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The Effect of Types of Microorganism and Temperature on Fiber Content of Fermented Rice Straw

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ABSTRACT

The aim of this research was to determine the NDF and ADF content of rice straw fermented with several types of microorganism at different temperatures. Rice straw was obtained from rice fields in Ishigaki District, Okinawa, Japan. The types of microbes used were *Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant Saccharomyces cerevisiae,* and *Aspergillus niger*. The mainplot was temperature (25, 35, 45°C) and the subplot was microorganism. Each treatment has 3 replications. There were two groups for controls, i.e. the control without molasses and the control with molasses. The rice straw fermented within 3 weeks. The ADF content in all temperature treatments showed that *S. cerevisiae* and *A. niger* treatments were higher ($P<0.01$) than that in other microorganism treatments. Hemicellulose content in *S. cerevisiae* and *A. niger* were tend to lower ($P<0.01$) compare to others microorganism treatments in all temperature treatments. The lowest hemicelluloses content of fermented rice straw are showed by *S. cerevisiae* and *A. niger* at 45°C. These finding suggested that the ADF content were high in *S. cerevisiae* and *A. niger* treatments, but showed decrease in hemicellulose and increase in cellulose and lignin.

Key words: rice straw, fermentation, ADF, NDF

Pengaruh perbedaan mikroorganisme dan suhu pada kandungan serat jerami padi fermentasi

ABSTRAK

Tujuan dari penelitian ini adalah mengetahui kandungan NDF dan ADF pada jerami padi yang difermentasi dengan beberapa mikroorganisme pada suhu yang berbeda. Jerami padi diperoleh dari Okinawa, Jepang. Mikroorganisme yang digunakan adalah Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant Saccharomyces cerevisiae, dan Aspergillus niger. Suhu sebagai petak utama (25, 35, 45°C) dan mikoroorganisme sebagai anak petak dengan setiap perlakuan mempunyai 3 ulangan. Ada dua kelompok control yaitu control tanpa penambahan molasses dan control dengan penambahan molasses. Fermentasi dilakukan selama 3 minggu. Kandungan ADF pada semua perlakuan suhu menunjukkan bahwa S. cerevisiae dan A. niger lebih tinggi dari pada perlakuan mikroorganisme. Kandungan hemiselulosa pada perlakuan S. cerevisiae dan A. niger cenderung lebih rendah dibandingkan dengan semua perlakuan mikroorganisme pada semua suhu. Kandungan hemiselulosa terendah dari jerami padi fermentasi ditunjukkan oleh S. cerevisiae dan A. niger pada suhu 45°C. Kesimpulan bahwa kandungan ADF tinggi pada perlakuan S. cerevisiae dan A. niger tetapi menunjukkan penurunan pada kandungan hemiselulosa dan meningkat pada selulosa dan lignin.

Kata kunci: jerami padi, fermentasi, ADF, NDF

INTRODUCTION

The use of agricultural waste, such as rice straw, as animal feed has been known along times ago, mainly during the dry season in the tropics area. This is because in the dry season, grasses are difficult to obtain in sufficient quantities to feed their livestock animal (i.e. cattle, goat, sheep). However, the nutritive value of crop residues is limited by low crude protein and low digestibility (Madrid *et al*., 1997) due to high fiber content (Van Soest, 2006). In nutrition, the term of fiber refers to the component of plant-derived foods and feedstuffs that are indigestible by mammalian enzyme systems (Moore and Hatfield, 1994). In forages commonly fed to livestock, fibers refer to the plant cell wall. The cell wall contents are resistant to enzymatic action and degradation are possible through microbial intervention in the rumen. Their break down depends on the proportion of different constituents such as lignin, hemicelluloses, cellulose and silica (Crowder and Chheda, 1982).

Van Soest (1967) separated cell wall content into a fiber fraction made up of an insoluble portion in neutral detergent fiber (NDF) and another insoluble portion in acid detergent (ADF) and lignin. The NDF, which is the residue after extraction with boiling neutral solution, consist mainly of lignin, cellulose and hemicelluloses and be regarded as a measure of the plant cell material. The ADF is the residue after refluxing with acid solution represents essentially the crude lignin and cellulose fractions of plant material but also includes silica. The determination of ADF is particularly useful for forages as there is a good statistical correlation between ADF and the digestibility (McDonald *et al*., 1994). Most predictions of forage nutritive value are based on estimated cell wall fractions, because the greatest determinant of the extent of digestion is the degree of lignifications and cell wall content (Mannetje and Jones, 2000).

The NDF and ADF of rice straw have been determined in many studies. Doyle *et al*. (1986) reported NDF content of rice straw are high (up to 86% of the dry matter) indicated that the more readily digestible components of the cell contents only presented in small amounts. Yulistiani *et al*. (2000) reported NDF content of rice straw varied from 65.3 to 75.0% ADF content varies from 48.5 to 60.1% dry matter basis, while Surahmanto (1991) reported, untreated rice straw contained 78.93% NDF, 20.28% hemicelluloses, 36.65% cellulose and 4.59% lignin. To improve nutritive value of rice straw, the treatment using microbes is one of promising method by decomposing fiber (lignin, hemicelluloses and cellulose) and increasing crude protein content. The quality of rice straw fermentation is affected by type of microbes and the environment (temperature). The effects of types of microorganism and temperatures on the fiber content of fermented rice straw are not well known. Therefore, the aim of this research was to determine the NDF and ADF content of rice straw fermented with several types of microorganism at different temperatures.

MATERIALS AND METHODS

Materials

Rice straw was obtained from rice fields in Ishigaki District, Okinawa, Japan. The types of microbes used were *Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant Saccharomyces cerevisiae,* and *Aspergillus niger.* Water was added to rice straw prior to fermentation. Molasses were added to rice straw 5% Dry Matter basis.

Split plot block randomized design was used in this experiment to study the effect of added different microbes and different temperature on the nutritive value of fermented rice straw. Treatments were assigned as mainplot and subplot. The mainplot was temperature (25, 35, 45°C) and the subplot was microorganism

(*Lactobacillus fermentum, Bacillus subtilis, Bacillus coagulant, Saccharomyces cerevisiae* and *Aspergillus niger*. Each treatment has 3 replications. There were two groups for controls, i.e. the control without molasses and the control with molasses. The nutritive values of rice straw as a material in this experiment are shown in Table 1.

Table 1. The Chemical Compositions of Rice Straw Material

Chemical composition	%DM
Dry Matter	89.73*
Crude Protein	2.83
Ash	17.41
Neutral Detergent Fiber	69.24
Acid Detergent Fiber	41.24
Acid Detergent Lignin	6.32

DM : Dry Matter; * in Fresh Matter

Methods

The methods used in the fermentation process were as follows: 1). Rice straws were chopped into small pieces at 1-2 cm length; 2). Water was added to the rice straws at ratio 2 : 3 (w/w); 3.) All types of microbes were cultivated in culture media agar specific for each types in plate and the occurred cell then were transferred to increase the cell numbers in culture media broth specific for each types in a 100 ml flasks to make microbes juice. The cell was then incubated for 3 days of each type; 4). The 5% (v/w) of the microbe juice and 5% (w/w) molasses were mixed with 1 kg of the rice straw based on dry matter. The control treatments were not mixed with molasses and the other one were mixed with molasses.; 5). Approximately 150 g of mixture were transferred into a plastic pouches; 6). Fermentation method: Fermentation bag (using plastic pouche) were sealed by vacuum sealer (anaerob) in groups of control, *L. fermentum*, *B. subtilis* and *B. coagulant*. Exception in group fermented with *S. cerevisiae* and *A. niger* the ferment plastic pouches were not sealed but were loosely closed (aerob).; 7). Rice straw bags were stored at 25, 35 and 45°C according to the treatment for 3 weeks.

After 3 weeks fermentation, the fermented materials were dried in a 60-70°C drying oven for 48 hours for determing water content. Dried samples were grounded prior to chemical composition analysis, NDF, ADF and ADL (AOAC, 1985).

Statistical Analysis

The model was $Y_{ijk} = \mu + \alpha_i + B_k + \delta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$. Where Y_{ijk} = observation from temperature *i*, receiving microorganism *j*, in replication *k*; μ = the overall of mean; B_k= the effect of block B (*k* = 1, 2, 3); α_i= the effect of temperature α (*i* = 25^{o}C, 35 ^{o}C and 45 ^{o}C); δ_i= error of temperature *I*; β_j= the effect of microorganism β (*j* = Control, Control+molasses, *L. fermentum*, *B. subtilis*, *B. coagulant*, *S. cerevisiae* and *A. niger*); $(\alpha\beta)_{ij}$= the interaction effect between temperature α and microorganism β; ε_{ijk} = error of temperature *i*, microorganism *j* and block *k.* The Least Significant Different was performed to determine the significant differences among treatments and when interaction between temperature and microorganisms were found (Sastrosupadi, 2000).

RESULT AND DISCUSSION

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of fermented rice straw are presented in Table 2 and Table 3. The NDF content was not affected ($P>0.05$) by temperatures, microorganisms and the interaction between temperatures and microorganisms, but the ADF content was influenced ($P<0.01$) by temperatures, microorganisms and its interaction. There were no temperatures effect ($P>0.05$) on hemicellulose, cellulose and lignin content of fermented rice straw, but these were an effect of microorganism ($P<0.05$). The hemicellulose was affected ($P<0.05$) by interaction between microorganism and temperature.

Table 2. Neutral Detergent Fiber Content of Fermented Rice Straw.

Microorganism	Temperature			
	25°C	35°C	45°C	Mean
	----------%DM---------			
Control	64.74	64.56	66.79	65.36
Control+ molasses	60.90	59.67	61.93	61.88
L. fermentum	62.88	61.98	61.72	62.19
B. subtilis	62.91	62.47	64.06	63.15
B. coagulans	64.46	61.90	62.53	62.97
S. cerevisiae	65.57	65.01	62.49	62.19
A. niger	65.93	63.11	63.95	64.33
Mean	63.91	63.12	62.42	

DM: Dry Matter

Table 3. Acid Detergent Fiber Content of Fermented Rice Straw.

Microorganism	Temperature		
	25°C	35°C	45°C
	----------% DM----------		
Control	39.98^{b}	39.11^{bc}	39.70^{b}
Control+ molasses	37.52^{c}	37.08^{c}	38.99^{b}
L. fermentum	38.19^{bc}	39.18^{bc}	38.74^{b}
B. subtilis	38.05^{bc}	39.60^{b}	38.05^{b}
B. coagulans	39.11^{bc}	39.61^{b}	37.90^{b}
S. cerevisiae	44.14^{a}	43.16^{a}	46.51^{a}
A. niger	44.36^{a}	42.15^{a}	46.82^{a}

Different letters in the same column are different (P<0.01)

DM: Dry Matter

Table 4. Hemicellulose Content of Fermented Rice Straw.

Microorganism	Temperature		
	25°C	35°C	45°C
	----------%DM----------		
Control	24.76^{ab}	25.45^{ab}	27.08^{a}
Control+ molasses	23.38^{ab}	29.05^{a}	22.95^{b}
L. fermentum	24.69^{ab}	22.80^{b}	22.97^{b}
B. subtilis	24.86^{ab}	22.87^{b}	26.01^{ab}
B. coagulans	25.36^{a}	22.29^{b}	24.63^{ab}
S. cerevisiae	21.43^{b}	21.85^{b}	15.98^{c}
A. niger	21.57^{ab}	20.96^{bc}	17.14^{c}

Different letters in the same column are different (P<0.01)

DM: Dry Matter

The ADF content in all temperature treatments showed that *S. cerevisiae* and *A. niger* treatments were higher (P<0.01) than that in other microorganism treatments. It could be explained by the cellulose and lignin content in ADF were higher than other temperature treatments (Table 5, Table 6 and Illustration 1), while ADF is a portion of plant fiber including the cellulose and lignin from cell walls and variable amount of xilans and other components (Jung, 1997).

Hemicellulose content in *S. cerevisiae* and *A. niger* were tend to lower (P<0.01) others microorganism treatments in all temperature treatments (Table 4). This result suggested that *S. cerevisiae* and *A. niger* produced lignocellulases enzyme to breakdown lignocelluloses into cellulose and lignin. This suggestion was supported by the study of Vijaya and Singaracharya (2005), who proved *A. niger* to be potential producers of enzyme involved in fermentation of lignocellulases. This is showed by the higher (P<0.01) cellulose and lignin content at those treatments (Table 5 and Table 6). The decreased hemicelluloses content increased cellulose and lignin levels (Givens *et al*., 1988; Mason *et al*., 1988). The lowest hemicelluloses content of fermented rice straw are showed by *S. cerevisiae* and *A. niger* at 45°C (Illustration 2). These indicated that those fungi may produce enzyme to decreased hemicellulose at 45°C.

Table 5. Cellulose Content of Fermented Rice Straw.

Microorganism	Temperature			
	25°C	35°C	45°C	Mean
	----------%DM----------			
Control	34.93	34.05	34.30	34.43^{b}
Control+ molasses	32.61	32.40	33.73	32.91^{b}
L. fermentum	33.57	34.25	33.46	33.76^{b}
B. subtilis	32.32	34.84	32.90	33.35^{b}
B. coagulans	34.09	32.43	32.81	33.11^{b}
S. cerevisiae	34.05	36.04	37.99	36.48^{a}
A. niger	36.99	34.87	38.87	36.91^{a}
Mean	34.27	34.12	34.87	

Means in the same column with different letters are different (P<0.01)

Table 6. Lignin Content of Fermented Rice Straw.

Microorganism	Temperatu	
	25°C	35°C
	----------%DM	
Control	5.04[c]	5.06[b]
Control+molasses	4.91[c]	4.68[b]
L. fermentum	4.62[c]	4.93[b]
B. subtilis	5.73[c]	4.76[b]
B. coagulans	5.02[c]	7.18[a]
S. cerevisiae	8.73[a]	7.13[a]
A. niger	7.37[b]	7.28[a]

Different letters in the same column are different (P<0.01)
DM: Dry Matter

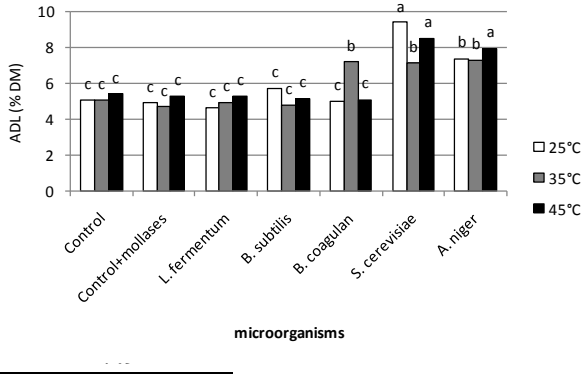

Illustration 3. The LSD test for lignin content of fermented rice straw. The different letters (a,b,c) indicates different (P<0.05)

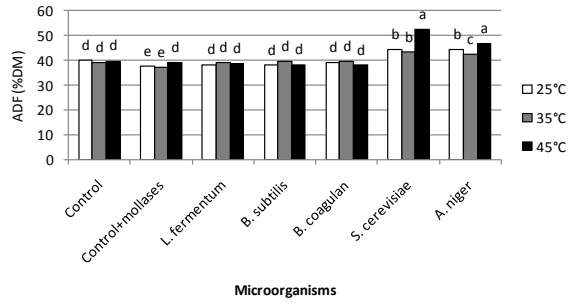

Illustration 1. The LSD test for ADF content of fermented rice straw. The different letters (a,b,c,d) indicates different (P<0.05)

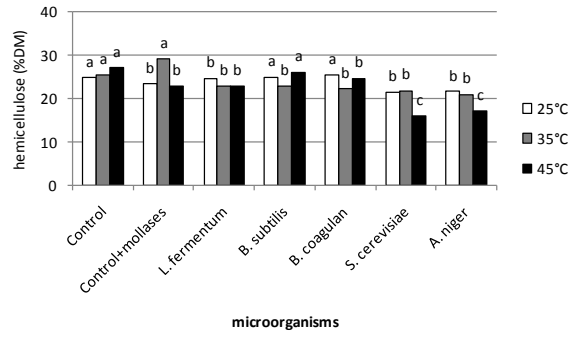

Illustration 2. The LSD test for hemicellulose content of fermented rice straw. The different letters (a,b,c) indicates different (P<0.05)

CONCLUSION

The ADF content were high in *S. cerevisiae* and *A. niger* treatments, but showed decrease in hemicellulose and increase in cellulose and lignin. It can be concluded that rice straw fermented with microorganism can improved its nutritive value. Moreover, fermentation time and possibility on usage as animal feed has been not studied in this study. Further investigation is required.

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