Nutritive Evaluation of Pineapple Peel Fermented by Cellulolytic Microbe and Lactic Acid Bacteria by In Vitro Gas Production Technique

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ABSTRACT: The research was conducted to evaluate the nutritive value of pineapple peel fermented by rumen cellulolytic microbes and followed by addition of lactic acid bacteria (LAB) at the 4th, 8th, 12nd and 16th days of fermentation. Cellulolytic microbes was added as much as 5% of dry matter (DM) into 300 g of pineapple peel with three replicates for each treatment. Fermentation without addition of cellulolytic microbes and LAB was carried out as control . Fermentation was done up to 21 d and the sample of fermented pineapple peel were taken out for physical quality measurements including odor, color, the presence of fungi, as well as the lactic acid content. The sample was dried at 55°C, and then prepared for chemical composition analysis including dry matter (DM), organic matter (OM), crude fibre (CF), crude protein (CP), extract ether (EE) and nitrogen free extract (NFE). The samples were also prepared for evaluation of kinetic fermentation by in vitro gas production technique proposed by Menke and Steingass. The data obtained were analyzed by analysis of variance using one way design and continued by Duncan's new multiple range test to examine the differences among the mean values. The result showed that pineapple peel fermented by cellulolytic microbes with addition of LAB in all treatment as well as the control had brown colour, and without fungus. The more acidic odor were found in fermented pineapple added with cellulolytic microbes and LAB, due to lactic acid content which increased significantly. Cellulolytic microbes and LAB addition didn't affect DM and OM content, while the content of CF tended to decrease. The treatments increased NFE as well as CP content, and decreased EE content significantly. Gas production, and the values of a, b, c fractions were not affected by addition of cellulolytic microbes and LAB. It could be concluded that lactic acid bacteria which was added after 12 days of cellulolytic fermentation) gave the best nutritive quality of fermented pineapple peel.

Keywords: Pineapple peel, Cellulolytic microbes, Lactic acid bacteria, Fermentation, In vitro gas production.

INTRODUCTION

In spite of the large amounts of industrial by product and agricultural waste, the problems of inadequate nutrition and prohibitive cost of conventional feedstuff during dry season remained unsolved. It is driving some farmers to find alternative feedstuffs. One of those materials which interested to be considered was pineapple peel. The problems of pineapple peel as animal feed are high water content, and low digestibility due to high fibre content. As a compound of Total Mix Fiber which was produced from agricultural byproduct as an alternative roughage feed, pineapple peel silage contained 23.98% of crude fibre (Maneerat *et al*., 2013). It was expected the nutritive quality of pineapple silage could be increased by reducing crude fiber content, therefore addition of cellulolytic microbes along with lactic acid bacteria (LAB) should be considered in the pineapple peel fermentation.

MATERIALS AND METHODS

Microbes Preparation

Donor Animal. In this experiment, 2 head of rumen fistulated Ongole crossbred cattle were used as the donor animal to get the rumen fluid for a source of fibrolytic microbes needed for fermentation as well as for in vitro gas production technique.

Microbes enrichment. Rumen fluid were collected from the both donor animals early in the morning before feeding time, composited, prepared and kept anaerobically in waterbath at 39ºC. A quantity of rumen fluid samples were taken out and subjected into enzymes assays (carboxymethyl cellulase/ CMC-ase) which were done in duplicate (Halliwel *et al.*,1985). Rumen fluid as much as 10% of medium volume was pipeted into glass fermenter which was already filled with enrichment medium based on Omelianski (1902) cit. Skinner (1971). Fermentation was done anaerobically at 39°C for 7 days.

Rumen cellulolytic microbes cultivation. The inoculum grown in the enrichment media was taken out, and it was added into the glass fermenter filled with growing medium as much as 10% from the total medium which used 4 g cellulose as substrate (Omelianski (1902) cit. by Skinner (1971). The fermenters was kept anaerobically at 39°C for 7 days. The culture was ready to be applied for pineapple fermentation immediately after enzymes assays.

Pineapple fermentation

After growing for 7 days, the cellulolytic culture as much as 5% DM was mixed with 300 g of air-dried pineapple peel, incubated anaerobically at room temperature and followed by addition of lactic acid bacteria (LAB) at the 4th, 8th , 12th and 16th days of fermentation. Fermentation without cellulolytic microbes and LAB addition was carried out as control . Fermentation was done up to 21 days.

Evaluation of fermented pineapple peel quality

At the end of this fermentation period the glass-silos were opened for sampling. The fermented pineapple peel samples were taken out and examined for their physical quality including odor, colour, texture, the presence of fungi and lactic acid content following Baker and Summerson method (Hawk, 1976). Content of dry matter was directly determined in the fresh fermented samples prior to 55°C drying for fermentation end products measurements. The dried sample was then analyzed for dry matter (DM), organic matter (OM), crude fibre (CF), crude protein (CP), extract ether (EE) and nitrogen free extract (NFE) content (AOAC Intl., 2005). The samples were also prepared for evaluation of kinetic fermentation by in vitro gas production technique proposed by Menke and Steingass (1988).

Statistical Analysis

Data obtain were analyzed by analysis of variance using one way design and the means were compared by Duncan's Multiple Range Test (Rosner, 1990).

RESULT AND DISCUSSION

Specific activity of Carboxy methyl cellulase (CMC-ase) in the rumen fluid was 0.08 U/mg. The specific activity of CMC-ase increased as the inoculums transferred to the enrichment media and also during cultivation in the growing media (0.16 and 1.74 U/mg) respectively. Physical quality of fermented pineapple peel was shown in Table 1.

Table 1. Physical quality and lactic acid content of pineapple peel fermented by cellulolytic bacteria and different time of lactic acid bacteria addition

Parameter	Without addition of inoculum (P0)	The day of lactic acid bacteria addition			
		4 (P1)	8 (P2)	12 (P3)	16 (P3)
Odour	Slightly acid	Acid	Acid	Acid	Acid
Colour	Brown	Brown	Brown	Brown	Brown
Texture	Rough	rough	smooth	smooth	smooth
Presence of fungi	-	-	-	-	-
Lactic acid (%)	0.03^{c}	0.11^{a}	0.10^{a}	0.07^{ab}	0.05^{bc}

^{abc}: Means within the same row with different superscript letters differ significantly (P<0.01)

The addition of cellulolytic microbes and LAB in the fermentation of pineapple peel significantly increased lactic acid content (P<0.01), therefore the odor became more acid compared with control. There were no fungi found in all treatments and the color of all fermented material remained the same, while the texture of fermented material became smoother as the effect of cellulolytic microbe and LAB addition. The increasing of acidity which was shown by the decreasing of pH and the change of odor to be more acid also reported by Yusiati et al. (2011) when cellulolytic microbes was added into the coffee pulp fermentation. The addition of LAB at the 4th day of fermentation gave the highest lactic acid content of fermented material. It was about 3.67 times lactic acid content of the control. Lactic acid content decreased by the addition time of LAB due to the shortage of LAB resident time in the fermentation media.

Chemical compositions of pineapple peel fermented by cellulolytic microbes and lactic acid bacteria addition were presented in Table 2.

Tabel 2. Chemical composition of fermented pineapple peel (%DM)

Parameter	Without addition of inoculum (P0)	The day of lactic acid bacteria addition			
		4th (P1)	8th (P2)	12th (P3)	16th (P3)
Dry matterns	47.64	46.66	46.21	46.48	46.08
Organic matterns	92.16	91.72	91.55	91.76	91.70
Crude protein	7.16^{z}	7.78^{xy}	7.88^{x}	7.63^{xy}	7.42^{yz}
Ether extract	11.10^{a}	11.19^{a}	11.41^{a}	7.94^{b}	9.51^{ab}
Crude fiberns	17.19	17.06	16.82	16.35	16.42
Nitrogen free extract	56.70^{b}	55.76^{b}	55.43^{b}	59.85^{a}	58.32^{ab}

^{abc} : Means within the same row with different superscript letters differ significantly (P<0.05)
^{xyz} : Means within the same row with different superscript letters differ significantly (P<0.01)
^{ns} : Not significant

Lactic acid bacteria addition into cellulolytic fermentation of pineapple peel did not give significant effect on dry matter and organic matter content. Nitrogen free extract (NFE) increased significantly when LAB was added after 12 days of cellulolytic fermentation. The increasing of NFE might be as an effect of the crude fiber content which have a tendency (P<0.07) to decrease. The increasing of cellulolytic fermentation time prior to LAB addition gave extended time to the

cellulolytic microbes to degrade the fiber content of pineapple peel and converted it to glucose which is component of NFE.

In vitro degradation of fermented pineapple waste

Data on gas production are given in Table3. It indicated, there was no significant effect of cellulolytic and LAB addition on total gas production, gas produced from a and b fraction as well as the rate of gas production (c values). Hanim *et al.* (2010) reported the same finding that addition of cellulolytic inoculums did not give any effect on a, b and c values of fermented cocoa pod, although its CF content decreased significantly.

Table 3. Total gas production, fraction a , b and, c value of fermented pine apple peel

Parameter (ml/300 mg DM)	without addition of inoculum (P0)	The day of Lactic Acid Bacteria addition			
		4th (P1)	8th (P2)	12th (P3)	16th (P3)
Gas Volume^{ns}	80.529	84.384	82.961	83.080	82.747
a fraction^{ns}	-0.425	-1.501	-0.693	-1.829	-2.153
b fraction^{ns}	83.077	89.649	87.776	87.669	88.219
c value (ml/h)^{ns}	0.055	0.052	0.051	0.054	0.052

^{ns} : non significant

Total gas volume and gas produced from insoluble fraction in this present study were higher compared with the gas produced by cellulolytic fermented caccao pod. It seem to be an effect of lower CF content of fermented pineapple peel compared with CF content of fermented caccao pod (16.35-17.19% vs. 22.28%). The increasing of NFE in fermented pineapple with addition of LAB at 12th days of fermentation, followed by the increasing of a fraction value.

Yusiati *et al.* (2010) reported that fermentation using 5% fibrolytic inoculums originally from rumen fluid with CMC-ase activity 4.71 U/mg and xylanase 0.028 U/mg, increased OM and DM in vitro digestibility of palm kernel cake, although CF content was not decrease. It seem that increasing of fermented pineapple peel digestibility could be expected by increasing the level of inoculums as well as by applying mix inoculums such as cellulolytic and xylanolytic.

CONCLUSION

The addition of cellulolytic microbes originally from the rumen fluid as much as 5% and lactic acid bacteria 2.5% after 12 days cellulolytic fermentation is the best way to increase the nutritive quality of fermented pineapple peel.

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