysis, hydrolyzed samples were filtered on cheese cloth, cooled down, centrifuged at 3000 rpm and filtered on 0.22 µm Millipore nylon membranes. Sugars produced were then determined at  $t_0$  and at 72 h reaction and measured as reducing sugars with the DNS method (Miller, 1959).

## Results and discussion

The initial solubles content of RS was 22.15 %. Table 1 shows that the ammonia treatment significantly increased solubles content of rice straw ( $P < .001$ ), mainly with moisture content (up to 47.17 %). Moisture appears to help the ammonia penetrate the biomass for reaction. In addition, moisture contributes to increase reaction temperature since ammonia dissolution in water is exothermic. Solubles increased with treatment time ( $P < .001$ ). The increase in solubles has a great impact on ruminant feeding since they supply immediate energy and nitrogen compounds for the rumen microorganisms. Hemicellulose content of the control (27.63 %) decreased with the PDA treatment and accounts for most of the increase in solubles content. Cellulose and lignin content of the control were 45.17 and 5.05 %, respectively. Cellulose decreased mainly in the 35 % and 60 % moisture-samples treated for 20 min ( $P < .05$ ) whereas lignin remained unchanged.

**Table 1. Carbohydrate fractions and stigar yield of untreated and PDA-treated rice straw Samples.**

Moisture content, %	Treatment time, min	Solubles %	Hemicellulose %	Cellulose %	Sugar yield ing/g DM
15	untreated	22.15 <sup>a</sup>	27.63 <sup>a</sup>	45.17 <sup>ab</sup>	82 <sup>a</sup>
15	20	28.18 <sup>b</sup>	23.11 <sup>b</sup>	43.66 <sup>bc</sup>	221 <sup>b</sup>
35	0 <sup>1</sup>	29.76 <sup>b</sup>	18.82 <sup>c</sup>	46.37 <sup>a</sup>	232 <sup>b</sup>
35	6	36.54 <sup>c</sup>	16.33 <sup>d</sup>	42.08 <sup>dc</sup>	407 <sup>c</sup>
35	20	41.62 <sup>d</sup>	12.55 <sup>e</sup>	40.78 <sup>d</sup>	483 <sup>d</sup>
60	20	49.31 <sup>e</sup>	10.40 <sup>f</sup>	35.24 <sup>e</sup>	536 <sup>e</sup>
	SEM	0.549	0.275	0.559	14.34

<sup>1</sup>subjected to treatment only during the delivery of the ammonia. a, b, c: Means in the same column not sharing a common letter are significantly different ( $P < .05$ ).

Sugar production increased significantly ( $P < .001$ ) for PDA-treated samples compared to the control, and was greater with higher moisture contents and increased times. The best PDA condition, 60 % moisture content for a 20 min treatment, increased the sugar yield by 6.5 times to 67 % of theoretical. The control RS had only 10 % hydrolysis, indicating that RS is resistant to enzymes which relates to its very low ruminal digestibility (Ferrer *et al.*, 1997). The high increase in sugar yield reflects the effect of the ammonia treatment on increasing the susceptibility of cellulose and hemicellulose to enzymatic hydrolysis. PDA treated RS provides an alternative source of sugars for animal feeding and may replace starch in appropriate applications.

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