Chemical composition and in vitro gas production of cassava peel silage using different preservatives

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Abstract. This study aimed to evaluate the effects of using different types and levels of preservatives on chemical composition and in vitro gas production (IVGP) of cassava peel silage. The following treatments were applied: T0: cassava peel without preservative; T1: T0 + molasses 2%; T2: T0 + molasses 4%; T3: T0 + rice bran 2%; T4: T0 + rice bran 4%; T5: T0 + cassava meal 2% and T6:T0 + cassava meal 4% with 3 replications arranged in a completely randomized design (CRD) for the chemical composition variable and randomized block design (RBD) for IVGP variable. The results showed that treatments significantly affected (P<0.01) DM, OM, and EE contents, significantly affected (P<0.05) IVGP but did not significantly affect CP, CF contents, potential, and rate of gas production (P>0.05). Treatment T5 seems to have higher values of IVGP and ME (159.1±7.19 ml/500 mgDM; 11.1±0.36MJ/kg DM, respectively) compared to the other treatments that might have related to a higher OM available for in vitro fermentation process. It can be concluded that the use of cassava meal at 2% level can be considered as the most ideal preservative to use to produce a high-quality cassava peel silage.

1. Introduction
Cassava peel is skinned tuber that are abundant available but has not been optimally utilized for ruminant feed. The availability of cassava peel is highly determined by the increase in cassava production. Indonesia has a cassava harvest area of 949,916 hectares with a production of 21,801,415 tons of cassava or a productivity of 22.95 tons/ha in year 2015 [1]. The percentage of cassava peel ranges from 8-25% of whole tubers, and those stuffs can be used as basal diet and energy source for ruminantst [2]. [3] reported that cassava peel contains DM (29.20%), and CP (5.72% DM), so it can be assumed that the potential availability of cassava peel as much as 5.74 tonnes/ha in fresh form or equivalent to 1.676.08 kgDM/ha.

It has been reported that the main constraint in using cassava peel is the presence of HCN ranging from 50.8[4] to 67.74 mg/kg [5]. HCN or commonly known as cyanide acid, is an anti-nutritional substance found in cassava product that can harm livestock if consumed in excess amounts. In addition, fresh cassava peel contains high water and is considered as a perishable food [6]. Based on these conditions, it is necessary to pre-treated the fresh cassava peel, for example by silage making. Fermentation process during silage making reduced HCN content the content of anti-nutritional as reported by[7] that the HCN content of fresh cassava peel was 580.93 ppm and after the fermentation process for 21 days the HCN content reduced to 1.16 ppm or down to 99.8% and increase the digestibility value of 25.29% to 54.75%.

The present study aimed to evaluate the effects using different types of preservatives on nutritive value and rumen fermentation of cassava peel silage executed an in vitro gas production technique.
2. Materials and methods

2.1. Location and time
This study was conducted at the Animal Nutrition and Feed Laboratory, Faculty of Animal Science, Universitas Brawijaya, in August – November 2019.

2.2. Materials
The materials in this study were cassava peels (*Manihot utilissima* Crantz.) obtained from the waste of a cassava tuber processing factory located in Boko Village, Asrikaton, Malang, East Java. The preservatives consisted of molasses, rice bran, and cassava meal were bought from the animal feed shop Ampeldento, Karangploso. The chemical composition of the ingredients used can be seen in Table 1. The following treatments were applied in study:

- T0: cassava peel without preservative
- T1: T0 + molasses 2%
- T2: T0 + molasses 4%
- T3: T0 + rice bran 2%
- T4: T0 + rice bran 4%
- T5: T0 + cassava meal 2%
- T6: T0 + cassava meal 4%

**Table 1. Chemical composition of the ingredients used.**

| Ingredients          | DM(%) | OM(%) | CP(%) | EE(%) | CF(%) | NFE(%) |
|----------------------|-------|-------|-------|-------|-------|--------|
| Cassava peel         | 34.67 | 95.17 | 6.46  | 0.70  | 11.33 | 76.61  |
| Molasses             | 77.70 | 89.00 | 4.20  | 0.20  | 7.70  | 76.90  |
| Rice bran            | 91.24 | 87.63 | 10.65 | 8.99  | 13.88 | 53.24  |
| Cassava meal         | 87.22 | 94.33 | 3.15  | 0.34  | 8.64  | 82.15  |

Note: Nutrition and Animal Feed Laboratory, Faculty of Animal Sciences, Universitas Brawijaya (2019).

2.3. Sample preparation
Materials of cassava peels were obtained from cassava cracker factory. They were chopped into pieces of 3-5 cm in size, washed to eliminate the soils that stick on the cassava peels, and the wilted for one night to reduce its water content until the DM matter reaches approximately 30-35%. The silage was made by weighing 5 kg cassava peels added with preservatives according to the treatments scenarios. Cassava peels and preservatives for each treatment were put into a bucket and mixed until homogen. The mixture for each treatment was then put into a plastic silo and compacted to establish anaerobic condition and stored for 21 days. At the end of the ensiling process, each treatment was checked for temperature and pH. Samples of silage were taken as much as 300 gram and were then oven-dried at 60°C to obtain air-dry matter, ground and prepared for proximate analysis, used for *in vitro* gas evaluation.

2.4. Taking of rumen fluid
Rumen fluids were obtained from the goats and sheep abattoir. Thermos bottle, thermometer, pH meter, and gloves were used. The bottle was filled with hot water (39-40°C) resemblance to the rumen temperature. The hot water in the bottle was poured out and replaced with rumen digesta until the bottle was fully filled. The bottle containing the rumen digesta was closed tightly and brought to the laboratory as soon as possible.
2.5. Chemical composition analysis
Samples of silage were subjected to proximate analysis following the procedure of [8] to determine the nutrient contents such as DM, OM, CP, EE, and CF.

2.6. In vitro gas production analysis
The in vitro gas production was done following the procedure of [9]. Approximately 500 mg dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 mL. The syringes were prewarmed at 39°C before the injection of 50 mL rumen fluid buffer mixture into each syringe. The syringe was incubated in a water bath at 39°C for 48 hours. Gas production at the time intervals 2, 4, 6, 8, 10, 12, 18, 24 and 48 were measured to determine the kinetics of the gas produced. Meanwhile, the potential and rate of gas production are calculated by the equation according to the equation described by [9] below,

\[ Y = b (1 - e^{-ct}) \]

Where Y: gas produced at the time 't' (ml/500mg DM), b: potential of gas production (ml/500mg DM), c: rate of gas production (ml/hour), t: incubation time (hour), and e: exponential.

Metabolizable energy (ME) value were estimated using equation described by [10] as follows:

\[ ME (MJ/kg DM) = 2.20 + 0.136 \text{GP} + 0.057 \text{CP} + 0.0029 \text{CF}^2 \]

Where GP: 24 h net gas production (ml/200mg DM), CP: crude protein (% DM), and CF: crude fat (% DM)

2.7. Statistical analysis
The data obtained were tabulated using Microsoft Excel then analyzed using analysis of variance (ANOVA) with a completely randomized design (CRD) to evaluate the effect of the treatment on chemical composition. Meanwhile, in vitro gas production parameters used a randomized block design (RBD) model. The potential and rate of gas production were obtained by the NEWAY method. If the data showed differences in treatment, it was continued with Duncan's Multiple Range Test.

3. Results and discussions

3.1. Chemical composition
The result of the chemical composition of cassava peel silage with different preservatives is presented in Table 2. There were significant effect (P<0.01) of treatments on DM, OM, and EE contents, but CP and CF contents were not affected significantly by treatments (P>0.05).

| Treatment | Chemical composition (%) |
|-----------|--------------------------|
|           | DM | OM | CP | EE | CF |
|           | (%DM) |     |    |    |    |
| T0        | 33.48 ± 0.711ab | 94.61 ± 0.384b | 6.20 ± 0.235 | 1.51 ± 0.294b | 10.62 ± 0.549 |
| T1        | 33.54 ± 0.386ab | 94.73 ± 0.035b | 6.70 ± 0.637 | 1.25 ± 0.053ab | 10.13 ± 0.799 |
| T2        | 34.25 ± 0.526bc | 94.47 ± 0.139ab | 6.27 ± 0.160 | 1.26 ± 0.033ab | 9.36 ± 0.663 |
| T3        | 33.40 ± 0.949ab | 94.82 ± 0.163b | 6.38 ± 0.149 | 1.37 ± 0.184b | 10.80 ± 0.340 |
| T4        | 32.36 ± 0.543a  | 94.30 ± 0.087ab | 6.26 ± 0.207 | 1.16 ± 0.024ab | 10.34 ± 0.522 |
| T5        | 34.31 ± 0.386bc | 94.58 ± 0.314b | 6.38 ± 0.067 | 0.85 ± 0.164a  | 10.37 ± 0.646 |
| T6        | 35.25 ± 0.130c  | 93.97 ± 0.274a  | 6.49 ± 0.087 | 1.08 ± 0.215ab | 10.16 ± 0.111 |

Note:*) Different superscript in the same column showed the significant effect at p<0.01.
The DM content of cassava peel silage in this study ranged from 32.36 - 35.21%. The highest value was observed in T6 (cassava meal 4%), whilst the lowest value was found in T4 (rice bran 4%). This study showed that the value of DM was lower than that of [11], who reported that cassava peel silage had a chemical composition in DM (65.11%), CP (4.50%), EE (0.85%) and CF (17.21%). These differences in the chemical composition might have been due to cassava peel materials and the types of preservatives used. OM contents of silage were considered high ranging from 93.97% in T6 to 94.82% in T3 and this indicated that there were plenty of substrates available for fermentation by rumen microbes.

3.2. In vitro gas production (IVGP)

Table 3. Cumulative gas production and predicted ME value.

| Perlakuan | Cumulative gas production (ml/500 mg DM) | ME (MJ/kg DM) |
|-----------|-----------------------------------------|---------------|
| T0        | 150.3 ± 4.96a                           | 10.5 ± 0.46a  |
| T1        | 152.1 ± 4.58ab                          | 10.6 ± 0.27ab |
| T2        | 154.2 ± 7.49abc                         | 10.8 ± 0.34ab |
| T3        | 153.6 ± 4.08ab                          | 10.7 ± 0.21ab |
| T4        | 153.5 ± 5.32ab                          | 10.8 ± 0.20bc |
| T5        | 159.1 ± 7.19c                           | 11.1 ± 0.36c  |
| T6        | 156.3 ± 3.54bc                          | 10.9 ± 0.07bc |

Note: *) Different superscript in the same column showed the significant effect at p<0.05.

Statistical analysis showed that treatments have significant effects on cumulative gas production (P<0.05). The highest gas production measured at 48 hours incubation was recorded in T5 (159.1 ml/500 mg DM) and not significantly different with T6 (156.3 ml/500 mg DM). Meanwhile, the lowest gas production was recorded in T0 (150.3 ml/500 mg DM), but that value was not different significantly with those obtained for T2 (154.2 ml/500 mgDM), T3 (153.6 ml/500 mgDM) and T4 (153.5 ml/500 mgDM). Gas production is a measure of OM being fermented in the rumen and energetic feed resources tend to produce higher gas production at a specific time of incubation. Figure 1. shows changes in the rate of gas production for each treatment.

![Rate of gas production 48-hours incubation](image.png)

**Figure 1.** Rate of gas production 48-hours incubation.
The potential value of gas production can be interpreted as a parameter of the part of OM that can be fermented in the rumen \((b\text{ value})\), whilst, the rate of gas production \((c)\) can be interpreted as the hourly fermentation rate of feed. The results of the calculation of the value of the gas production fermentation parameter can be seen in Table 4.

**Table 4.** Value \(b\) and \(c\) IVGP of cassava peel silage incubation 48 hours.

| Treatments | Value \(b\) (ml/500 mgDM) | Value \(c\) (ml/hour) |
|------------|----------------------------|----------------------|
| T0         | 181.7 ± 8.10               | 0.069 ± 0.0095       |
| T1         | 182.6 ± 7.47               | 0.071 ± 0.0079       |
| T2         | 185.4 ± 8.80               | 0.070 ± 0.0074       |
| T3         | 185.1 ± 6.92               | 0.071 ± 0.0078       |
| T4         | 185.0 ± 7.24               | 0.071 ± 0.0088       |
| T5         | 190.1 ± 9.86               | 0.071 ± 0.0084       |
| T6         | 187.5 ± 2.57               | 0.071 ± 0.0076       |

Statistical analysis showed that treatments did not give significant effects (\(P> 0.05\)) on “\(b\)” and “\(c\)” values. The highest “\(b\)” value was recorded in T5 (190.1 ml/500 mg DM) followed by T6, T2, T3, T4, T1 and T0 (187.5; 185.4; 185.1; 185.0; 182.6 and 181.7 ml/500 mg DM, respectively). Similar trends were also seen in “\(c\)” and these evidents imply that potential gas production and the rate of gas production are influenced by nutrient contents, especially OM available for microbial fermentation in the rumen. In this study silage processed using different types of preservatives showed almost similar nutrient contents (ie DM, OM, CP, EE and CF), it is therefore realistic that their fermentation product parameters observed such as gas production and rate of gas production were not significantly different. Predicted ME values as seen in Table 3. shows that a higher cumulative gas production of cassava peel silage processed using cassava meal preservatives (T5 and T6) has contributed to the higher ME values.

4. Conclusions

Based on the result of this study, it is concluded that the use of cassava meal (2% and 4%) were able to produce cassava peel silage with higher nutritive value (ME value) and gas production.

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