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Chemical Composition And Surface Images Of Untreated And Urea-Treated Rice Straw As Influenced By Days After Threshing

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Abstract
The purpose of this study was to evaluate the chemical composition and changes in the surface structure of rice straw as affected by the number of days of exposure to the field prior to processing. A total of 24 packs of rice straw samples collected at 1 day after threshing (DAT), 4 DAT, and 7 DAT field exposure untreated and treated with urea for 10 days were analyzed for its chemical changes using proximate analysis and surface structure of the fiber compounds through scanning electron microscopy (SEM). Results (%DM basis) showed that 1DAT untreated rice straw obtained the highest dry matter (DM) concentration at 94.61%. Seven DAT untreated rice straw contain the highest amount of Ash at 20.92%. Likewise, 1 DAT and 7 DAT untreated rice straw contains higher levels of crude protein (CP) at 9.60% and 9.79%, respectively. Ether extract (EE) was observed highest in 1DAT untreated rice straw at 1.97%. Considerable breakdown of cellulose, hemicellulose, and lignin fractions of rice straw was attained in 4DAT and 7DAT untreated, and 1 to 7 DAT urea-treated rice straw (UTRS) as evident by its lower NDF, ADF, and ADL values. Changes in the surface structures of microfibers efficiently captured using SEM showed the differences in the morphology of the fibers as influenced by number of days exposed to the field prior to treatment. Therefore, in order to optimize the protocol to improve the feeding and nutritional quality of rice straw, it is suggested that the number of days of exposure to the field be considered prior to processing. Likewise, UTRS is an effective method to disintegrate the complex fibrous compounds of the cell wall of rice straw.

Keywords: chemical composition, rice straw samples, fiber compounds, rice straw (UTRS), complex fibrous compounds.

INTRODUCTION
Rice straw is one of the most abundant crop by-products, especially in the tropics. The volume reaches approximately 800 to 1000 million tons/year in Asia (PSU, 2020). For decades, it has been widely used in industries, from bioenergy production, pulp and paper production, soil ameliorant and fertilizer, mulching material, and litter material in barns and cages, to feeds for ruminant animals, particularly cattle. In subsistence farms, rice production comes along with livestock production as a source of nutrients and income for the family. After harvesting the grains, it leaves copious amounts of straws in the paddy fields, which are then plowed back during land preparation for replanting of rice. Although it is a low-cost and practical nutrient source for cattle (IRRI, 2018), its utilization as feed is minimal due to the high fiber fractions such as NDF, ADF, lignin, and silica levels with low levels of crude protein that results in its low intake, digestibility, and palatability (Ravi et al 2019; Sarnklong et al 2010). Several experiments have been focused on the

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investigation to improve the chemical composition and nutritive quality of rice straw as animal feed. Methods to disintegrate the lignocellulosic bonds of cell walls in rice straw include physical, chemical, biological, or a combination of two or more of these techniques have been employed. A common technique used to improve the nutrient content of rice straw as feed for cattle is ammoniation. Ammoniation is carried out using urea. This method is more practical to the smallholder farmers, which contributes majorly to the total animal population in developing countries. Urea treatment revealed improvements in crude protein content, digestibility, and feed intake of rice straw. Moreover, it significantly reduced the NDF and ADF content and increased the in vitro dry matter and organic matter digestibility of rice straw (Yalchi et al 2009). However, this improvement depends on several factors, such as climatic conditions, farm management, and harvest time. Considering the labor force of the households and the bulk of workloads done during the harvesting of the grains, commonly, the straws are left in the field for a few days until the major tasks in the grain processing are finished. Different studies in the past have focused on the role of ammoniation in the improvement of the nutritional quality and digestibility of rice straw, including its effect on the rumen microflora, and the production performance of the animal. Nevertheless, the effect of environmental factors, such as the length of exposure of the rice straw to the environment before processing has not been elucidated. To optimize the process of ammoniation of rice straw using urea, and maximize its feeding potential and nutrient quality, considering the number of days that the straw is exposed in the field, it is important to examine the changes in the chemical composition and surface structure of rice straw before and after urea treatment. Scanning electron microscopy (SEM) allows targeted analysis of sample surfaces. It provides a higher image resolution for characterizing microstructures like fiber fractions. It is a quick, nondestructive determination of the modification of the surface structure of the sample (Sleeman and Carter, 2005). Hence, it is an efficient method to use in the analysis of the changes in the surface structure of the rice straw. In order to optimize an effective procedure to improve the nutrient quality of rice straw as feed for cattle, it is important to understand the effect of field exposure (measured by the number of days after threshing) on the changes to its chemical composition and surface structure, hence this study was conducted.

OBJECTIVES

This study aimed to evaluate the chemical composition and changes in the surface structure of rice straw as affected by the number of days of exposure to the field prior to processing.

METHODOLOGY

Collection and preparation of rice straw

Rice straws from the same variety of rice planted in the same season with a rain-fed irrigation system were collected manually at one (1), four (4), and seven (7) days after threshing (DAT). They were chopped to approximately a handspan in length to facilitate processing.

Preparation, application of the urea solution, and storage of the urea-treated rice straw

Urea solution was prepared using the technology applied at the University of the Philippines Research Development and Extension (UPRDE, 2018), wherein 4 grams of urea was dissolved in 100-millimeter water, obtaining a 4% urea solution. Ten liters (10L) of the urea solution was sprayed evenly onto the 10 kgs of chopped rice straw. The urea-treated rice straw (UTRS) was placed in polyethylene plastic bags tied closely with rubber bands and stored in a cool dark place at room temperature for ten days. While for untreated rice straw, 10L of water was sprayed into the 10 kgs of rice straw. Twenty-four packs of untreated and treated rice straw were prepared.

Treatment Design and Statistical Analysis

The study was laid out in 2x3 factorial in a Completely Randomized Design with processing method (untreated and Urea-treated rice straw or UTRS) as Factor A and the number of days exposure to the field or days after threshing as Factor B. Data obtained was analyzed using analysis of variance (ANOVA). Significant differences in means were compared using Tukey's HSD test at a 5% level of significance using Minitab Statistical Software.

Sample preparation for SEM-EDS

A representative sample from the different treatments and replicates was reduced to 200mm in diameter. A Hitachi SU3500 model SEM equipped with Bruker Quantax Xflash 6|30 was used to observe the morphological features of all the samples. The samples were mounted on an aluminum specimen stub using carbon tape. An arrowed line was clicked and dragged to select the position of the line scan on the area of interest where EDS spectral information was derived from the drawn line.

RESULTS AND DISCUSSION

The chemical composition of untreated and urea-treated rice straw processed on different days after threshing presented in Table 1 shows a significant interaction between processing and the number of days after threshing. Results showed that untreated rice straw at 1 DAT had the highest DM content of 94.61%. The highest Ash content (20.92%) was obtained in untreated rice straw at 7 DAT although means were comparable to untreated 1 DAT (20.81%). The highest concentration of CP was observed in untreated rice straw in 7 DAT and 1 DAT (9.79% and 9.60%, respectively). A noticeable percentage of EE was noted in untreated rice straw at 4 DAT (1.97%). Although no significant interaction was found in CF, the NDF, ADF, and ADL fractions of fiber showed an interaction effect. NDF was highest at 1 DAT and 4 DAT UTRS (71.02% and 69.29%), while the highest percentage of ADF was shown in 4 DAT UTRS at 52.55%. ADL was noted highest in 7 DAT untreated rice straw at 5.64%. The number of days after threshing and processing revealed no significant interaction effect for the NPN content of the rice straw. On the other hand, Table 2 shows the main effects of the processing of rice straw and the number of days after threshing as factors to the changes in its chemical composition and surface morphological structures. It showed that the DM content of UTRS was significantly higher (93.16%) than in untreated rice straw (92.91%). A higher level of DM was obtained in 1DAT rice straw at 93.78% compared to other days, while the highest Ash content was observed in untreated rice straw (20.67%) compared to UTRS (19.31%). It was found highest at 7 DAT (20.63%). Comparing the CP of untreated rice straw and UTRS, higher CP content was found in untreated rice straw at 9.48% than 8.39% in UTRS. The highest CP content of rice straw was observed in 1 DAT and 7 DAT at 9.24% and 9.21%, respectively. EE was found highest in 4 DAT at 1.73%, although this value is comparable to 1 DAT with 1.25% EE. The result of the CF content was observed to be higher in untreated rice straw at 29.64% than 26.90% in UTRS. The NDF concentration was noted higher in UTRS at 68.67% than in untreated rice straw at 64.50%. Although no significant difference was observed in the ADF fractions of untreated and UTRS, the highest ADF content was found in 4 DAT at 50.51%. The highest ADL content was shown in untreated rice straw at 4.97%. It was noticeably highest in 7 DAT at 5.16%. NPN content was obviously higher in untreated rice straw than in UTRS (0.24% and 0.16%, respectively). The highest NPN value was obtained at 1 DAT at 0.21%. Naturally, rice straw has a significant amount of DM composed of higher concentrations of fiber such as cellulose (32-47%), hemicellulose (19-27%), and lignin (5-24%) (Huang et al. 2020; Singh et al. 2021). Exposure of rice straw to environmental conditions promotes the biochemical decomposition of its highly recalcitrant compounds, such as the fiber fractions (Vitousek et al. 1994; Gregorich et al. 2017; Guo et al. 2018). In the present study, the collection of rice straw at 1 DAT and kept untreated for 10 days had short exposure to the environmental condition that may cause its fast decomposition, thus maintaining the compactness and intact DM content. Silica is the abundant element present in rice straw, and the primary component of the ash at 14.65 % (Mohamed and Taher, 2006). Khaleghian et al. (2017) revealed that alkali treatment such as the application of sodium carbonate, could effectively remove silica from the rice straw. This observation supported the result of the present investigation wherein the highest amount of Ash was observed in 7DAT untreated rice straw. During this period, there was an initial decomposition of other fiber fractions leaving a copious amount of silica, a hard element to decompose, resulting in its higher concentration upon measurement. The higher levels of CP in 1 DAT and 7 DAT untreated rice straw could be due to the gradual degradation of cell wall components that protects the cell contents including protein compounds inIact after

Chemical composition (*%DM basis*)	Untreated			UTRS			*P-Value* (*AXB*)
	1DAT	4DAT	7DAT	1DAT	4DAT	7DAT	
DM	94.61^{A}	92.54^{D}	91.5800^{E}	92.95^{C}	92.87^{C}	93.66^{B}	0.000
Ash	20.81^{AB}	20.28^{B}	20.92^{A}	18.89^{C}	18.73^{C}	20.33^{B}	0.000
CP	9.60^{A}	9.05^{B}	9.79^{A}	8.89^{B}	7.65^{C}	8.64^{B}	0.031
EE	0.99^{B}	1.97^{A}	0.99^{B}	1.50^{AB}	1.49^{AB}	0.85^{B}	0.054
CF	29.49	29.86	29.57	27.44	26.68	26.60	0.249
NDF	71.02^{A}	69.29^{A}	65.71^{B}	64.48^{B}	65.01^{B}	64.02^{B}	0.002
ADF	52.55^{A}	48.46^{C}	50.49^{B}	48.19^{C}	49.20^{BC}	48.46^{C}	0.000
ADL	5.64^{A}	4.85^{B}	4.43^{C}	3.91^{D}	3.97^{D}	4.67^{BC}	0.011
NPN	0.25^{A}	0.17^{AB}	0.19^{AB}	0.18^{AB}	0.19^{AB}	0.11^{B}	0.079

Table 1. Interaction effect of the chemical composition of treated and untreated rice straw treated on different days after threshing.

DM: Dry matter; OM: Organic matter; CP: Crude protein; EE: Ether extract; CF: Crude fiber; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; ADL: Acid detergent lignin; UTRS: Urea treated rice straw; Factor A: handling; Factor B: DAT-Days after threshing: 1, 4, 7; Means with different letter superscripts within the row are significant at $p<0.05$.

Chemical composition (*%DM basis*)	FACTOR A		FACTOR B			*P-Value* (*A*)	*P-Value* (*B*)
	UNTREATED	UTRS	1DAT	4DAT	7DAT		
DM	92.91^{B}	93.16^{A}	93.78^{A}	92.70^{B}	92.62^{B}	0.000	0.000
Ash	20.67^{A}	19.31^{B}	19.85^{B}	19.51^{B}	20.63^{A}	0.000	0.000
CP	9.48^{A}	8.39^{B}	9.24^{A}	8.35^{B}	9.21^{A}	0.000	0.000
EE	1.31^{A}	1.28^{A}	1.25^{AB}	1.73^{A}	0.92^{B}	0.841	0.002
CF	29.64^{A}	26.90^{B}	28.46^{A}	28.27^{A}	28.08^{A}	0.000	0.561
NDF	68.67^{A}	64.50^{B}	67.75^{A}	67.15^{A}	64.86^{B}	0.000	0.000
ADF	49.38^{A}	49.76^{A}	48.69^{B}	50.51^{A}	49.52^{B}	0.197	0.000
ADL	4.97^{A}	4.18^{B}	4.17^{C}	4.41^{B}	5.16^{A}	0.000	0.000
NPN	0.24^{A}	0.16^{B}	0.21^{A}	0.18^{AB}	0.15^{B}	0.036	0.025

Table 2. Chemical composition of untreated and urea-treated rice straw processed at different days after threshing.

DM: Dry matter; OM: Organic matter; CP: Crude protein; EE: Ether extract; CF: Crude fiber; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; ADL: Acid detergent lignin; UTRS: Urea-treated rice straw; Factor A: handling; Factor B: DAT-Days after threshing: 1, 4, 7; Means with different letter superscripts within the row are significant at $p<0.05$.

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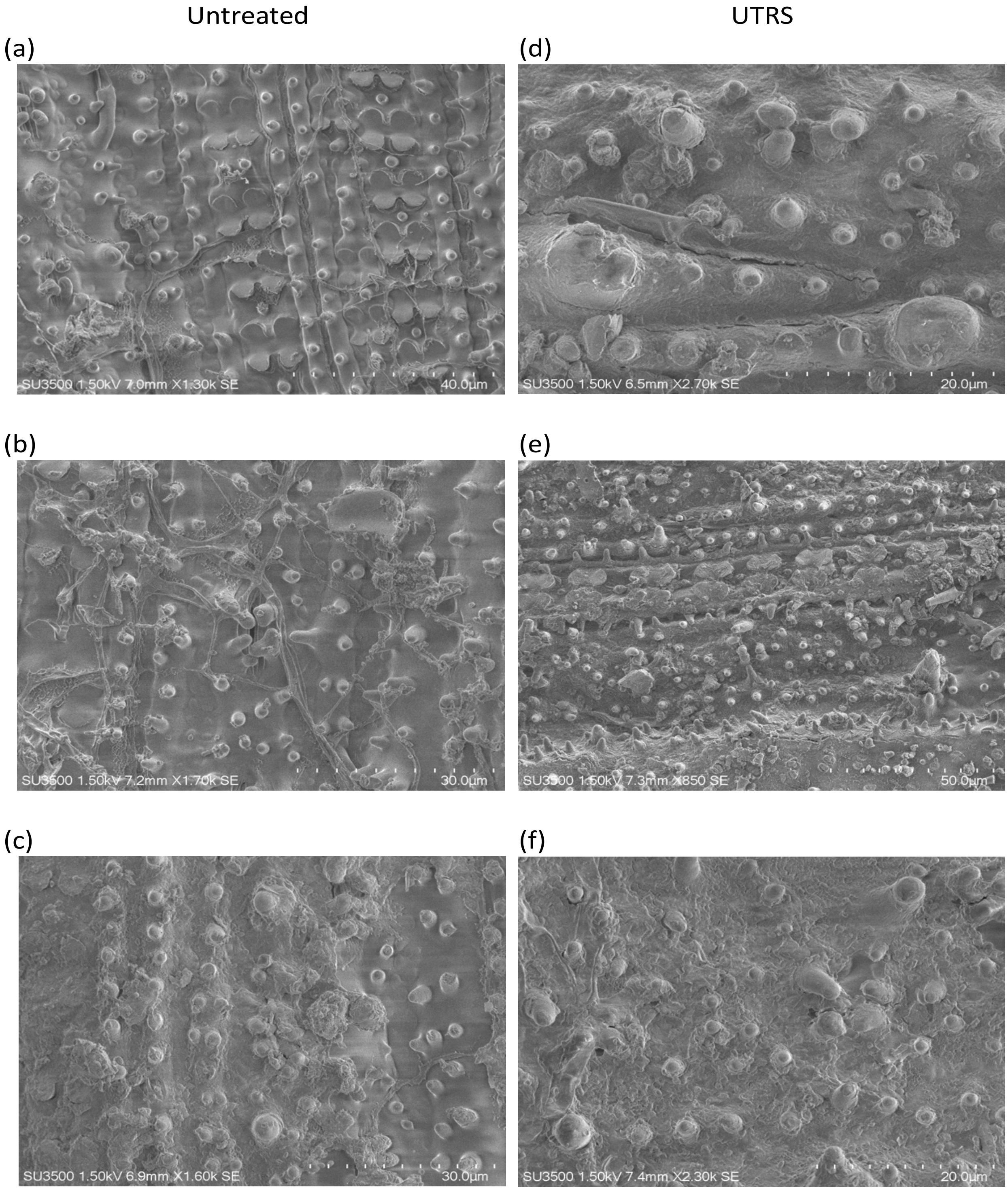

Figure 1. SEM surface images of rice straw before (a-1DAT, b-4DAT, c-7DAT) and after urea treatment (d-f) in 1.50K electron beam intensity.

fermentation. This is supported by the findings of Vadiveloo (1986), untreated rice straw contained higher CP. Although, urea was found to be effective in increasing the crude protein content of rice straw (Aquino et al. 2019; Wanapat 1985; Schiere and Ibrahim 1989), nitrogen losses occurred during treatment preparation, opening the silo, and even during the collection of samples for analysis (Jayasuryia and Pearce 1983). Nguyen et al. (2001), also revealed that two-thirds of the urea-N applied during straw treatment volatilizes during storage and aeration. The higher content of EE in 4 DAT untreated rice straw corroborated to the result of Vadiveloo (1986), wherein substantial reduction of CP, Crude fat followed after alkali treatment. Degradation of the fiber fractions of the cell wall such as the NDF, ADF, and ADL in the present study corroborated the findings of Wanapat et al (2013), Zaman and Owen (1995), and Vadiveloo (1986), which showed a decrease in the NDF levels of rice straw as a result of fermentation with urea.

Conclusion

Days of exposure to the field and processing method can influence the chemical composition and surface structure of the rice straw. Based on the findings of the study, it is concluded that 1 DAT contains a remarkable amount of DM, and EE, as well as Ash in 7DAT untreated rice straw. In terms of maintaining a considerable amount of CP, 1DAT, and 7DAT untreated were found to be the best. Furthermore, an extensive breakdown of cellulose, hemicellulose, and lignin fractions of rice straw was attained when rice straw was left in the field for 4DAT and 7DAT untreated, and 1-7 DAT treated with urea as evident by its lower NDF, ADF, and ADL values. Imaging the modification in the surface structures of microfibers was efficiently captured using SEM. Therefore, in order to optimize the protocol to improve the feeding and nutritional quality of rice straw, it is suggested that the number of days of exposure to the field of the rice straw be considered prior to processing. Likewise, UTRS is an effective method to disintegrate the complex fibrous compounds of the cell wall of rice straw. Thorough handling during UTRS making is advised to avoid nitrogen losses, thus maintaining a high level of CP.

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