

IMPROVING THE NUTRITIONAL VALUES OF SORGHUM GRAIN USING *HERICIUM* SPP. AND *STROPHARIA RUGOSOANNULATA*

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ABSTRACT

Sorghum (*Sorghum bicolor* (L.) Moench) is an important cereal that can be used as a less expensive alternative to corn as animal fodder. Sorghum seeds were aseptically impregnated with the mycelia of various *Hericium* spp. and *Stropharia rugosoannulata* strains to develop a novel method of enriching the nutritional value of animal feed using mushrooms. The moisture, total fat, crude ash, crude protein and carbohydrate content of sorghum grains varied according to mushroom species. Generally, fermentation with *Hericium* spp. increased the crude protein and total carbohydrates of the grains, while *S. rugosoannulata* strains increased crude ash and protein content. Fermentation with *Hericium* spp. increased the antioxidant activity of sorghum. These results indicate the potential of using mushroom mycelia as an additive to improve the nutritional value of sorghum grains for use as animal feed.

Key words: proximate analysis, antioxidant activity, mushroom, fermentation, supplementation

INTRODUCTION

Yellow corn is the most common cereal grain used as animal feed in the Philippines (Mateo and Carandang 2006). However, these crops are also used primarily for human consumption and industrial purposes. In addition, it has a higher production cost due to its higher water and fertilizer requirement compared to sorghum (Attah 2002). Consequently, for the past years, sorghum has been used as a more economical alternative for corn as feed ingredient (Pasquali et al. 2016). Accordingly, these grains also share comparable levels of crude protein and metabolizable energy with corn. Sorghum grains even contain higher percent ash and fiber (Olomu 1995). In relation to this, world sorghum production has increased from 57.7 million tons (mt) in 2006 to 63.9 mt in 2016 (FAOStat), making sorghum the fifth most cultivated cereal crop worldwide following wheat, corn, rice, and barley (Gualtieri and Rapaccini 1990). Africa is the largest producer of sorghum contributing to 41.6% of world production, followed by America (37.4%), and Asia (16.1%). Production in the Philippines has also increased significantly from 87 tons in 2006 to 616 tons after over a decade (FAOStat). Sorghum is used for its several functions. These grains can be used as food (Kulamarva et al. 2009), feedstock for biofuel production, fertilizer, and commonly as animal feeds (Taylor et al. 2006).

The formulation of animals feed with adequate nutritional value is a major concern in both livestock and poultry production owing to the increasing cost of feed and feed ingredients (Oyedeki and Atteh 2003). The chemical composition of animal feed determines its nutritional value (Sniffen et al. 1992). Protein is an essential component of animal feeds necessary for growth and development

(Cheeke 2005). Plants are the most common source of dietary proteins in animals, particularly cereals such as maize, soybean, wheat, and sorghum (Ravindran 2013). However, plants provide deficient amino acids, thus, a supplementary source of amino acids such as animal protein may be necessary (Akhter et al. 2008). Although the protein supplied from animals provides a balanced amount of essential amino acids, this source is expensive for commercial production and has a potential risk for disease transmission associated with animal sources (Denton et al. 2005). Other supplementary protein sources also include synthetic amino acids (Cmiljanić et al. 2005), processed plant proteins (Palacios et al. 2004), animal and blood by-products such as spray-dried plasma (Stein 1996). Natural and synthetic antioxidants are used as additives to commercial feeds to lessen oxidative stress during feed production and storage, thereby preserving important nutrients like vitamins (Calabotta and Shermer 1985). Several sources of natural antioxidants as feed ingredient include many plants such as cereal crops (Castillo et al. 2013). However, protein and antioxidants are expensive feed ingredients; thus, naturally enriching animal feeds is important to lessen production cost and improve animal performance. Mushrooms are known for their nutritional and medicinal properties and are excellent sources of protein, essential amino acids, carbohydrates, fiber, minerals, and vitamins (Jiskani 2001; Chang and Buswell 1996). The mycelium and fruiting bodies of numerous edible mushrooms are also known to contain antioxidant compounds (Sánchez 2017). Two types of mushrooms, *Hericium* spp. and *Stropharia rugosoannulata* strains are well-known for their nutritional value and bioactive compounds (Wong et al. 2009; Niedzielski et al. 2017). *Hericium* spp. and *S. rugosoannulata* strains were used for fermentation in our desire to develop a low-cost and natural method of improving the nutritional value of sorghum

MATERIALS AND METHODS

Source of pure culture. Cultures of four *Hericium* spp. (*H. americanum*, *H. corraloides*, *H. erinaceus*, and *Hericum* sp.) and three *S. rugosoannulata* strains were obtained from the Easygrow Mushrooms Inc., Farmington Hills, Michigan, USA. Mycelial discs of these cultures were transferred aseptically into PDA culture plates and incubated until complete mycelial colonization.

Preparation and inoculation of sorghum grains. One kg of sorghum grains was washed thoroughly and boiled for about 30 minutes, then drained. Approximately 50 g of sorghum grains was dispensed into each 500-ml glass catsup bottle, plugged with cotton and covered with paper. These were then sterilized at 15 psi, 121°C for 30 minutes using an autoclave (Reyes et al. 2009). After cooling down, these bottles were inoculated with 10 mm mycelial disk of *Hericium* spp. and *S. rugosoannulata* strains with three replicates for each strain, and incubated at room temperature until full mycelial ramification for about seven days. Meanwhile, two control treatments were used, namely oven dried grains and autoclaved sorghum grains. The first control was oven dried for 24 hours while the second was autoclaved at 15 psi, 121°C for 30 minutes.

Preparation of extract. After complete mycelial colonization, sorghum grains were dislodged from the bottles then oven dried for several hours. The dried samples were pulverized using a blender and sieved using a metal sieve. One gram of each pulverized sample was mixed with 10 mL absolute ethanol in a conical centrifuge tube. These were placed on a shaker for 12 hours continuously and centrifuged for 15 minutes. The extracted solutions were decanted into a test tube with cover.

Proximate analysis. Ethanol extracts of fermented sorghum were analyzed for proximate composition including total fat, crude ash and crude protein following the standard methods by the Association of Official Analytical Chemists (AOAC 1995). Moisture content of grains was obtained after seven days of fermentation with mycelia. Total carbohydrate content was calculated by subtracting the contents of moisture, ash, fat, and protein from 100 and expressed as a percentage of dry mass.

Determination of antioxidant activity. Antioxidant activity of sorghum grains fermented with *Hericium* spp. and *S. rugosoannulata* strains was determined by means of Trolox equivalent antioxidant capacity (TEAC) using DPPH radical scavenging assay using the method developed by Brand-Williams et al. (1995) with slight modifications.

Statistical analysis. The data obtained were analyzed using one-way analysis of variance (ANOVA) and significance of differences between treatment means were compared using Tukey HSD test at 95% least significant difference ($p < 0.05$). SPSS version 20 was used to perform these calculations.

RESULTS AND DISCUSSION

Proximate composition analysis. The effect of solid-state fermentation on the proximate composition of sorghum grains using mushroom mycelia was evaluated and presented in Table 1.

Moisture content of sorghum was decreased by 0.24-1.32% after supplementation with *Hericium* spp. and *S. rugosoannulata* strains. Similarly, total fat content also decreased in grains fermented with *Hericium* spp., but no significant difference was observed in grains with *S. rugosoannulata* strains. Interestingly, the highest level of crude ash was recorded in sorghum with mycelia of *S. rugosoannulata* (2.15-2.40 %) which were statistically higher than the unfermented grains (1.90%). Meanwhile, crude protein levels increased by 2.25-2.85% in grains impregnated with all the three strains of *S. rugosoannulata*. Likewise, protein improved with *H. corraloides*, *H. erinaceus* and *Hericium* sp. No significant difference was observed in carbohydrate content among treatments.

Table 1. Proximate composition of sorghum grains with *Hericium* spp. and *S. rugosoannulata* strains expressed in percentage.

Treatment	Moisture (%)	Total Fat (%)	Crude Ash (%)	Crude Protein (%)	Total Carbohydrates (%)
Control 1 (oven dried)	9.72±0.41 ^a	2.70±0.38 ^a	1.90±0.00 ^b	9.80±0.14 ^e	75.88
Control 2 (autoclaved)	9.68±0.68 ^a	2.96±0.25 ^a	1.90±0.00 ^b	10.50±0.42 ^{de}	74.96
<i>H. americanum</i>	8.40±0.06 ^d	1.74±0.10 ^b	1.75±0.07 ^b	9.70±0.14 ^e	78.41
<i>H. corraloides</i>	8.72±0.06 ^d	1.11±0.01 ^b	1.95±0.07 ^b	11.05±0.21 ^{cd}	77.17
<i>H. erinaceus</i>	9.48±0.08 ^{ab}	1.34±0.19 ^b	1.95±0.07 ^b	11.30±0.00 ^{bcd}	75.93
<i>Hericium</i> sp.	9.33±0.09 ^{abc}	1.47±0.01 ^b	1.75±0.07 ^b	10.20±0.14 ^{de}	77.25
<i>S. rugosoannulata</i> 01	8.88±0.00 ^{bcd}	2.74±0.38 ^a	2.15±0.21 ^{ab}	12.25±0.07 ^{ab}	73.98
<i>S. rugosoannulata</i> 02	8.87±0.05 ^{bcd}	2.98±0.09 ^a	2.15±0.21 ^{ab}	12.05±0.21 ^{abc}	73.95
<i>S. rugosoannulata</i> 03	8.98±0.13 ^{bcd}	2.86±0.00 ^a	2.40±0.00 ^a	12.65±0.64 ^a	73.11

Values presented are means and SD

Treatment means in each column with different letter of superscript are significantly different from each other at 5% level of significance using Tukey's HSD test.

The decrease in moisture content of grains recorded after fermentation can be attributed to the water absorption activity of mycelia from the substrate to support growth (Van Nieuwenhuijzen and Oei 2005). Several cereal crops including sorghum contain anti-nutritional factors (ANFs) such as tannin and dhurrin, which can result to reduced palatability and protein digestibility. These ANFs can be removed through several processes such as heat treatment causing the protein level to increase (Oke et al. 2004; Adeyemo and Longe 2007; Etuk et al. 2012). Autoclaving the sorghum at 121°C for 30 minutes for solid-state fermentation of mushroom mycelia influenced the increase in protein content of the grains compared to the oven dried sorghum. Meanwhile, autoclaved control increased

slightly the protein level but showed no significant difference with the oven dried control. Further supplementation of mushroom mycelia significantly increased the protein of the grains. *S. rugosoannulata* strains improved the protein by 2.25-2.85%. Similar findings showed that several heating methods, including autoclaving, reduced the tannin level in winged bean, cowpeas, and legumes. This reduction in tannin resulted in increased protein digestibility (Kadam et al. 1987; Umoren et al. 1997; Hefnawy 2011).

Chemical composition of mushrooms varies according to species (Manjunathan et al. 2011). Among the *Hericium* spp. used in this study, *H. erinaceus* is the most studied. Its fruiting body is known as a good source of carbohydrates (76.5% on dry weight), protein (18.8% DW), ash (7.52% DW), fiber (7.10% DW), and fats (2.01% DW) (Sharif et al. 2016). These mushrooms also contain several amino acids, substantial amounts of potassium and phosphorus, and aroma substances (Eisenhut et al. 1995). *S. rugosoannulata* contains 6.04 g ash, 25.89 g crude protein, 3.72 g crude fat, and 64.35 g carbohydrates per 100 g dry weight (Liu et al. 2012). The nutritional composition of these mushrooms contributed to the increased value of fat, ash, protein, and carbohydrates in sorghum after seven days of fermentation.

Antioxidant activity. The mycelium and fruiting bodies of numerous edible mushrooms contain antioxidant compounds such as phenolics, flavonoids, polysaccharides, tocopherols, glycosides, carotenoids, ergothioneine and ascorbic acid (Sánchez 2017). The role of antioxidants on improving animal nutrition and health has been well documented (Lykkesfeldt and Svendsen, 2007; Surai, 2007; Celi, 2011). This study attempted to evaluate the influence of fermenting sorghum with mycelia on its antioxidant activity (Table 2).

Table 2. Antioxidant activity of sorghum grains fermented with *Hericium* spp. and *S. rugosoannulata* strains expressed as mg of Trolox equivalent (Teq) per g.

Treatments	mg Teq/g
Control 1 (oven dried)	2.53±0.05 ^e
Control 2 (autoclaved)	2.84±0.05 ^d
<i>H. americanum</i>	3.37±0.06 ^a
<i>H. corraloides</i>	3.41±0.06 ^a
<i>H. erinaceus</i>	3.04±0.04 ^c
<i>Hericium</i> sp.	3.22±0.06 ^b
<i>S. rugosoannulata</i> 01	2.44±0.04 ^{ef}
<i>S. rugosoannulata</i> 02	2.33±0.02 ^f
<i>S. rugosoannulata</i> 03	2.34±0.02 ^f

Values presented are means and SD

Treatment means in each column with different letter of superscript are significantly different from each other at 5% level of significance using Tukey's HSD test.

Grains impregnated with *H. americanum* and *H. corraloides* both had the highest antioxidant activity (3.37-3.41 mg Teq/g) among treatments followed by *Hericium* sp. and *H. erinaceus*. Some studies reported that both fruiting body and mycelium extracts of *H. erinaceus* possess antioxidant activity using different methods (Mujić et al. 2010; Wong et al.). While *S. rugosoannulata* also exhibits antioxidant activity (Liu et al. 2012), fermentation with its mycelia have slightly decreased the antioxidant activity of sorghum in comparison with the controls.

CONCLUSION

The formulation of nutritive animal feed is crucial in improving animal performance; however, this process can be expensive. This study used *Hericium* spp. and *S. rugosoannulata* strains as enrichment for sorghum grains. The findings suggest that fermentation of sorghum using *Hericium* spp. increase protein, carbohydrates, and antioxidant activity, while *S. rugosoannulata* strains improved ash and protein contents. Fermentation of sorghum using mycelia can therefore be a low-cost and natural alternative to enhance the nutritional value as a potential ingredient for animal feed. Analysis for anti-nutritional factors before and after fermentation is recommended to further support their role in improving protein digestibility.

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