Research Article

Improvement of *In vitro* Digestibility and Gas Production of Sugar Palm Peel Silage using Fermented Napier Grass Juice

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Abstract

The objective of this study was to improve sugar palm peel silage using fermented Napier grass juice. Fermented Napier grass juice without sugar and with 1% glucose, 1% sucrose, 3% glucose, 3% sucrose, 5% glucose and 5% sucrose were prepared. Results showed that pH values of the fermented juices were 3.94 - 5.72. The highest total sugar content (136.30 mg/ml) was obtained from fermentation with 5% sucrose. In addition, fermentation without sugar gave higher lactic acid bacteria (15.17×10^6 cfu/ml) and aerobic bacteria content (19.23×10^7 cfu/ml) than other treatments while fermentation with 3% glucose gave the highest yeast and fungi count (22.37×10^7 cfu/ml). Therefore, the fermented juice without sugar was further used to improve the fermented sugar palm peel. Sugar palm peel silages without fermented juice and with 1% fermented juice and 1% molasses were prepared. Results showed that adding 1% fermented juice gave the lowest pH at day 7 and 14. Adding 1% molasses resulted in higher crude protein and lower hemicellulose and cellulose contents than other treatments. *In vitro* dry matter digestibility of samples with 1% fermented juice and 1% molasses at day 7 were significantly higher than sample without fermented juice. Similarly, gas production of sugar palm silages was significantly different among treatment. Gas production of sugar palm peel silages was highest at day 7. Based on the results, it can be concluded that fermented Napier grass juice was suitable for improving sugar palm peel silage.

Key Words: Sugar palm peel; Epiphytic lactic bacteria; Fermented plant juice of Napier grass

Introduction

In Thailand, some area lacks of feed during dry season (in March - April). Agricultural by - products are usually feed to ruminant animals in the form of roughage such as rice straw. Moreover, various kind of raw material such as fresh grass or residues in the form of silages are feed to ruminant animal in dry season. Sugar palm or Asian Palmyra palm (*Borassus flabillifer*, Linn) is widely available in Phetchaburi Province, Thailand. The chief product of the palmyra palm is the palmyra sap, which has sucrose as the main sugar (Naknean et al., 2010). This coconut - like fruit and seed can be eaten raw, and the industrial scale processing of this fruit yields plentiful of fibrous sugar palm peels as one of the by-products (Rungrodnimitchai, 2011) which is rich in sucrose (Naknean et al., 2010). Saenphoom et al. (2016) reported that fermented sugar palm peel with pineapple peel in 2:1 ratio at 14 - day had low pH and produced low fiber content and high digestibility silage. Successful silage production needs epiphytic

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lactic acid bacteria (LAB) and water soluble carbohydrate (WSC) to produce sufficient lactic acid for rapid pH reduction (McDonald et al., 1991; Bureenok, 2011). Fermented juice of epiphytic lactic acid bacteria (FJLB) is an alternative method to usage of silage additive for improving the fermentative quality of tropical grass silages due to improved crude protein digestibility and increase population of cellulolytic bacteria (Bureenok et al., 2011). Therefore, the objective of this study was to assess quality of fermented plant juice of Napier grass and the improvement of sugar palm peel silage using fermented plant juice of epiphytic lactic acid bacteria.

Materials and Methods

Fermented juice of epiphytic lactic acid bacteria preparation

Fresh Napier grass (25 g) was macerated in distilled water (500 ml) using a blender. The mixture was filtered through two layers cheesecloth and the obtained juice was transferred into a glass bottle. The juice was added with glucose and sucrose at 0, 1, 3 and 5%. The bottle was capped and incubated at 30°C for 24 h to prepare fermented juice of epiphytic lactic acid bacteria (FJLB).

Sugar palm peel silage preparation

Sugar palm peel was obtained from a local dessert shop in Phetchaburi Province, Thailand. The peels were chopped with a forage cutter approximately 2 - 3 cm lengths and mixed with the silage additives. The chopped sugar palm peels were added without FJLB (T1), 1% FJLB (T2) which contained 1.5×10^7 cfu/ml of total lactic acid bacteria (LAB) and 1% commercial molasses (T3). The silages were fermented at room temperature (30 °C) under anaerobic condition by using airtight plastic pouches. Sample was collected for 3, 7, 14 and 21 days.

pH and total sugar measurement

The pH of FJLB and silage samples was measured using pH meter (Cyberscan, Eutech instrument, Singapore). For total sugar content, 1 ml of FJLB sample was rapidly mixed with 3 ml of concentrated sulfuric acid and mixed for 30 sec. The temperature of mixture rises rapidly within 10 - 15 sec after addition of sulfuric acid. After that, the solution was cooled in ice for 2 min to bring it to room temperature. Finally, the absorbance was measured at 315 nm using UV spectrophotometer (Model Libra S22) (Ammar et al., 2013)

Microbial count

Microbial counts were measured by the plate count method. Lactic acid bacteria (LAB) was counted on plate count agar containing Bactobasilus MRS agar. Aerobic bacteria, fungi and yeast were counted on nutrient agar and potato dextrose agar, respectively. The agar plates were held in an incubator at 35-37°C for 18-24 h. Colonies were counted as viable numbers of microbial as Colony forming unit/ml (CFU/ml) (Kozaki et al., 1992).

Chemical compositions

The silage samples were dried in hot air oven at 60°C for 48 hr. The proximate analysis of dried samples for dry matter (DM), crude protein (CP), ash, ether extract (EE) were determined as according to AOAC (1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Van Soest et al., 1991). Hemicellulose was calculated as NDF-ADF and cellulose as ADF - ADL. Gross energy (GE) was determined by bomb calorimeter.

In vitro dry matter digestibility and gas production

Two cattle fed on a roughage diet were used as the donors of rumen fluid. Rumen digesta was collected from each animal via the ruminal fistula before morning feed. The rumen digesta was homogenized, and strained through filter cloth (Sommart et al., 2000) after which 660 ml of the resulting rumen fluid was added to the reduced medium (39 °C). The reduced medium was prepared as described by Makkar et al. (1995). Approximately 0.5 g of dried sample was transferred into 50 ml serum bottle (Makkar et al., 1995). The bottles were pre - warmed in a water bath at 39 °C for about 1 h. prior to injection 40 ml of rumen medium (using a 50 ml syringe). The bottles were stoppered with rubber stoppers, crimp sealed and incubated in a water bath at 39 °C. The bottles were gently shaken for 30 min after the start of incubation and then at three hour intervals for 12 h.

Gas production was measured by recording the amount of gas volume after incubation using a 100 ml glass syringe connected to incubation bottle with a 23 gauge and 1.5 inch needle. Readings of gas production was recorded at 12, 24, 48 and 72 h. after incubation periods. *In vitro* dry matter digestibility (IVDMD) was estimated after the last gas measurement by drying the bottle content at 100 - 105 °C.

Statistical analysis

Data was subjected to analysis of variance using Proc. ANOVA (SAS, 1998). Treatment combinations means were statistically compared using Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). A significance level of $P < 0.05$ was used to differentiate between means.

Results and Discussion

FJLB and Silage pH

pH value of FJLB was not significantly different among treatment ($P > 0.05$). The pH value of FJLB was in the range of 3.94 - 5.72. Agreement with previous studied of Chanprecha et al. (2016) reported that value of FJLB from grass (Napier grass, Ruzi grass and Guinea grass) was in the range of 4.78 - 3.29. pH value of sugar palm peel silages was not significantly different among treatment ($P > 0.05$) except day-3. The pH value of sugar palm peel silage was in the range of 3.39-3.59 which T2 (1% FJLB) has lower pH value at day-7, 14 and 21 as compared to other treatments (Figure 1). This result was similar with Saenphoom et al. (2016) reported that the pH of sugar palm fermented with pineapple peel approximately 3.2 - 3.4 which slightly acid. The recommended pH of silage should have a pH less 4.5 (McDonald et al., 1991). However, Bureenok et al. (2011) found that Ruzi grass silage treated with molasses had lower pH than no additive and FJLB. The mixture silage of fresh rice straw, tofu waste and cassava waste with FJLB had lower pH value than silage non supplemented with FJLB because addition of FJLB at ensiling ensured a rapid fermentation (Santoso et al., 2014).

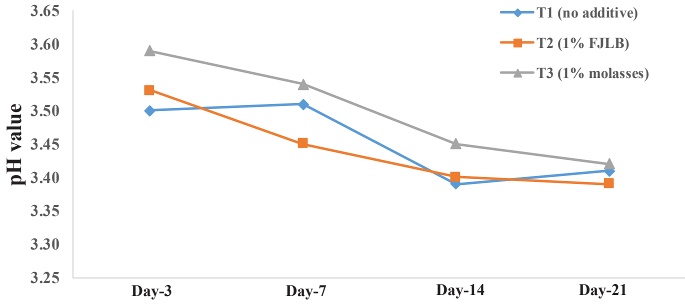


Figure 1 pH value of sugar palm peel silage

Total sugar content

Total sugar content of FJLB was significantly different among treatments ($P < 0.05$) (Figure 2). Total sugar content of T7 (5% sucrose) was higher (136.30 mg/ml) at h - 18 than other treatments ($P < 0.01$) because bacteria can break down polysaccharide and released sugar. According to Chanprecha et al. (2016) found that addition of glucose in FJLB had higher sugar (68.04 mg/ml) than no add sugar (0.97 mg/ml).

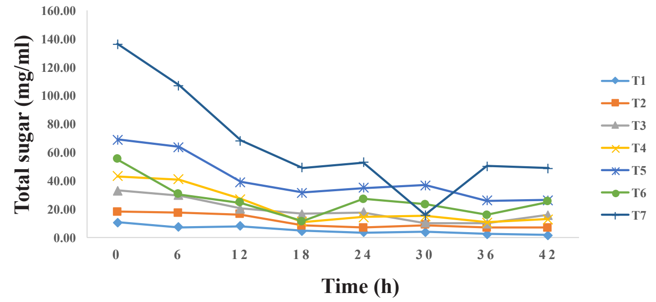


Figure 2 Total sugar content of FJLB T1 = FJLB without sugar, T2 = FJLB with 1% glucose, T3 = FJLB with 1% sucrose, T4 = FJLB with 3% glucose, T5 = FJLB with 3% sucrose, T6 = FJLB with 5% glucose, T7 = FJLB with 5% sucrose

Microbial count

Difference in microbial count of FJLB was significant among treatments ($P < 0.05$) (Figure 3). Lactic acid bacteria (LAB) content of T1 (non - sugar) was higher (15.17×10^6 cfu/ml) than other treatments ($P < 0.05$). Aerobic bacteria content of T1 (non-sugar) and T3 (1% sucrose) was higher (19.23×10^7 cfu/ml and

17.40 × 10^7 cfu/ml) than other treatments (P < 0.01). Yeast and fungi contents of T4 (3% glucose) were the highest (22.37 × 10^7 cfu/ml). However, T1 (non-sugar) showed the highest lactic acid bacteria content which is suitable to improve the fermented sugar palm peel. Conversely, previous study of Chanprecha et al. (2016) reported that FJLB with 2% glucose had higher LAB (7.91 log (cfu/ml)) than without sugar (6.57 log (cfu/ml)).

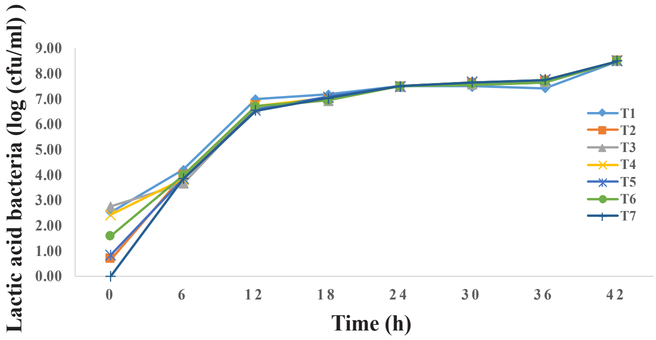


Figure 3 Lactic acid bacteria of FJLB T1 = FJLB without sugar, T2 = FJLB with 1% glucose, T3 = FJLB with 1% sucrose, T4 = FJLB with 3% glucose, T5 = FJLB with 3% sucrose, T6 = FJLB with 5% glucose, T7 = FJLB with 5% sucrose

Chemical compositions

Chemical compositions of sugar palm peel silages were significantly different among treatments (P < 0.05) (Table 1 and 2). At day-7, sugar palm peel silages were high crude protein and fiber content such as hemicellulose and cellulose. T3 (1% molasses) had higher crude protein and lower NDF, ADF, ADF, hemicellulose and cellulose than other treatment. According to previous study by Saenphoom et al. (2016), it was reported that fermented sugar palm peel with pineapple peel in 1:2 ration had lower NDF, ADF and ADL than 2:1 and 1:1 ratio which lower fiber content may be attributed to the higher acidity of pineapple. Pasebani et al. (2011) found that guinea grass silage with epiphytic lactic acid bacteria had effectively increased crude protein and decrease NDF and ADF. Correspondingly, Yahaya et al. (2004) found that FJLB silage had low ADF and ADL content due to the decrease in pH value induced by the fermentation of silage by these lactic acid bacteria. In addition, Hill et al. (2001) found that molasses reduced NDF content in silage.

Table 1 Chemical compositions (%) of sugar palm peel silage at day-3 and 7

Item (%)	Day-3			SEM	Day-7			SEM
	T1	T2	T3		T1	T2	T3	
Moisture	82.53	82.48	82.96	0.15	80.15^{a}	82.26^{a}	82.23^{a}	0.30
Ash	4.17^{a}	4.62^{b}	5.64^{c}	0.07	4.57^{d}	4.64^{d}	5.61^{e}	0.07
Crude protein	4.13	4.53	4.76	0.13	5.09	5.23	5.32	0.08
Ether extract	0.70^{a}	0.45^{b}	0.46^{b}	0.01	0.59^{e}	0.60^{e}	0.89^{d}	0.02
NDF	52.12^{c}	57.80^{a}	54.15^{b}	0.28	57.17^{a}	57.16^{a}	51.51^{b}	0.14
ADF	32.66^{c}	37.02^{a}	34.53^{b}	0.19	37.65^{a}	37.01^{b}	34.51^{c}	0.07
ADL	5.25d^{e}	6.31^{d}	4.74^{e}	0.25	6.01	5.15	4.06	0.34
Hemicellulose	18.68^{b}	19.48^{a}	18.79^{b}	0.07	19.19^{de}	19.61^{d}	17.30^{e}	0.32
Cellulose	27.52^{e}	28.97^{de}	29.87^{d}	0.32	30.47	32.43	31.52	0.57
GE (kcal/g)	4,085	4,084	4,037	16.22	4,086^{e}	4,201^{d}	4,029^{e}	17.86

^{a,b,c} Values on the same row with different superscripts differ significantly (P < 0.01).d,e Values on the same row with different superscripts differ significantly (P < 0.05).T1 = without FJLB, T2 = 1% FJLB, T3 = 1% molasses, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, GE = Gross energy

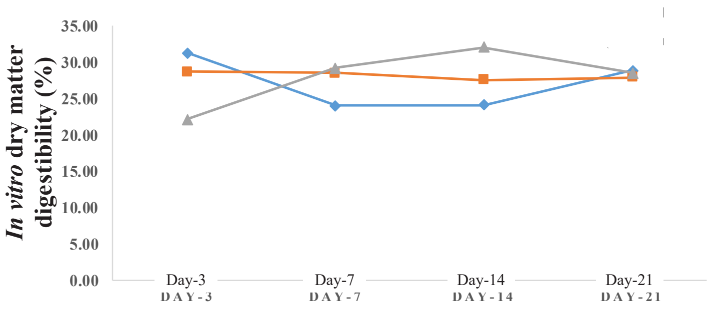
Table 2 Chemical compositions (%) of sugar palm peel silage at day - 14 and 21

Item (%)	Day-14			SEM	Day-21			SEM
	T1	T2	T3		T1	T2	T3	
Moisture	81.60	81.60	82.49	0.28	80.17	81.18	80.96	0.30
Ash	4.85[e]	4.80[e]	5.70[d]	0.06	5.59[e]	5.50[e]	6.51[d]	0.07
Crude protein	3.46[b]	4.85[a]	5.19[a]	0.10	3.19	3.12	3.18	0.08
Ether extract	0.45[c]	0.70[b]	1.39[a]	0.02	1.55[d]	1.68[d]	1.16[e]	0.02
NDF	58.00[a]	57.84[a]	55.40[b]	0.16	59.28[a]	58.72[a]	53.38[b]	0.14
ADF	39.20[a]	38.50[b]	36.19[c]	0.09	37.38[a]	36.28[a]	35.05[b]	0.07
ADL	5.67	5.61	4.92	0.23	6.33[d]	5.79[d]	3.94[e]	0.34
Hemicellulose	19.32	17.21	18.83	0.71	21.28[d]	20.59[de]	19.42[e]	0.32
Cellulose	33.75[d]	32.89[de]	31.61[e]	0.23	30.55	31.36	30.04	0.57
GE(kcal/g)	4,145	4,176	4,071	18.04	4,165	4,216	4,185	17.86

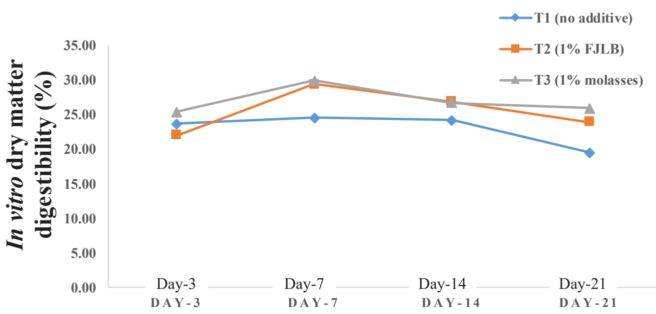
[a,b,c] Values on the same row with different superscripts differ significantly ($P < 0.01$).d,e Values on the same row with different superscripts differ significantly ($P < 0.05$). T1 = without FJLB, T2 = 1% FJLB, T3 = 1% molasses, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, ADL = Acid detergent lignin, GE = Gross energy

***In vitro* dry matter digestibility**

In vitro dry matter digestibility (IVDMD) of sugar palm peel silages are shown in Figure 4. The results showed that the IVDMD of T2 (1% FJLB) and T3 (1% molasses) at day - 7 was significantly higher than T1 (no additive) ($P < 0.05$) due to high acid in of which acidic property of the silage may induce partial acid hydrolysis of fiber. Shultz et al. (1974) reported that silage treated with various additives (alkaline, acid, enzyme, molasses, urea and limestone) had higher IVDMD than untreated silage. Yahaya et al. (2004) found that addition of FJLB increased digestibility of DM and NDF compared with control and silage treated with acetic acid. Bureenok et al. (2011) reported that FJLB silage cannot increase digestibility of dry matter but increased crude protein digestibility compared with no additive. However, this result was not similar to the previous study by Santoso et al. (2014) which reported that the mixture silage of fresh rice straw, tofu waste and cassava waste with FJLB had no effect on IVDMD.



At 24 h after incubation time



At 72 h after incubation time

Figure 4 *In vitro* dry matter digestibility (%) of sugar palm peel silage at 24 and 72 h after incubation times

Gas production

Gas production of sugar palm peel silages were significantly different among treatments (P < 0.05) with gas production of sugar palm peel silages was the highest at day - 7 (Table 3). T2 (1% FJLB) had higher gas production than other treatment. According to Pereira et al. (2013), silage presented high gas volume (28.16 ml), as they are one of the highest contributors to fibrous carbohydrate, which represent sources of fast available energy for initial microbial growth of ruminal organism.

Table 3 Gas production (ml) of sugar palm peel silage

Incubation times (h)	Fermentation times (day)	Treatments			SEM
		T1	T2	T3	
12 h	3	49.70^{a}	37.80^{c}	43.30^{b}	0.17
	7	52.50^{a}	53.20^{a}	49.70^{b}	0.18
	14	39.00^{b}	48.80^{a}	37.20^{c}	0.21
	21	39.70^{c}	51.30^{a}	48.30^{b}	0.29
24 h	3	80.50^{b}	73.80^{a}	67.20^{b}	0.26
	7	77.80^{c}	82.80^{b}	76.30^{a}	0.22
	14	64.20^{c}	73.00^{b}	76.50^{a}	0.11
	21	66.30^{b}	64.80^{b}	79.00^{a}	0.27
48 h	3	90.30^{b}	96.20^{a}	96.30^{a}	0.21
	7	104.20^{c}	101.50^{b}	100.20^{a}	0.19
	14	89.20	99.80	100.00	0.40
	21	90.80	89.20	99.70	0.19
72 h	3	107.50	105.8	108.30	0.43
	7	113.30	113.20	113.20	0.17
	14	98.50^{c}	105.70^{a}	101.70^{b}	0.33
	21	103.10^{b}	104.00^{b}	106.70^{a}	0.20

[a,b,c] Values on the same row with different superscripts differ significantly (P < 0.01). SEM = Standard error of mean T1 = without FJLB, T2 = 1% FJLB, T3 = 1% molasses

Conclusions

In this study, we found that FJLB without sugar had the highest lactic acid bacteria content which is suitable to improve the fermented sugar palm peel. Moreover, sugar palm peel silage treated with FJLB could increase digestibility due to lower fiber content and lower pH. Addition of FJLB at ensiling ensured a rapid fermentation and improved quality of silage.

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