Chemical composition and in vitro gas production of fermented cassava pulp with different types of supplements

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ABSTRACT

The effect of different additives on chemical composition, in vitro gas production and dry matter (DM) disappearance of cassava pulp was investigated. The experiment used the completely randomized design with 2 × 2 × 2 factorial arrangement of treatments with control. The control treatment was cassava pulp fermentation without any supplementation. Factor A was a type of microbes including yeast (Saccharomyces cerevisiae) or a mixture of microbes (effective microorganisms (EM)), factor B was supplementation of molasses and urea (MU), and factor C was supplementation of a mixture of exogenous enzyme. It was found that the interaction between factors A and C was significant for crude protein (CP), non-protein nitrogen and non-structural carbohydrate (NSC) contents. MU supplementation increased the CP content of fermented cassava pulp; however, EM and MU addition resulted in a significantly lower NSC and higher neutral detergent fibre fractions than the control. The supplementation showed higher gas production when compared with un-supplemented group, particularly when MU were added. However, DM disappearance was not affected by type of microbe, urea–molasses addition or exogenous enzyme supplement. Thus, the nutritive value of fermented cassava pulp with yeast and EM was improved by MU supplementation but not by exogenous enzyme addition.

1. Introduction

Recently, the price of animal feedstuffs has dramatically increased, although several industrial by-products have been found as substitutes. Cassava pulp has widespread use in the nutrition of many livestock species, such as swine, rabbit, poultry and ruminants. Based on the literature review, cassava pulp comprises 15.8–23.4% dry matter (DM) with 1.2–2.8% crude protein (CP), 55.0–74.4% nitrogen-free extract, 0.1–2.4% fat, 17.9–24.0% crude fibre and 1.7–2.8% ash, on DM basis (Yimmongkol 2009). This industrial by-product is a source of non-forage fibre which has the potential to improve the productivity and health of dairy and beef cattle as well as to control feed costs (Bradford & Mullins 2012). This was confirmed by Bekamp (2011) who surveyed dairy farmers in Muaklek district, Saraburi Province of Thailand, and found wide use of cassava pulp as a feed supplement.

Value-added cassava waste products, including ethanol, organic acid, bio gas and high fructose syrup, are obtained from enzyme utilization followed by microbial fermentation. An improved nutrient composition of cassava pulp by fermentation with microorganisms has been reported (Aro et al. 2008; Khampa et al. 2009). The protein content of cassava pulp was increased from 1.2–2.8% of DM to 12.1% DM by fermentation with Saccharomyces cerevisiae (Khampa et al. 2009). However, Aro et al. (2008) found that the CP content of cassava pulp was increased nearly 7% by microbial fermentation (a combination of fungi and bacteria) with additional biodegradation of anti-nutritional components. Nutrient components of fermented cassava pulp were further increased by supplementation with other substances. The addition of molasses and urea (MU) increases CP though reduces fibre in yeast-fermented cassava pulp (Khampa et al. 2010). Alternatively, enzymatic treatments, such as cellulase/pectinase hydrolysis (Sriroth et al. 2000) and alpha-amylose/cel lulose (Dissaro 2000), can disrupt the fibrous structure of pulp, allowing more starch granules to be fermented by microorganisms during the fermentation process. In addition, the fermentation of cassava pulp not only changes nutrient composition but also results in preservation of the fermented products, detoxification of anti-nutritional factors, improvement in the array of aromas, flavours and textures, and reduced time of degradation (Steinkraus 1995; Aro et al. 2008; Gurbuz 2009). Therefore, the aim of this study was to investigate the effect of different additives on chemical composition, in vitro gas production and DM disappearance of cassava pulp.

2. Materials and methods

2.1. Experimental design and treatment

The experiment was conducted under the control and advice of the Office of Experimental Field and Central Laboratory, Faculty of Agriculture, Ubon Ratchathani University, Warinchamrap, Thailand.
Ubon Ratchathani, Thailand. The experiment followed the completely randomized design with $2 \times 2 \times 2$ factorial arrangement of treatments with control. The control treatment involved cassava pulp fermentation without any supplementation. Factor A was a type of microorganisms including yeast (Saccharomyces cerevisiae) or a mixture of microbes (effective microorganisms (EM), KYUSEI Co., Ltd., Thailand), factor B was supplementation of MU, and factor C was supplementation of a mixture of exogenous enzyme (Asia Star Animal Health Co., Ltd., Thailand) containing beta-glucanase, xylanase, cellulase, amylase, pectinase, mannanase and phytase. Therefore, nine treatments were T1, control (fermented with no supplementation); T2, fermented with yeast (Yeast); T3, fermented with EM (EM); T4, fermented with yeast, molasses and urea (Yeast + MU); T5, fermented with EM, molasses and urea (EM + MU); T6, fermented with yeast and enzyme (Yeast + Enz); T7, fermented with EM and enzyme (EM + Enz), T8, fermented with yeast, molasses and urea, and enzyme (Yeast + MU + Enz); and T9, fermented with EM, molasses and urea, and enzyme (EM + MU + Enz). Three replicates were performed for all treatments.

### 2.2. Fermentation of cassava pulp

Cassava pulp was collected from an industrial factory for cassava manufacture (Eiamsiri Co., Ltd., Thailand) located in Nayia District, Ubon Ratchathani Province. Fresh cassava pulp samples were taken and immediately frozen (~20°C) prior ensiling. Cassava pulp samples were well mixed with 0.5 kg/tonne of exogenous enzyme for 12 h before fermentation with microbes. Fermentation of cassava pulp with microorganisms (yeast or EM) was conducted according to the method of Khampa et al. (2009). The rates of yeast, EM, urea and molasses addition were 0.33 g, 25 ml, 3.3 kg and 4.2 kg per 100 kg of dry cassava pulp, respectively. Samples of fermented cassava pulp were collected on day 21 of incubation. Fresh and fermented cassava pulp samples were analysed for DM (Method #930.15) and ash (Method #923.03) and CP contents (Method #976.06) by the proximate analysis procedure (AOAC 1995). Fibre contents (Method #923.03) and CP contents (Method #976.06) by the AOAC 1995. Fibre contents were determined (neutral detergent fibre, aNDFom; and acid detergent fibre, ADFom) using the detergent analysis method (Van Soest et al. 1991). Non-protein nitrogen (NPN) in fermented cassava pulp was measured according to the method of Awolumate (1983). In addition, non-structural carbohydrate (NSC) was calculated by: OM – aNDFom – CP, as assumed that ether extract fraction was less than 2.4% DM (Yimmongkol 2009).

### 2.3. Gas production technique

Two male, Thai-native × Brahman crossbred beef cattle (350 ± 40 kg body weight (BW)) were used as rumen fluid donors. The animals were individually penned, clean fresh water and mineral blocks were offered as free choice. The animals were fed with rice straw as a roughage on ad libitum basis and concentrate (14% CP, 2.5 Mcal/kg of ME, consisting of: 70% cassava chip, 7% rice bran meal, 7% coconut meal, 10% palm kernel meal, 1% sulphur, 2% urea, 1% mineral premix and 1% salt) was fed at 0.5% BW in two equal portions, at 7.00 am and at 4.00 pm. The animals were given the diets for 7 days before rumen fluid was collected. The rumen fluid was obtained from each animal via rumen fistula, before morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks. A strict anaerobic condition was maintained during rumen fluid collection according to the method of Menke et al. (1979). Artificial saliva was prepared according to Menke and Steingass (1988; Gürbüz 2007). The artificial saliva and rumen fluid were mixed at a 2:1 ratio to produce a rumen inoculation mixture. Three bottles containing only rumen inoculation mixture were used as blank. Mean gas production of the blank samples was subtracted from each measurement to give the net gas production. The 100 ml glass tubes with 200 mg of fermented cassava pulp sample were pre-warmed in a water bath at 39°C before filling with 60 mL of rumen inoculation mixture. Thirty minutes after the start of incubation, the tube was gently mixed and then mixed three times every three hours. The volume of gas production was recorded on 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60 and 72 h of incubation. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows:

$$y = a + b(1 - e^{-ct}),$$

where ‘$y$’ is the gas produced at time ‘$t$’; ‘$a$’ is the gas production from the immediately soluble fraction; ‘$b$’ is the gas production from the insoluble fraction; ‘$c$’ is the gas production rate constant for the insoluble fraction (b); and ‘$t$’ is incubation time; and $P(a + b)$ is the potential extent of gas production. At 24 h post inoculation, a set of samples were taken to determine in vitro digestibility according to Van Soest and Robertson (1985).

### 2.4. Statistical analysis

All data were subjected to analysis of variance according the general linear model of SAS (2006). Orthogonal contrast was used to investigate the effect of supplementations while the following model was used for factorial comparison: $Y_{ij} = \mu + A_i + B_j + C_k + AB_{ij} + AC_{ik} + BC_{jk} + ABC_{ijk} + \varepsilon_{ijk}$. Differences among mean were tested by Tukey’s test with $p < .05$ was accepted as representing statistically significant differences.

### 3. Results

#### 3.1. Chemical composition

Untreated cassava pulp contained 175, 981, 45, 23, 579, 357 and 123 g/kg of DM, OM, CP, NPN, NSC, aNDFom and ADFom, respectively, which was comparable with the control. The supplementation treatments (T2–T9) had a lower DM proportion but were higher in CP and aNDFom contents when compared with the control (T1) ($P < .05$), particularly when MU were added. Although the interaction between type of microbe (A) and enzyme supplementation (C) was statistically significant ($p < .05$) for CP, NPN and NSC contents, fermented cassava pulp with yeast or EM and enzyme added were not significantly different ($p > .05$) (Table 1). Fermentation of cassava pulp with yeast and molasses with urea (Yeast + MU), with yeast or EM plus molasses with urea and enzyme (Yeast + MU + Enz and EM + MU + Enz) had a higher CP percentage than...
other treatments (*p < .05). Besides, cassava pulp fermented with EM and MU had significantly lower NSC but higher aNDFom fractions than the control (*p < .05).

### 3.2. In vitro fermentation

Kinetics of in vitro gas production and DM disappearance of fermented cassava pulp are given in Table 2. The interaction between MU supplementation (B) and enzyme supplementation (C) on gas produced from soluble fraction (a) and rate of gas production (c) was found to be statistically significant (*p < .05). The supplementation resulted in higher gas production kinetics including a, c and potential of gas production (P) when compared with un-supplemented group (*p < .05) particularly when MU were added (*p < .05). In addition, gas produced from soluble fraction (a) was increased by supplementation with MU (T4, T5) or with enzyme (T6, T7) but not with the combinations of these additives (T8, T9). Dry matter disappearance was not affected by type of microbe, MU addition or exogenous enzyme supplementation (*p > .05).

### 4. Discussion

Cassava pulp with a CP content of 1.6–5.3% DM was reported by Djuma’ali et al. (2011) which was comparable to 45 g/kg DM found in this study. This may be due to the variety of cassava, rate of fertilizer application and other crop management factors, as well as starch extraction methodology of each factory. Fakin et al. (2012) found significantly different CP contents between various cassava genotypes (1.81–4.53% DM). Production of cassava starch results in 10–25% of the original processed root DW being solid waste depending on starch processing technology (Thai Tapioca Starch Association, TTSA). Therefore, percentage nutritive value of cassava pulp could vary. The increase in CP content of fermented cassava pulp could be due to supplementation of 3.3% DM of urea. This agrees with Khampa et al. (2009) who found that urea supplementation caused an increase in CP content of yeast-fermented cassava pulp from 2.4% to 14.1% DM. Moreover, Thongkratok et al. (2010) showed that the protein content of microorganism-fermented cassava pulp was increased from 2.6% to 21.2% DM by adding urea at 1.25% fresh basis. Urea is composed of 46.0% of nitrogen; consequently, the addition of 3.3% urea must result in 9.5% CP content of fermented cassava pulp. Crude protein content in fermented cassava pulp in the present study was found to increase from 2.4% DM to 14.1% DM. However, Aro et al. (2008) reviewed chemical composition of through microbial inoculation and

### Table 1. Chemical composition of fermented cassava pulp (g/kg DM).

| Treatment | DM | OM | CP | NPN | NSC | NDF | ADF |
|-----------|----|----|----|-----|-----|-----|-----|
| T1 Control | 183 | 976 | 48<sup>b</sup> | 3.2<sup>b</sup> | 611<sup>a</sup> | 318<sup>b</sup> | 102 |
| T2 Yeast | 162 | 977 | 50<sup>b</sup> | 2.4<sup>b</sup> | 537<sup>ab</sup> | 389<sup>ab</sup> | 131 |
| T3 EM | 168 | 976 | 78<sup>b</sup> | 14<sup>b</sup> | 480<sup>abc</sup> | 418<sup>ab</sup> | 140 |
| T4 Yeast + MU | 160 | 974 | 133<sup>a</sup> | 67<sup>ab</sup> | 392<sup>abc</sup> | 448<sup>ab</sup> | 165 |
| T5 EM + MU | 146 | 976 | 178<sup>a</sup> | 106<sup>a</sup> | 281<sup>c</sup> | 517<sup>a</sup> | 156 |
| T6 Yeast + Enz | 160 | 975 | 77<sup>b</sup> | 2.4<sup>b</sup> | 490<sup>abc</sup> | 407<sup>ab</sup> | 134 |
| T7 EM + Enz | 160 | 977 | 58<sup>b</sup> | 8.2<sup>b</sup> | 521<sup>a</sup> | 398<sup>b</sup> | 139 |
| T8 Yeast + MU + Enz | 150 | 973 | 169<sup>a</sup> | 10.2<sup>a</sup> | 338<sup>abc</sup> | 466<sup>ab</sup> | 169 |
| T9 EM + MU + Enz | 146 | 973 | 167<sup>a</sup> | 9.8<sup>b</sup> | 448<sup>ab</sup> | 358<sup>ab</sup> | 120 |
| SEM | 3.40 | 0.84 | 10.6 | 17.2 | 15.4 | 15.6 | 16.2 |

Control, un-supplement; yeast, Saccharomyces cerevisiae; EM, effective microorganisms; MU, molasses and urea supplement; Enz, a mixture of enzymes; Control vs. supplements, T1 vs. T2–T9; DM, dry matter; OM, organic matter; CP, crude protein; NPN, non-protein nitrogen; NSC, calculated non-structural carbohydrate; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of the mean.

<sup>a</sup>Values differ at a significance level of (*p < .05; ns, not statistically significant.

<sup>b</sup>*p < .05; ns, not statistically significant.
found that proportion of fibre of fermented cassava pulp was decreased while NSC proportion could be increased. Therefore, the decrease in rapidly degradable carbohydrate during fermentation of cassava pulp through microbial inoculation could be expected.

The present study was not to investigate fermentation quality and end-products of fermented cassava pulp; however, improving of colour and odour by molasses addition and increasing of fermentation pH by urea supplementation were found in our pilot study. Maneerat et al. (2015) stated that greater dietary intake in fattening steer received rice straw with molasses when compared with other agro-industrial by-products could be due to the better palatability. Urea addition increased pH and digestibility (Demirel et al. 2003) as well as efficiency of microbial net protein synthesis of corn cultivation (Robertson et al. 2006). McDonald et al. (1991) revealed that WSC is a limiting factor in producing good-quality fermented products to serves as a carbon source for microorganisms. Molasses and exogenous enzyme addition were used as WSC providing techniques for cassava pulp fermentation in this present study.

The increase in gas produced from soluble fraction (a), rate of gas production (c) and potential of gas production (P) could result from MU addition. Sugar cane molasses (a rich source of sucrose) and urea (a nitrogen source) are totally soluble and degraded in the rumen by microorganisms. Paengkoum and Bunnakit (2012) reported that the mean gas production was significantly higher in mixture of cassava pulp and urea than in control. On the other hand, it has been shown that feeding fermented cassava pulp improves rumen fermentation. Bacterial and fungal zoospore populations in the rumen of crossbred Native cattle increased when fed with yeast-fermented cassava pulp (Khampa et al. 2011). Ammonia concentration in the rumen which is a nitrogen source for microbes increased with urea addition. However, excess ammonia in the rumen would probably be lost in manure and contribute to nitrogen pollution (Kebreab et al. 2001).

In this study, yeast (Saccharomyces cerevisiae) and EM gave comparable result for characteristic of fermented cassava pulp. However, several groups of microbe in EM, such as filamentous fungi, fermenting microorganisms and lactic acid bacteria, could have a great effect on the fermentation process. Aro (2008) reported that chemical composition of fermented cassava pulp fluctuated when different types of microorganism were applied. Moreover, the quality of grass silage, such as pH, DM loss and acid composition varied when different species or strains of microbes were inoculated (Cai et al. 1998). In addition, one of the most useful measurements of silage quality is its pH value which was not measured in the present study. Optimizing pH (3.5–4.2) of silage could reduce nutritional losses particularly due to volatilizing of short-chain fatty acids (King 2009).

In terms of enzyme supplementation, the high yield of glucose indicates the potential use of enzymatic-hydrothermally treated cassava pulp has been reported by Djuma’ali et al. (2011). Siroth et al. (2000) also have reported that enzymatic treatment of cassava pulp increases the susceptibility of starch molecules to alpha-amylase hydrolysis thereby increases the amount of reducing sugar for further fermentation by microorganisms. The lack of enzyme effects in this study could be due to the differences in the fermenting condition. Beauchemin et al. (2002) reviewed that temperature, pH, ionic strength, substrate concentration and substrate type are the factors that affect the activity of an enzyme. A temperature of approximately 60°C and a pH between 4 and 5 are the optimal conditions for most commercial enzymes (Coughlan 1985). The present study found that DM disappearance did not differ between control (T1) and supplementation groups (T2–T9) which agreed with Kaewwongsa et al. (2011) who revealed that DM and organic matter disappearance of fermented cassava pulp did not differ between levels of yeast supplementation. However, gas production increased without an increase in DM disappearance suggesting decreased amount of VFAs. Therefore, exploration of the amount and proportions of VFAs which are end-products of microbial fermentation

### Table 2. Kinetic of gas production and DM disappearance (g/kg DM) of fermented cassava pulp.

| Treatment                  | Kinetic of gas production | DM disappearance |
|----------------------------|---------------------------|------------------|
|                           | a  | b     | c     | P   |    |
| Control                   | 2.0 | 0.043 | 80.1  | 82.1 | 741 |
| Yeast                     | 2.5 | 0.038 | 91.8  | 94.3 | 820 |
| EM                        | 2.2 | 0.040 | 91.8  | 94.1 | 800 |
| Yeast + MU                | 4.9 | 0.052 | 99.2  | 104.2| 769 |
| EM + MU                   | 5.2 | 0.056 | 95.1  | 100.3| 810 |
| Yeast + Enz               | 5.4 | 0.046 | 88.0  | 93.4 | 774 |
| EM + Enz                  | 6.7 | 0.043 | 89.3  | 96.0 | 759 |
| Yeast + MU + Enz          | 4.2 | 0.050 | 93.4  | 98.5 | 767 |
| EM + MU + Enz             | 1.7 | 0.054 | 107.2 | 108.9| 792 |
| SEM                       | 0.4 | 2.4   | 0.005 | 2.4  | 16.0|

Control, un-supplement; yeast, Saccharomyces cerevisiae; EM, effective microorganisms; MU, molasses and urea supplement; Enz, a mixture of enzymes; Control vs. supplements, T1 vs. T2–T9; a, the gas production from the soluble fraction; b, the gas production from the degradable fraction; c, the gas production rate; P, the potential extent of gas production; DM, dry matter; SEM, standard error of the mean.

**a**< .05; **ns** not statistically significant.

**Values differ at a significance level of ***P*** < .05.  
*SEM values differ at a significance level of ***P*** < .05.  
*SEM values differ at a significance level of ***P*** < .05.
would be useful as the predictor of nutritional utility of ruminant diet.

5. Conclusions
The results of the study indicated that cassava pulp could be fermented with yeast (Saccharomyces cerevisiae) or EM in order to be conserved and used as animal feed. Addition of MU may improve the nutritive value of fermented cassava pulp although exogenous enzyme supplementation appears to be ineffective. Utilization studies of fermented cassava pulp supplemented with urea, molasses and exogenous enzyme in digestion trials should be conducted for assessing the effect of this product on the performance of livestock.

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