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Effects of Fermentation on the Nutritional Composition, Mineral Content and Physical Characteristics of Banana Leaves

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Abstract. Banana leaves are one of the most abundant agriculture wastes in Malaysia. However, it is not suitable to be given directly to ruminant as feed and need to be pre-treated. Therefore, the objectives of this study were to evaluate the nutritional composition of banana leaves fermented with different additives including molasses, effective microorganisms (EM) and urea on the nutritional composition, mineral content and physical characteristics. Samples of banana leaves were divided into three treatment groups; control (no additives), treatment 1 (3% of molasses + 3% EM + 0.5% urea) and treatment 2 (5% of molasses + 4% EM + 1% urea). Samples were fermented for 21 and 35 days. The pH value, nutritional composition, mineral content and physical characteristics were determined and subjected to analysis of variance. There is significance different ($P<0.05$) in pH value for treated samples fermented for 35 days. Moisture and crude protein content were significantly higher ($P<0.05$) for both treatment groups, fermented for 21 and 35 days. The aroma for treated samples were pleasantly acidic compared to the control. In conclusion, fermentation for 21 days with molasses, EM and urea improves the nutritional composition of banana leaves, therefore it can be considered as an appropriate pre-treatment method to produce an alternative material for animal feed.

1. Introduction

Banana or scientifically known as *Musa* is from the *Musaceae* family and can be found grown in tropical and subtropical areas such as Hawaii, Malaysia, Thailand, Indonesia and Philippines. This plant does not have growing season and it is high potential to be available throughout the year [1]. It is planted 35,156 hectares in Malaysia with 376,690 metric tonne per year of the production [2]. The plant took up to nine until twelve months to grow before the fruits can be harvested. Upon harvested, the banana plant will become banana by-products that can be divided into two which are banana crop residue (leaves and pseudo-stems) and the classification and packaging process wastes (bananas and bunch) [3]. The residue from the cutting down of banana plant often wasted. Typically, it is composted and act as natural fertilizers to the soil [4] and create an additional environmental burden when delivered to the local landfill [1].

Banana crop residue which are the leaves, has high potential as one of the sources as animal feed, especially to ruminants, such as cattle, sheep, goats and buffalo [5]. The ruminant industry in Malaysia is growing in recent years but the major constraint is the lack of feed availability in the country [6]. Ruminant production relies on feeds comprising of fodder and crop residues [7]. In livestock production, feed cost contributes 70% of the total cost and the cost is expected to rise as the time goes by [8, 9, 10]. Thus, by using banana crop residue as an alternative feed for ruminant can reduce feed cost in ruminant production. Ruminants are able to digest the dry matter of banana leaves and pseudo-stems. As for the leaves, it is 65% digestibility and 75% digestibility for the pseudo-stems. It is recommended to include urea and highly digestible forage as supplements for the leaves and pseudo-stems to meet the maintenance requirements of ruminants [11]. In addition, it could provide additional energy for ruminant and gives benefit including reducing expenses [4]. Moreover, it can be source of fibre in ruminant feed and provide energy [12]. Unfortunately, banana leaves contain low dry matter and high moisture content [3], hence not suitable to give directly to ruminant as feed. To improve the quality, it can be pre-treated by ensiled it using fermentation process with addition of additives and supplementation to rise the dry matter content and lower the moisture content. Local farmers can make their own silage using local banana crop residue rather than depending on imported feed [13]. Silage can last longer and could be one of the solutions to reduce the dependency towards imported feed and grazing land that has poor management. Therefore, this study focused on the effects of fermentation on the nutritional composition, mineral content and physical characteristics of banana leaves.

2. Materials and Methods

2.1. Experimental Design and Preparation of the Samples

Banana leaves of variety *Berangan* were obtained from Agro Techno Park, Universiti Malaysia Kelantan, Jeli Campus and transported to the laboratory in clean polyethylene bags. The samples were separated from the midrib (the middle part that divide the banana leaves), chopped to approximately 2cm, wilting and were assigned to three treatment groups; control, treatment one and two. There were no additives added in the silage for control. Treatment one and two were added with three different additives which were molasses, effective microorganisms (EM) and urea. Treatment one has 3% of molasses, 3% of EM and 0.5% of urea that were mixed with fresh banana leaves. Meanwhile, treatment two has 5% of molasses, 4% of EM and 1% of urea that were mixed with fresh banana leaves. There were three replications for all control and treatment samples with the amount of banana leaves used for each are 200 g. All treatment samples were compacted into 2-quart glass jars and were degassed, sealed and kept at ambient temperature for 21 and 35 days.

2.2. pH Determination

The pH of the samples was determined according to the method of [14]. Two grams of sample was mixed in 20 ml distilled water and allowed to stand for 15 minutes and filtered with Whatman filter paper. The pH of the silage was determined by LAQUAtwin Compact pH Meter by Horiba.

2.3. Chemical Analysis

All samples were analysed using proximate analysis according to Association of Official Analytical Chemists (AOAC) International [14] to determine dry matter (DM) (Method 934.01) by oven drying at 70˚C for 48 h, crude protein (CP) (Method 2001.11) conducted using Kjeldahl method and involved three steps which are digestion, distillation and titration, crude fibre (CF), ether extract (EE) (Method 954.02) was carried out using a Soxtec 2055, Fat Extraction System machine by FOSS, USA and ash was determine after incinerated for 3 h at 550˚C.

2.4. Mineral Analysis

In this research, two elements that were analysed were calcium (Ca) and iron (Fe). The determination of calcium and iron content in the sample, it was performed using Atomic Absorption Spectroscopy (AAS). The mineral method is a continuation from the ash method based on [15]. Calcium and iron of each sample were calculated using the standard calibration that were obtained after it were analysed using Atomic Absorption Spectrometry (AAS).

2.5. Physical Characteristics Analysis

Physical analysis is analysis that is done based on the look and appearance of the sample, by five senses which are sight, smell, hearing, taste and touch. All samples were physically examined by their physical appearance and texture, colour, mould presence and aroma. The parameter is based on a manual by [16].

2.6. Data Analysis

Data from the experiment was analysed by using analysis of variance (ANOVA), SPSS version 23 software package with significance different set at $P<0.05$. The differences between treatment were analysed using Duncan multiple range test of the same package.

3. Results and Discussion

3.1. pH Value of Fermented Banana Leaves

The different in pH of each sample after 21 and 35 days of fermentation are varying. Table 1 shows the pH value of the fermented banana leaves based on their mean value and standard error after being fermented for 21 days and 35 days. The highest pH value was sample from treatment two that was fermented for 35 days, 8.8±0.09, and the lowest was sample from treatment two that was fermented for 21 days, 3.6±0.50. The pH value for all sample are within the pH range for 21 days of fermentation. Duponte et. al [1] reported pH of 5.6 for fermented banana stalk (control) and pH of 5.7 for the fermented banana stalk (treated) when the silage achieved 21 days of fermentation. At this phase, pH is lowered from 6.0 to 3.8 to 5.0 because lactic acid bacteria in the silage use sugar from soluble carbohydrates available from forages and molasses to produce organic acids. When the pH is lowered, it inhibits the enzyme activity and the cell growth. After the silage exceed 21 days of fermentation, the pH increases up to 7.0 pH [17]. During this phase, a large number of clostridia bacteria is grown as these bacteria are favoured by humid condition with high pH value. Besides, lactic acid bacteria slowly decrease as clostridia bacteria are responsible for the production of CO2 and butyric acid [18]. Anaerobic bacteria will not produce lactic acid and start producing butyric acid. This led to sour silage and pH value can be above 5.0. During this phase, mould and yeast start to active.

Table 1. The comparison of pH value between fermented banana leaves.

	Fermented Banana Leaves					
	21 Days			35 Days		
	Control	T1	T2	Control	T1	T2
pH Value	3.8±0.44^{a}	5.4±0.35^{a}	3.6±0.50^{a}	6.9±0.06^{a}	8.8±0.09^{c}	8.0±0.20^{b}

Notes: ^{abc} means in the same row with different superscript are significantly different ($p<0.05$), SE-Standard error.
Control; no additives, T1; 3% molasses+3% EM + 0.5% urea, T2; 5% molasses+4% EM+1% urea.

3.2. Chemical Composition and Mineral Content of Fermented Banana Leaves

The comparison of nutritional composition between control and treatment samples of fermented banana leaves is shown in Table 2. The composition that were analyzed including dry matter (DM), moisture, crude protein (CP), crude fibre (CF), ether extract (EE) and ash. The mineral that were analysed were calcium (Ca) and iron (Fe) and the comparison leaves is shown in Table 2. From the results, banana leaves that fermented with additives contain low Ca content compared to that were fermented without additives. Meanwhile, Fe content in all sample was similar.

Table 2. The comparison of chemical composition between fresh and fermented banana leaves.

Composition (%)	Fermented Banana Leaves					
	21 Days			35 Days		
	Control	T1	T2	Control	T1	T2
DM	33.95±0.09^{c}	32.54±0.27^{b}	31.45±0.02^{a}	35.35±0.69^{b}	33.91±0.46^{b}	29.75±1.02^{a}
Moisture	66.05±0.09^{a}	67.45±0.27^{b}	68.55±0.02^{c}	64.65±0.69^{a}	66.09±0.46^{a}	70.25±1.02^{b}
CP	11.52±0.11^{a}	15.44±1.47^{b}	16.66±0.18^{b}	14.59±0.16^{a}	16.46±0.29^{b}	16.70±0.22^{b}
CF	27.73	32.55	31.67	30.37	31.70	31.30
EE	4.15±0.27^{a}	3.99±0.24^{a}	4.41±0.14^{a}	5.35±0.05^{b}	4.77±0.07^{ab}	4.64±0.25^{a}
Ash	20.52±0.19^{a}	20.42±0.19^{a}	20.22±0.19^{a}	12.91±0.53^{a}	11.94±0.00^{a}	12.20±0.30^{a}
Calcium (Ca)	0.14±0.00^{b}	0.10±0.00^{a}	0.10±0.00^{a}	0.13±0.00^{c}	0.11±0.00^{a}	0.11±0.00^{b}
Iron (Fe)	0.03±0.00^{a}	0.02±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}

Notes: ^{abc} means in the same row with different superscript are significantly different ($p<0.05$), SE-Standard error.
Control; no additives, T1; 3% molasses+3% EM + 0.5% urea, T2; 5% molasses+4% EM+1% urea.

The DM, CF and ash reported by [11] were 82.3%, 15.6% and 38.8% respectively, which were contrast with the results obtained for this study. However, the CP and EE content were 12.5% and 5.9% respectively, there were ranged with the current results. The DM content in all fermented samples were a bit higher than the nutrients requirements needed by ruminants, however, the CP content was in the range which are 7% to 18% [19]. The fresh banana stalk contained higher dry matter content and lower moisture content than the other fermented banana stalk [1]. During the fermentation process, high moisture content is produced [20]. Hence, this explained why all fermented banana leaves sample had high moisture content than fresh leave samples as they undergo fermentation process. the DM content in the silage is lower as the inclusion of molasses is higher compared to the DM content in silage with no molasses [21]. The addition of molasses lead to rich nutrition for material and abundant of other deteriorating bacteria [22]. The silages with higher lactic acid concentrations tend to be higher nutritive, palatable and sweet smelling, which results in greater nutrient intake [23]. Additives as molasses contain

high levels of soluble carbohydrate, are likely to be able to produce sufficiently high levels of lactic acid. Therefore, this will help in improving the fermentation and quality of silage [24].

3.3. Physical Characteristics of Fermented Banana Leaves

The results of physical characteristics of fermented banana leaves in terms of texture, colour, aroma and mould presence are shown in Table 3. The texture of fermented banana leaves was firm and dry for control and samples in T1 fermented for 21 days. While for samples of T1 fermented for 35 days and T2 for both 21 and 35 days was slightly wet. The silage texture is affected by the water content of the material at the beginning of the ensilage process [25]. The colour identified from all samples were yellowish brown. The good quality of silage should be bright green to yellow or brownish depending on the material used [26].

Table 3. The comparison of field assessment between fermented banana leaves.

Physical Characteristic	Fermented Banana Leaves					
	21 Days			35 Days		
	Control	T1	T2	Control	T1	T2
Texture	Firm and dry	Firm and dry	Firm and slightly wet	Firm and dry	Firm and slightly wet	Firm and slightly wet
Colour	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown	Yellowish brown
Aroma	Pleasantly acidic, very fruity and sweet aroma	Pleasantly acidic, slightly fruity and sweet aroma	Pleasantly acidic, slightly fruity and sweet aroma	Very fruity and sweet aroma	Pleasantly acidic, slightly fruity and sweet aroma	Pleasantly acidic, very fruity and sweet aroma
Mould Presence	No	No	No	Yes	No	No

The aroma presence from fermented banana leaves is acidic for all samples.

The smell of acids arises due to the formation of acids, especially lactic acid fermentation conducted by lactic acid bacteria during the silage process takes place [27]. Mould presence were observed only in control samples fermented for 35 days. Overall presence of mould considered as very low. Good technique applied during silo filling lead to quick achievement of anaerobic condition and caused minimum growth of mould and fungi [28].

4. Conclusion

Considering the pH value, chemical composition and physical characteristics, fermentation for 21 days with additives as molasses, EM and urea had considerable impact on nutritional composition of banana leaves which suggested that additives were necessary when the banana leaves were ensiled. As the fermented banana leaves contained different amount of chemical composition, hence, they can be suggested for different uses and needs. Therefore, it can be considered as an appropriate pre-treatment method to produce an alternative material for animal feed.

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