The effects of fermentation time and em4 dose on nutrient content of kepok’s peel as animal feed

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Abstract. Kepok banana peel is a waste product from the processing of kepok banana which can be used as an alternative in animal feed. Low crude protein and high crude fiber become the main problems in the utilization of kepok’s peels. One way to improve its quality is by fermentation. The starter of fermentation used is EM4. The purpose of this research was to determine the effect of dose and length of fermentation against nutritional content of kepok’s peels. This research was designed using completely randomized design factorial with 3 replications and continued using the duncan test. The kepok’s peels were given EM4 at doses of 1, 3, and 5% and then fermented for 3, 5, and 7 days. The results of this research showed that the dose of 5% and 5 day fermentation time were optimal and efficient to increase water and crude protein content and reduce levels of dry matter and crude fiber of banana peel. There was no interaction between dosage and fermentation time for each research parameter.

1. Introduction
Kepok’s peel is a waste material from the results of the processing of food products made from kepok banana. Kepok’s peel is known to be used as animal feed. The nutritional content of kepok’s peels are quite complete, such as carbohydrates, fats, proteins, calcium, phosphorus, iron, B vitamins, vitamin C, and water [1]. However, the use of kepok banana peel as animal feed has problems such as low crude protein and high crude fiber. Fermentation is one way to improve the quality of nutrients from animal feed ingredients. Fermentation is the process to degrade organic compounds into simpler compounds by using the microbes.

Effective microorganism (Em4) is one of the fermentation starters that can be used to improve the nutritional quality of kepok’s peels. The results of previous research on fermentation of coconut pulp using EM4 showed that the microbes in EM4 were able to increase crude protein and reduce crude fiber in fermented coconut pulp [2]. [3] The use of EM4 can increase crude protein content in vegetable waste. From these researches, different dosage levels are needed in order to increase the nutrient content of food crop waste that is kepok banana peels. [4] It is stated that EM4 is a mixed culture of various beneficial microorganisms especially Lactobacillus, photosynthetic bacteria, actinomycetes, yeast and fungi so that it can be used as a fermentation starter.

The low quality of nutrition is a main problem in the utilization of food crop and agricultural waste as animal feed. Fermentation method is a solution to improve the nutritional value of agricultural
waste, and ingredients that are not efficient to be used as animal feed ingredients. Fermentation in legumes can improve the nutritional content and functional components compared to the original ingredients [6]. One of the fermentation starters that can be used to improve the nutritional quality of agricultural waste, especially kepok’s peel waste which is cheap and easy to obtain is by using EM4.

2. Methods
This research was carried out experimentally using a completely randomized design (CRD) factorial pattern with 2 factors, each factor consists of 3 levels with 3 replications followed by Duncan’s test to find out the level / level in a significantly different sample. The material used was 1 kg of kepok’s peels for each test and then given a dose of EM4 with different levels of 1% (D1), 3% (D2), and 5% (D3). Then, it was fermented for 3 days (L1), 5 days (L2), and 7 days (L3). The treatment in each research was carried out with 3 replications so that as a whole produced 27 treatment combinations namely 3 x 3 x 3 unit experiments. Sampling test parameters and methods were as follows:

2.1 Water and dried material
Water in proximate analysis is all liquid that evaporates on heating for some time at a temperature of 105-110 °C. Measurement of water by the oven method can be searched by using the formula [5]. Meanwhile, dried material can be calculated by subtracting 100% of the percentage of water content obtained [7].

2.2 Crude fiber
Crude fiber is part of the diet consisting of components of cellulose, hemicellulose, and lignin. It can be searched by using the formula [7].

2.3 Crude protein
Crude protein is a complex organic compound composed of elements of carbon, hydrogen, and oxygen, but all proteins contain nitrogen. Crude protein measurement by method of Kjedhal semimikro can be calculated by using the formula [7].

3. Results and Discussion
Improving the nutritional quality of feeding ingredients can be used in various ways, one of them is by fermentation technology. This fermentation utilizes the work of microorganisms to degrade organic compounds into simpler compounds. The results obtained are as follows:

3.1 Water and dried material
The results of the Kepok’s peel samples tested were as follows:

| Dose of EM4 (D) | Duration of fermentation (L) | Mean |
|----------------|-----------------------------|------|
|                | L1 (3 days) | L2 (5 days) | L3 (7 days) |      |
| D1 (1 %)       | 4.59       | 5.54       | 6.90       | 5.68c|
| D2 (3 %)       | 5.69       | 6.58       | 7.55       | 6.60b|
| D3 (5 %)       | 6.69       | 7.62       | 8.99       | 7.77A|
| Mean           | 5.66c      | 6.58b      | 7.81a      |      |

Note: Different superscripts in the same row and column shows a very significant differences (P <0.01).

Based on the results of diversity analysis, It was shown that the EM4 dose and fermentation time showed a very significant difference (P <0.01) on changes in water content in fermented kepok’s peels. However, there was no interaction between the two factors. Based on the results of Duncan's mean range test (DMRT), it was known to increase in the water content of the fermented kepok’s peel. Based on the EM4 dose used, the highest water content was found in em4 concentration of 5 % (D3) namely 7.77 % and the lowest water content was found in em4 concentration of 1 % (D1) namely
5.68%. Meanwhile, when viewed from the fermentation time, the highest water content was found in 7 days fermentation (L3) namely 7.81% and the lowest water content was found in 3 days fermentation (L1) namely 5.66%.

The higher dose given EM4 and the longer fermentation time will increase the water content on a kepok’s peel fermentation. This is presumably because the higher doses given EM4 and the longer the fermentation time, the microbes that work will be more and more and growing that will carry out the metabolism of carbohydrates on a kepok’s peel. The side effect of carbohydrate metabolism are water and steam. According to Murray et al. [8] carbohydrate metabolism was separated into aerobic and non aerobic phases. The aerobic phase of the main product was pyruvic acid and the anaerobic phase of the main product as lactic acid. This results undergoes further oxidation to carbon dioxide (CO$_2$) and water (H$_2$O).

After the water content was known, the dry matter can be calculated. The results of the dried material in the samples tested were as follows:

| Table 2. Dry matter (%) |
|------------------------|
| Doe of EM$_4$ (D) | Duration of fermentation (L) | Mean |
| L$_1$ (3 days) | L$_2$ (5 days) | L$_3$ (7 days) | |
| D$_1$ (1%) | 95.41 | 94.46 | 93.10 | 94.32$^A$ |
| D$_2$ (3%) | 94.31 | 93.42 | 92.45 | 93.40$^B$ |
| D$_3$ (5%) | 93.31 | 92.40 | 91.01 | 92.23$^C$ |
| Mean | 94.34$^a$ | 93.42$^b$ | 92.19$^c$ |

Note: Different superscripts in the same row and column show very significant differences (P <0.01).

Based on the results of diversity analysis, It was shown that the EM4 dose and fermentation time had a very significant effect (P <0.01) on changes in the levels of dry matter on the fermented kepok’s peel. But there as no interaction between the two factors. Based on the results of Duncan's mean range test (DMRT), it was found that there was a decrease in the dry matter of the fermented kepok’s peel. Based on the dose of EM4 used, the highest decrease dry matter was found in em4 concentration of 5 % (D3) namely 92.23% and the lowest decrease in dry matter was found in em4 concentration of 1 % (D1) namely 94.32%. Meanwhile in terms of fermentation time, the highest decrease in dry matter was found in 7 days fermentation (L3) namely 92.19% and the lowest decrease in dry matter was found in 3 days fermentation (L1) namely 94.34%.

The higher EM4 dose and the longer fermentation time will decrease dry matter. This is presumably because the higher the EM4 dose and the duration fermentation time, more microbes will grow and develop along with the time of fermentation so that the water content produced will be more and the dry matter will decrease. According to Ohshima et al. [9], it was stated that the fermentation of a feed ingredient with lactic acid bacteria can reduce dry matter.

### 3.2 Crude fiber

The content of crude fiber of fermented kepok’s peels was as follows:

| Table 3. Crude fiber |
|---------------------|
| Doe of EM$_4$ (D) | Duration of fermentation (L) | Mean |
| L$_1$ (3 days) | L$_2$ (5 days) | L$_3$ (7 days) | |
| D$_1$ (1%) | 23.36 | 22.63 | 21.29 | 22.43$^A$ |
| D$_2$ (3%) | 22.51 | 21.30 | 20.44 | 21.42$^B$ |
| D$_3$ (5%) | 21.40 | 20.82 | 19.39 | 20.54$^C$ |
| Mean | 22.42$^a$ | 21.58$^b$ | 20.37$^c$ |

Note: Different superscripts in the same row and column shows a significant differences (P <0.01).

Analysis of diversity showed that the EM4 dose and fermentation time showed a very significant difference (P <0.01) on the decrease the level of crude fiber on the fermented of kepok’s peel.
However, there was no interaction between the two factors. Based on the results of Duncan's mean range test (DMRT), it was found that there was a decrease in the level of crude fiber on the fermented kepok’s peel. Based on the EM4 dose used, the highest decrease in crude fiber content was found in em4 concentration of 5% (D3) namely 20.54% and the lowest decrease in crude fiber content was found in em4 concentration of 1% (D1) namely 22.43%. Meanwhile, when viewed from the duration of fermentation, the highest decrease in crude fiber content was found in 7 days fermentation (L3) namely 20.37% and the lowest decrease in crude fiber content was found in 3 days fermentation (L1) namely 22.42%.

The higher dose of EM4 given and the longer fermentation time, will decrease the crude fiber content from kepok’s peel. This is presumably because EM4 contains cellulolytic bacteria that produces cellulase enzymes. This enzyme is able to degrade and stretch lignosellose and lignohemiselulosa bonds. Then, the more EM4 doses are given and the longer fermentation time, the more microbes will grow and develop along with the increase in fermentation time. According to Razie et al. [10] cellulose can be degraded by cellulase enzymes. These enzymes degrade insoluble cellulose molecules into mono or simple dissolved disaccharides so that they can be used by microbes as an energy source. Pasaribu et al. [11] the advantage of Lactobacillus in em4 in degrading crude fiber is that bacteria did not produce crude fiber in its activity, so it is more effective in reducing crude fiber than yeast and fungus.

### 3.3 Crude protein

The crude protein content of fermented kepok’s peels was as follows:

| Dose of EM4 (D) | Duration of fermentation (L) | Mean |
|----------------|-----------------------------|------|
|               | L₁ (3 days)                 | L₂ (5 days) | L₃ (7 days) |
| D₁ (1%)       | 7.88                        | 8.19          | 7.04          | 7.71<sup>C</sup> |
| D₂ (3%)       | 8.76                        | 9.18          | 8.12          | 8.69<sup>B</sup> |
| D₃ (5%)       | 9.24                        | 11.13         | 9.09          | 9.82<sup>A</sup> |
| Mean          | 8.63<sup>b</sup>            | 9.50<sup>a</sup> | 8.08<sup>b</sup> |

Note: Different superscripts in the same row and column shows a significant differences (P <0.01).

Based on the results of the diversity analysis, It was shown that the EM4 dose and fermentation time had a very significant difference (P <0.01) on changes in crude protein levels of fermented kepok’s peels. However, there was no interaction between the two factors. Based on the results of Duncan's mean range test (DMRT) it was found that there was a increase the level of crude protein on the fermented kepok’s peel. Based on the EM4 dose used, the highest increase in crude protein content was found in em4 concentration of 5% (D3) namely 9.82% and the lowest increase in crude protein content was found in em4 concentration of 1% (D1) namely 7.71%. Meanwhile, when viewed from the fermentation time, the highest increase in crude protein content was found in 5 days fermentation (L2) namely 9.50% and the lowest increase in crude protein content was found in 7 days fermentation (L3) namely 8.08%.

The higher EM4 dose given will increase the crude protein content of fermented kepok’s peel. This is presumably because the higher the EM4 doses are given, the more microbes that degrade organic matter into single cell proteins. It is known that the microbes that make up the body's cells are single cell proteins. Then, the more microbes that work will increase single cell protein. According to [12], the crude protein content after fermentation often increases due to microbes that have good reproductive growth, it can convert more cellulose and organic matter into single cell protein (SCP). The microbes during the fermentation process can release free amino acids and bioactive peptides [13]. The length of time of fermentation causes a decrease in crude protein content. This is presumably because microbes in the fermentation process convert protein compounds into volatile ammonia (NH₃) compounds. The longer the fermentation time, the more protein is converted into ammonia.
which then evaporates so that the crude protein decreases. Proteins are degraded into dipeptides and so on into NH$_3$ or N$_2$ compounds which are lost through evaporation [14]. Decreasing levels of crude protein caused by proteolytic activity by fungi [15].

4. Conclusion

The conclusion of this study is that the use of EM4 dose of 5% and 5 day fermentation time were optimal and efficient to increase water and crude protein content and reduce levels of dried material and crude fiber. There was no interaction between dosage and fermentation time for each research parameter. 5 % of em4 concentration and a 5 days of fermentation time are recommended to ferment of kepok’s banana peel.

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