Enhancement of the nutritional value of elephant grass (*Pennisetum purpureum* Schum.) for use as animal feeds and for xylanase production

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Elephant grass (*Pennisetum purpureum* Schum.) is one of the most important tropical forage crops currently under study. The present study evaluates the enhancement of nutritional value of elephant grass for use as animal feeds and xylanase production. Biochemical analysis were carried out on untreated elephant grass, autoclaved elephant grass, solid state fermented elephant grass and solid state fermented autoclaved elephant grass. The results showed that the solid state fermented elephant grass produced the highest increase in soluble proteins (13.6±0.8 and 20.4±0.9 mg g^{-1}), glucose levels (32.4 ±1.6 and 54.6±3.3 mg g^{-1}), and reducing sugar concentrations (89.2±1.7 and 114.4±7.9 mg g^{-1}) compared to the other groups of the study. Furthermore, the results obtained for total phenol content and total flavonoid were significantly higher in solid state fermented elephant grass and autoclaved solid state fermented elephant. Similarly, the free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl were potentially active in solid state fermented elephant grass. For xylanase production, solid state fermented elephant grass showed the highest enzymatic activity. These results indicated that solid state fermentation enhanced the nutritional value of elephant grass, which can also be used as substrate for the production of xylanase.

Key words: Elephant grass, animal feeds, solid-state fermentation, Xylanase, protein.

INTRODUCTION

Growing human population can result to the immense need to exploit existing livestock resources to meet animal protein requirements; this is impossible unless optimal fodder and forage production is ensured (Weishaupt et al., 2020). Sustainable livestock production is highly dependent on the availability of quality feed and forage resources (Negawo et al., 2017; Naah et al., 2019; Bakare et al., 2020). A major problem facing livestock producers in tropical countries is how to provide a proper nutrition for their animals, especially during the dry season when pasture and cereal residues are limiting in quantity and nutritional quality (Michalk et al., 2018; Kebede 2020).

Elephant grass (*Pennisetum purpureum* Schum.) is a monocot belonging to the family Poaceae (grass family) and genus *Pennisetum* (*Pennisetum* Rich. ex Pers.; fountain grass) (Negawo et al., 2017). Elephant grass, an excellent forage in the tropical and subtropical regions, is the major livestock feed in West Africa, particularly for cattle, dairy and sheep (Bakare et al., 2020). Due to the fact that most smallholder livestock producers predominantly own small and fragmented pieces of land, grasses such as elephant grass offer a best-fit alternative to other feed options, as these are high yielding forages which require a minimum amount of inputs and acreage (Negawo et al., 2017). With respect to advances in nutritional quality of elephant grass,

a number of opportunities exist to leverage the knowledge and advances seen in other fodder crops to the improvement of elephant grass and the benefit of livestock productivity (Simeão et al., 2021; Chandel et al., 2021). Lignin affects the digestion of cell-wall polysaccharides by interfering (as a physical barrier) with microbial enzymatic activity and therefore, developing low-lignin elephant grass lines could substantially improve its digestibility and nutritional quality for enhanced livestock productivity (Tan et al., 2019). Nutritional quality, palatability, and propagation by seed or vegetative organs are currently the main limitations, and the diseases elephant grass stunt and head smut are significantly challenging its production in some regions of Africa (Makini et al., 2019; Mutwedu et al., 2020). In order to improve the utilization of elephant grass, enhancement of the nutritive value through proper processing methods is necessary. The microbiological procedure of solid state fermentation (SSF) is one of the approaches employed to improve the nutritive value of animal feed as fermentation increases the bioavailability of nutrients (Olukomaiya et al., 2020; Egbune et al., 2021). This present study aimed at enhancing the nutritional value of elephant grass for use as animal feeds and for xylanase production.

MATERIALS AND METHODS

Substrate preparation for solid state fermentation

Elephant grass (*P. purpureum*) was harvested from Delta State University, Abraka, Delta state and was identified and authenticated in the Department of Botany. The voucher number (UBH-P545) was obtained from the University of Benin Herbarium, Edo State, Nigeria. The samples were pulverized using a commercial grinding machine (SM-1 Retsch GmbH 5667 HAAN) and stored at room temperature. *Rhizopus oligosporus* strains were obtained from Harmony Path. Ltd. laboratory located at Songhai in Amukpe, Sapele, Delta State.

Solid state fermentation of elephant grass (*P. purpureum*)

Solid state fermentation was carried out according to the method described by Ofuya and Nwajiuba (1990), at pH 6 in biofermenters using 50 mM phosphate buffers at room temperature for 72 h. One gram (1 g) of *R. oligosporus* (5.4×10^2 CFU) was homogenized in 10 ml of prepared phosphate at pH 6 in a bio fermenter. 10 g of the ground elephant grass was used in the homogenization step and allowed to ferment for a 72 h period at room temperature. Unfermented control samples (containing dried and ground elephant grass, devoid of any presence of molds; with buffer only, and without any cells) was prepared alongside the test samples as shown in Table 1. After fermentation, 6 g of the mixtures were withdrawn from each of the bio fermenter; 40 ml of distilled water was added prior to homogenization using mortar and pestle. 10 ml of the mixture was collected into a test tube and centrifuged for 10 minutes to get the crude extract. This supernatant (crude extract) was used as the crude extract or sample for the various assays which were carried out in triplicates (Table 1).

Table 1. Various pretreatment for elephant grass.

A	B	C	D
Untreated elephant grass (Control)	Autoclaved elephant grass	Solid state fermented (SSF) elephant grass using *Rhizopus oligosporus*	Solid state fermented (SSF) autoclaved elephant grass using *Rhizopus oligosporus*

Autoclaving was carried out in 500 ml Erlenmeyer flasks at 121°C for 20 min (0.12-MPa autoclave pressure).

Biochemical assays

Total soluble proteins were assayed following the procedure described by Gornall et al. (1949) employing bovine serum albumin as the standard. Glucose level was measured using the procedure described by the Randox glucose kit following the manufacturer instructions (Randox Laboratories Ltd, County Antrimm BT29 4QY,

United Kingdom). Reducing sugars were estimated using the 3,5-dinitrosalicylic acid (DNS) colorimetric technique (Miller, 1959). Antioxidant inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined following the procedure described by Hatano et al. (1988). Total phenol content was estimated by the procedure described by Singleton and Rossi (1965) using catechin as the standard. Total flavonoid contents were determined by colorimetry using a method described by Jia et al. (1999).

Xylanase assay

Xylanase activity was measured according to the method presented by Konig et al. (2002). At the desired fermentation time, the produced xylanase in each flask was extracted by adding 90 ml distilled water and mixing in a rotary shaker (100 rpm) at room temperature for 30 min. The samples were filtered and the filtrates were analyzed for xylanase activity. One unit of xylanase activity (U) was defined as the amount of the enzyme required to liberate 1 µmol of xylose per min under the assay conditions. The yields were expressed as U per gram dry substrate.

Statistical analysis

All data were subjected to statistical analysis. Values were reported as Mean ± Standard deviation and the experimental results were analyzed using analysis of variance (ANOVA) and also a Fischer test of least significance (LSD) was carried out to compare the various group means. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Changes in pH values of the various pretreatments of elephant grass are shown in Figure 1.

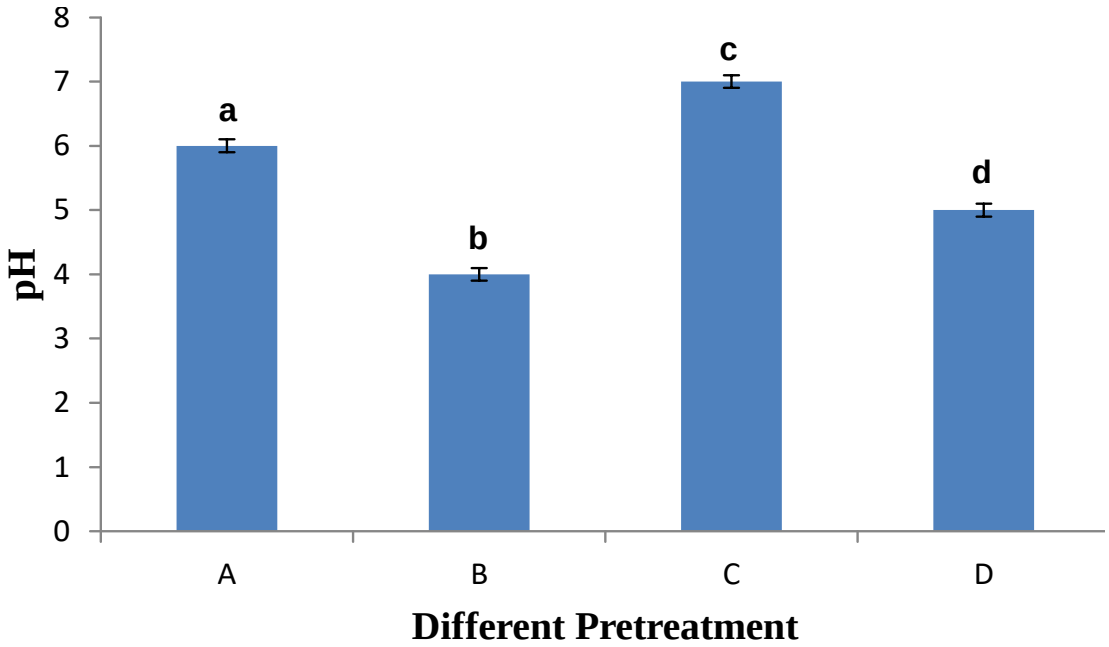

Figure 1. pH changes in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *Rhizopus oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *Rhizopus oligosporus.* Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$).

There was a decrease in pH for autoclaved and solid state fermented autoclaved elephant grass, while there was in increase in pH of SSF elephant grass. The initial pH of solid state fermented elephant grass increased from 6.0 to 7.0 at the end of the 72 h fermentation period which depicts that solid state fermentation of elephant grass favors neutral fermentation. Most

fungi and bacteria are favored by reactions near neutrality while a few are favored by an alkaline reaction. High pH inhibits many microorganisms and determines the types of organisms that grow in fermenting mass (Aganbi et al., 2020). In this study, the increase in pH during fermentation could have been contributed by the strong proteolysis of the fungi *R. oligosporus*. This report is similar with previous reports. Owens et al. (1997) reported that a rise in pH value from 4.5 to 8.5 was observed in the fermentation of soybeans with the development of strong ammoniacal odour because of the hydrolysis of seed protein and the metabolism of the resultant amino acids.

The results of the determination of the levels of soluble proteins in the various pretreatments of elephant grass are shown in Figure 2.

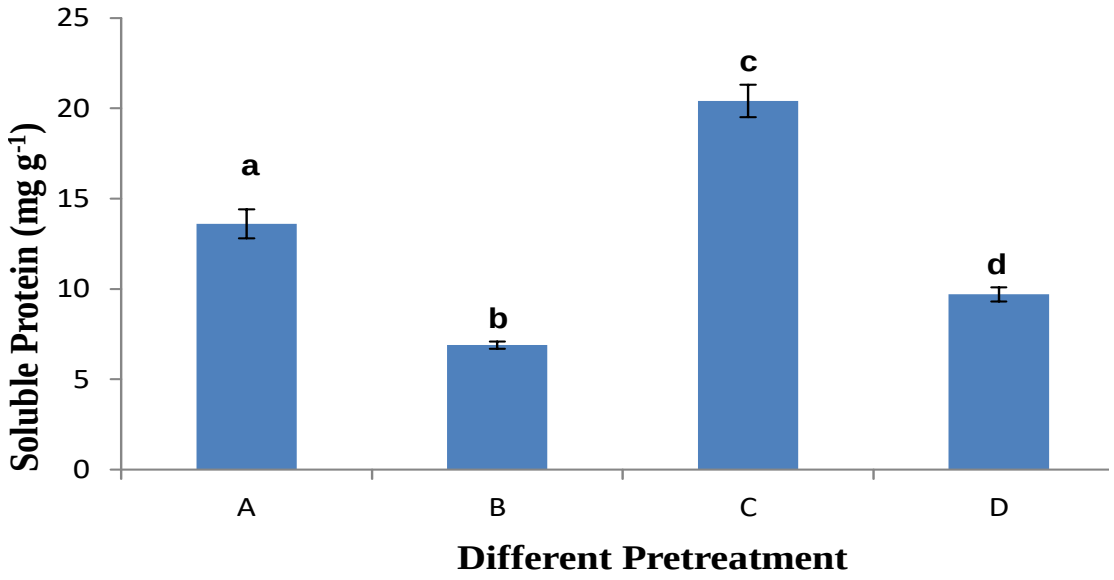

Figure 2. Level of soluble proteins in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *R. oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *R. oligosporus.* Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$).

There was a significant increase ($p<0.05$) in soluble proteins content from 13.9±0.8 to 20.4±0.9 mg g^{-1} of elephant grass fermented with *R. oligosporus* (SSF), while there was a total decline in the levels of soluble protein in other pretreatments. The surge in soluble protein of the solid state fermented elephant grass was as a result of fungal consortium having the ability to produce various extracellular enzymes extracellular enzymes (proteins) including cellulase, hemicellulase, and lignases into the substrate in an attempt to utilize carbon source (Sharma et al., 2017; Hawashi et al., 2019; Akassou and Groleau, 2019; Ong and Lee, 2021). The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles (Elyas et al., 2002). In addition, observed increment in protein content after fermentation was probably due to shift in dry matter content through depletion during fermentation by action of the fermenting microorganisms (Cai et al., 2019). The results are in line with the findings of Egbune et al. (2021) who noted that solid state fermentation of Pearl millet using the fungus *R. oligosporus* resulted in a significant increase ($p<0.05$) in soluble protein from 105.48 ± 0.36 (mg g^{-1}) in the unfermented control (0%) to 169.13 ± 1.01 (mg g^{-1}) at sample fermented at 10% inoculum.

The results of the determination of glucose concentration in the different pretreatments of elephant grass are shown in Figure 3. There was a significant increase ($p<0.05$) in glucose concentration of SSF

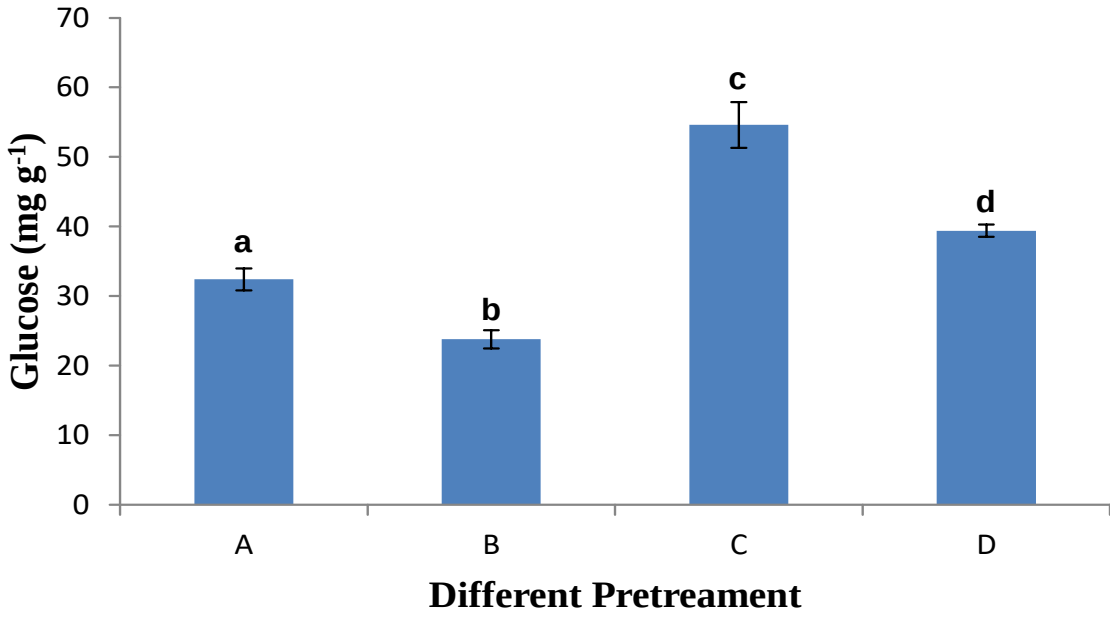

Figure 3. Glucose concentration in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *Rhizopus oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *Rhizopus oligosporus.* Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$).

elephant grass (54.6±3.3 mg g^{-1}) and SSF autoclaved elephant grass (39.4±0.9 mg g^{-1}) compared to the control (32.4±1.6 mg g^{-1}). The report of Anigboro et al. (2020) on *R. oligosporus* fermentation of maize (*Zea mays*) offal is in line with this study. In all fermentation processes, the sugar concentration showed an increase during the initial 12 h (Da Silva et al., 2019). This phenomenon can be associated with the hydrolysis of pectin, cellulose, sucrose, and other coffee pulp complexes carbohydrates, into monomers of glucose and fructose (Marques et al., 2016; Murthy and Naidu, 2011).

The results of the determination of reducing sugar concentration in the different pretreatments of elephant grass are shown in Figure 4. There was an observed increase ($p<0.05$) in solid state fermented elephant grass (114.4±7.9 mg g^{-1}) and solid state fermented autoclaved elephant grass (96±4.6 mg g^{-1}) compared to the control (89.2±1.7 mg g^{-1}). Autoclaved elephant grass had the least reducing sugar concentration (72.4±4.6 mg g^{-1}). Fermented elephant grass yielded more reducing sugar compared to the other pretreatment probably because the substrate was degraded and used as their source of carbon to liberate various enzymes during the process. Similar observations have been reported by Olanbiwoninu and Odunfa (2012) and Kolo et al. (2020).

The results of the determination of total phenolic content (TPC) in the different pretreatments of elephant grass are shown in Figure 5. There was no significant increase ($p > 0.05$) in Autoclaved elephant grass compared to the control; however, there was a significant increase ($p < 0.05$) in the SSF and SSF Autoclaved elephant grass. The increase in TPC may be validated by the proteolytic activities of fungi leading to the release of bound to free TPC. Phenolic compounds are secondary metabolites that have health-promoting benefits such as anticancer activities and antioxidant (Balasundram et al., 2006). Solid-state fermentation (SSF) has been successfully applied to enhance the nutritional and organoleptic qualities of food, and convert agro-industrial residues or plants for valuable phenolic compounds (Liu et al., 2017; Martins et al., 2011). Thus, SSF may support the conversion of bound to free phenolics, thus, improving their bioavailability (Dey et al., 2016).

The total flavonoid content (TFC) in the various pretreatment of elephant grass is shown in Figure 6. The TFC was improved in the solid state fermented and solid state fermented

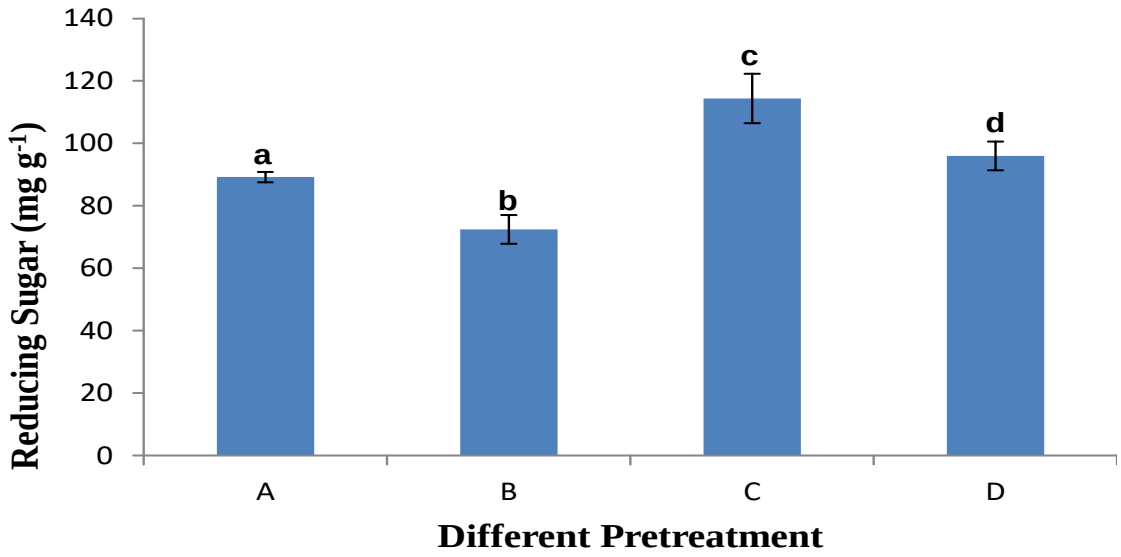

Figure 4. Reducing sugar concentration in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *R. oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *R. oligosporus.* Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$).

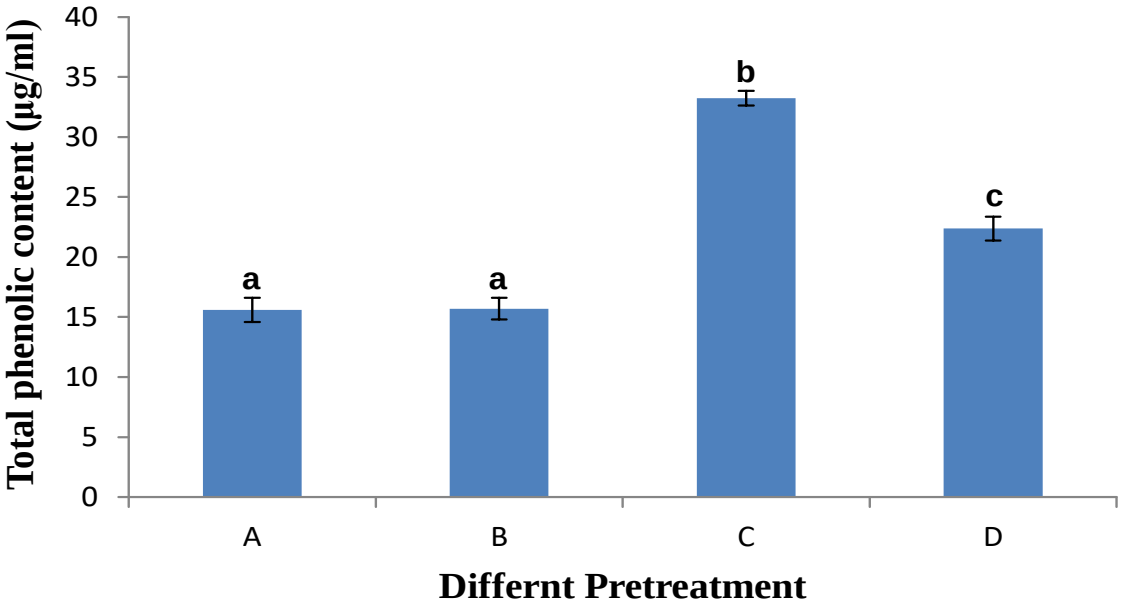

Figure 5. Total phenolic content in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *R. oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *R. oligosporus.* Values with superscripted letter a, b, c are significantly different from the control ($p < 0.05$).

autoclaved elephant grass (79.3±1 and 71.6±1.2 µg/ml). The highest TFC was obtained in solid state fermented elephant grass at 72 h fermentation, while the lowest TFC was obtained from the control (63.8±0.7 µg/ml), at 72 h. Phenolic compounds and flavonoids have generally been attributed to the antioxidant properties. Among the source of natural antioxidants, filamentous fungi have a great potential. Smith et al. (2015) showed that filamentous fungi are good sources of natural antioxidants. These filamentous fungi include *Aspergillus* sp., *Penicillium* sp., *Mucor* sp. and *Rhizopus* sp. (Okoli et al., 2006, 2007; Krnjaja et al., 2014; Vera et al., 2016).

Figure 7 shows the free radical scavenging activities in the different pretreatments of elephant grass. Elephant grass

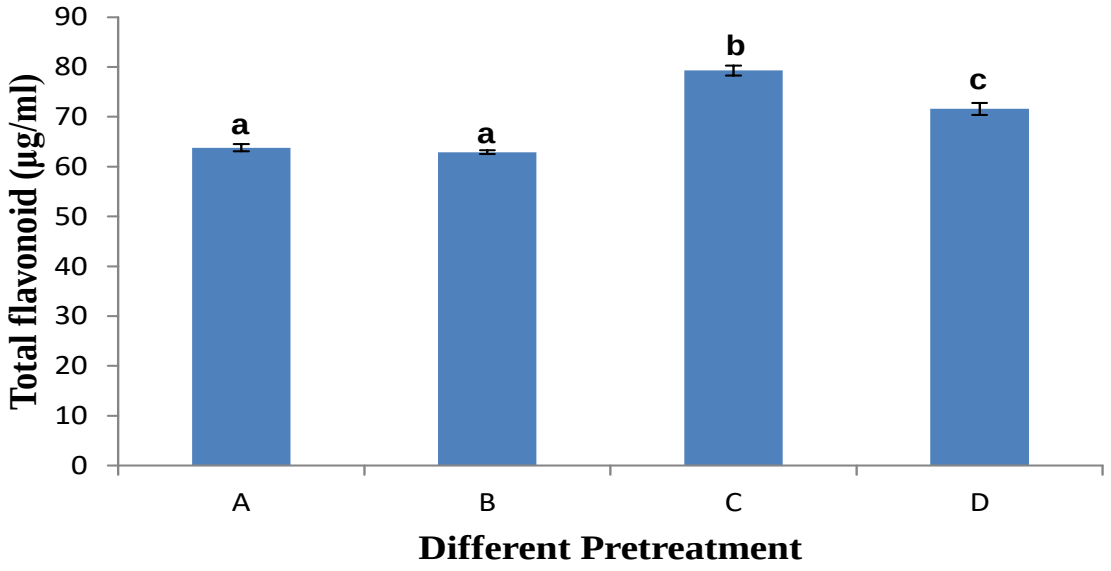

Figure 6. Total flavonoid content in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *R. oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *R. oligosporus.* Values with superscripted letter a, b, c are significantly different from the control ($p < 0.05$).

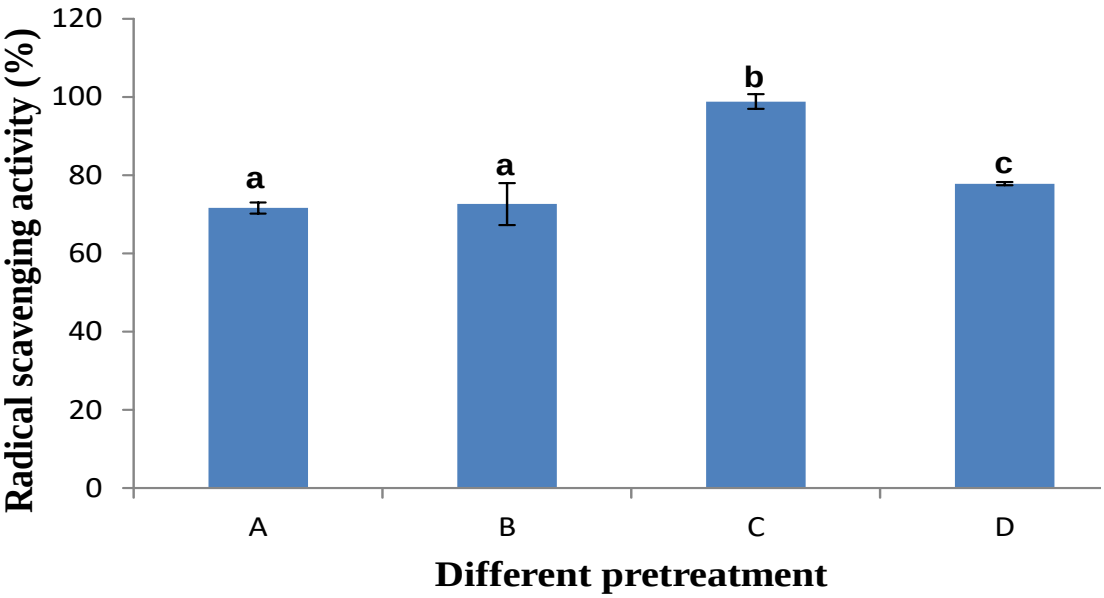

Figure 7. Free radical scavenging activities in the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *R. oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *R. oligosporus.* Values with superscripted letter a, b, c are significantly different from the control ($p < 0.05$).

subjected to SSF was more potentially active compared to the control. Compared with other pretreatments, the changes in the free radical scavenging ability of the solid state fermented elephant grass were mainly caused by the fermentation, and the changes might be related to the conversion of chemical components during fermentation and the production of secondary metabolites from fungus (Martins et al., 2011); some antioxidant components were produced during fermentation, especially some less polar compounds. In addition, fermentation induces the structural breakdown of plant cell walls, leading to the liberation or production of various antioxidant compounds (Hur et al., 2014).

The results of xylanase activity varied markedly with the different pretreatments of elephant grass in a range of 7-18 U/g (Figure 8).

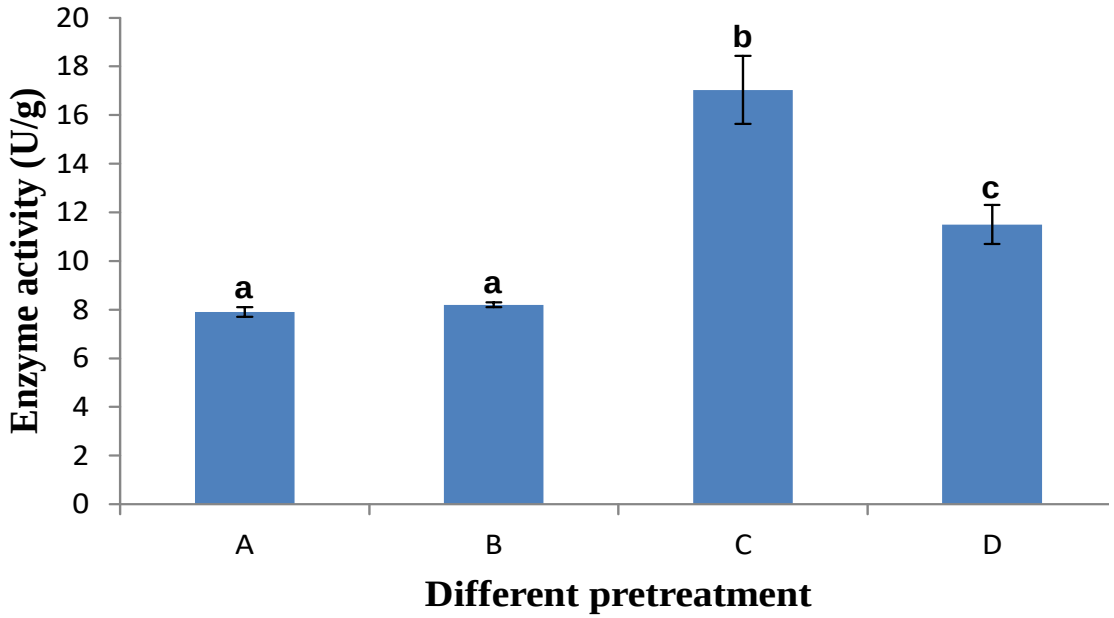

Figure 8. Xylanase activities of the different pretreatments of elephant grass. A: Untreated elephant grass (Control), B: Autoclaved elephant grass, C: Solid state fermented (SSF) elephant grass using *R. oligosporus,* D: Solid state fermented (SSF) autoclaved elephant grass using *R. oligosporus.* Values with superscripted letter a, b, c are significantly different from the control ($p < 0.05$).

The lowest value was obtained in the control, and the highest activity was achieved in solid state fermented elephant grass. This showed that the amount of nitrogen present in the control is too low to support good growth and enzyme production, and that the nitrogen source and its quantity affected xylanase production (Tai et al., 2019; Park et al., 2002). Xylanase is a class of enzymes that degrade the linear polysaccharide xylan into xylose, thus breaking down hemicellulose, one of the major components of plant cell walls. As such, it plays a major role in micro-organisms thriving on plant sources for the degradation of plant matter into usable nutrients. Xylanases are the key enzymes that degrade the xylosidic linkages in the xylan backbone of the biomass (Malgas and Pletschke, 2019; Javed et al., 2019). Filamentous fungi are producers of xylanolytic enzymes in medium being used for the purpose.

Conclusion

Solid state fermentation enhanced the nutritive value (proteins, glucose and reducing sugar) and antioxidant capacity of elephant grass. The significant increase in proteins and sugars indicated that the solid state fermented elephant grass could be excellent components for animal feed production.

Solid state fermented elephant grass could be a good source of the enzyme, xylanase.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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