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Cassava-based (*Manihot esculenta* Crantz) fermented energy-protein food for bovines

Ramos-Juárez, Jesús A.[1*]; Reyes-Cruz, Luis A.[1]; Bucio-Galindo, Adolfo[1]; Zaldívar-Cruz, Juan M.[1]; López-López, Rutilo[2†]

[1] Colegio de Postgraduados, Campus Tabasco, Carretera Libre Cárdenas-Coatzacoalcos km 21, Poblado C-27, Cárdenas, Tabasco, México, C. P. 86500.
[2] Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias, Campo Experimental Huimanguillo, Carretera Federal Huimanguillo-Cárdenas km 1, Huimanguillo, Tabasco, México, C, P. 86600.
* Correspondence: ramosj@colpos.mx

ABSTRACT

Objective: To assess the effect of different yeast levels and fermentation times on the chemical, fermentative, and microbiological composition of cassava in order to produce a cassava-based fermented food.

Design/Methodology/Approach: We used a completely randomized design with a factorial arrangement, three yeast inoculum levels (0, 5, and 10%), and five fermentation times (0, 1, 2, 3, and 4 days).

Results: We found significant interaction of the studied factors with the pH and crude protein (CP) variables. The highest pH values were obtained adding 10% yeast inoculum (YI) (8.67). CP values of 16.55% were found. No differences caused by the studied inoculum levels and fermentation times were found in true protein (TP) and the *in situ* degradation of dry matter (IDDM). TP and IDDM had 8% and 80% values, respectively.

Study Limitations/Implications: A behavior test with bovines must be conducted to demonstrate the potential of cassava-based fermented foods in meat and/or milk production.

Findings/Conclusions: The yeast inoculum and the fermentation days did not increase the TP in the cassava-based fermented food.

Key words: Protein, Tuber, Solid state fermentation.

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INTRODUCTION

Grasses are the basis of the diet in bovine production systems. However, since grass production and quality present seasonal variations, supplements are needed to complement its biomass and nutrient deficit and, consequently, to increase the production of meat and milk (Ramos *et al.*, 2018). The use of commercial concentrates is not always an option for small and midsized producers due to their high market cost. Therefore, it is necessary to search for alternative supplements based on raw materials that farmers can produce in their own farms and then subject them to a protein enrichment process.

Image by Brett Hondow at Pixabay

Cassava is a tropical crop used as food for both humans and animals. Its root (tuber) contains approximately 65% moisture. To use it as food, cassava is cut in 1-2 cm thick slices and then laid out in the sun to reduce its moisture content to 15 or 18%. The tuber has a significant content of high-digestibility starch (60-72%) and a low protein content (2.4%). Therefore, its use in supplements requires high quality protein ingredients (De Blas *et al.*, 2010), which are the most expensive ingredients in livestock diets.

Solid state fermentation (SSF) is a biotechnological process used to enrich fibrous products or by-products with low protein contents (Ramos *et al.*, 2018). Microorganisms (yeasts) are used to increase microbial biomass, which in turn increases the protein content of the fermented material (Jiménez-Alfaro *et al.*, 2020).

The objective of this work was to evaluate the effect of different yeast levels and fermentation times on the chemical, fermentative, and microbiological composition of cassava in order to produce a cassava-based fermented food.

MATERIALS AND METHODS

Site of the experiment

The experiment was conducted at the Colegio de Postgraduados, Campus Tabasco, Cárdenas municipality, Tabasco, Mexico (18° 00' N, 93° 30' W, at an altitude of 12 m).

Treatments and experimental design

We used a completely randomized experimental design with a 3×5 factorial arrangement, three levels of yeast inoculum (0, 5, and 10%), and five fermentation times (0, 1, 2, 3, and 4 days), with a total of 15 treatments and four replications per treatment (60 experimental units, Roux bottles).

Preparation of the yeast inoculum

The yeast inoculum (YI) was prepared through the liquid state fermentation process, using molasses (15%), soy paste (4%), polished rice (4%), mineral salts (0.5%), magnesium sulfate (0.32%), urea (0.48%), yeast (0.5%), and water (75.2%). The ingredients were mixed in a 2-L beaker that was shaken in a DBO 200 incubator (Novatech®, Mexico) for 5 minutes, three times a day for three days, at a temperature of 30 °C.

Preparation of the cassava-based food for fermentation

We used sugarcane, which we cut 24h before use (Elías *et al.*, 1990) and let stand in the shade. Afterwards, we grinded it in a PFA 3000 forage chopper (8 HP Power Cat, Mexico). We grinded sweet cassava bought in a public market in a LI-12A industrial blender (TAPISA®, Mexico). We mixed a 250-g sample according to the percentages shown in Table 1 and added the corresponding YI to each treatment (0, 5, and 10%). We determined the YI moisture and added water to adjust the initial dry matter (DM) of the feed to be fermented.

We then put the mix in 1-L Roux bottles (Pyrex®, USA) —whose mouths were covered with cotton to produce aerobic fermentation— and then placed the bottles in a DBO 200 incubator (Novatech®, México) at a temperature of 30 °C. Mineral salts (LAPISA®,

Table 1. Inclusion percentage of the ingredients to be fermented.

Ingredients	Content (%)
Cassava meal	62.2
Soybean meal	4.0
Urea	1.5
Sugarcane	30.0
Magnesium sulphate	0.3
Mineral salts	2.0

México) contained 12% P, 13% Ca, 15.6% Cl, 10.4% Na, 0.6% Mg, 0.3% S, 0.12% Zn, 0.12% Mn, 0.03% Cu, 50% Co, and 3.0% Se.

Fermentative analyses

After the different fermentation times (0, 1, 2, 3, and 4 d) had ended, the entire content of the Roux bottles was collected on a crystal plate and homogenized. We took a 10 g sample, to which we added 90 mL of distilled water; subsequently, the whole sample was shaken in an orbital shaker (VWR DS-500E®, USA) for 30 min. We immediately measured the pH with a potentiometer (Denver Instrument®, USA) and the °Brix in a 00700 handheld refractometer (ATAGO®, Japan).

Chemical analyses

We put the solute in a paper bag, weighed it, and then placed it in a forced air stove at 62 °C, until it reached a constant weight. Subsequently, we grinded it in a Model 4 mill (Thomas Wiley®, Germany) with a 2-mm sieve, in order to conduct the following analyses: moisture and crude protein (CP), following AOAC (2016); true protein (TP), according to Meir (1986); neutral detergent fiber (NDF) and acid detergent fiber (ADF), following Lourenco *et al*. (2017); and *in situ* degradation of dry matter (IDDM), according to Haile *et al*. (2017). We subtracted the moisture percentage from 100 to calculate dry matter (DM).

Microbiological analyses

We took a 10 g sample, mixed it with 90 mL peptone water (Official Mexican Standard NOM-110-SSA1-1994, 1994), and then shook it in an orbital shaker (VWR DS-500E®, USA) for 30 min. Afterwards, we filtered the samples with sterile gauze pads and made dilutions with 1 mL of the filtered material. The potato dextrose agar (PDA) medium was used to quantify the yeast, while the Man Rogosa and Sharpe (MRS) medium was used to quantify the lactic bacteria, with 10^{-5}, 10^{-6}, and 10^{-7} dilutions in duplicate, using the pour plate method. According to the fermentation days, 90×15 mm sterile Petri dishes were incubated in inverted position at 30 °C.

Statistical analysis

The resulting data were analyzed with ANOVA statistical tests in order to determine differences between treatments. Means were compared using Tukey's test with the SAS

software (2017), version 9.4. The separate effect of the studied factors was assessed with SAS's Slice tool when variable interaction was found.

RESULTS AND DISCUSSION

Fermentative analyses

Significant interaction was recorded between the studied factors in the pH and °Brix variables. The highest pH values were reached when 10% YI was added on fermentation days 0, 1, 2, and 3 (Figure 1).

The pH is decisive for yeast growth and microorganism development in SSF. In this work, pH values ranged from 6.14, with the addition of 0% YI, to 8.67, with the addition of 10% YI. A pH of 4.5 to 6.5 is considered optimal for yeast growth in the SSF process. Nevertheless, Elías *et al.* (1990) found that the optimal pH for yeast growth in the SSF-induced enrichment of sugarcane was 3.5 to 6.

No effect was found on the °Brix at the studied YI levels on fermentation days 0 and 3; however, the lowest °Brix values were found when 5 and 10% YI were added on fermentation days 1 and 2, with no difference between them (Figure 2).

The reduction in sugar concentration on fermentation days 1 and 2 (when 5 and 10% YI were added) could have indicated that yeasts were using sugar and that further growth was to be expected. However, this did not happen, probably as a result of the high pH value.

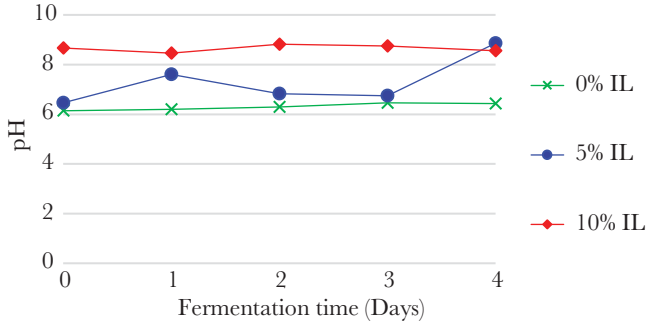

Figure 1. Effect of yeast inoculum (YI) levels and fermentation days on the pH of the fermented food.

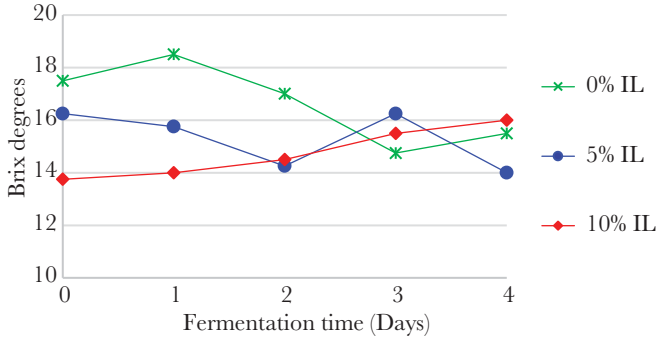

Figure 2. Effect of yeast inoculum (YI) levels and fermentation days on the °Brix of the fermented food.

Chemical analyses

We determined that the studied factors interacted with the CP variable. There were differences only on days 1 and 2. The highest CP values on day 1 were obtained with the addition of 0 and 10% YI, with no statistical difference between the two percentages. The highest CP values on day 3 were obtained with the addition of 5 and 10% YI, with no difference between them (Figure 3).

Cassava has a 2.4% CP concentration. In this work, values of up to 16.56% were reached at the end of the fermentation process; these values are within the range used in commercial supplements for bovines in the growth stage. This increase in CP is caused by the addition of urea (1.5%) and soy paste (4%). Similar data were reported for pineapple silages, using a yeast inoculum: CP levels increased from 8.7% to 18.5% (Rodríguez *et al.*, 2014).

Urea is the cheapest source of non-protein nitrogen (NPN) used in ruminant diets; if there is an available energy source, it can be used by rumen bacteria to synthetize amino acids. However, if it is not used correctly, animals run the risk of suffering intoxication, as a consequence of the fast transformation of urea into ammonia (NH_3), which increases the rumen pH and the permeability of the rumen walls to non-ionized NH_3 (McDonald *et al.*, 2013). Just as in the case of this study, an advantage of feed produced by SSF is the absence of urea-related intoxication risks when the food is eaten fresh, because, at the end of the SSF process, the NPN of urea becomes ionized ammonium $\left(NH_4^+\right)$, a substrate that can be used by rumen microorganisms and to which rumen walls are less permeable. In addition, voluntary consumption of the fermented product is slow (Ramos *et al.*, 2016).

Due to the initial addition of water, no differences were found in the DM content of the cassava-based fermented food between the different inoculum levels or the fermentation times (Table 2). DM varied between 33 and 34%; these data are similar to those reported by Rodríguez *et al.* (2014), who added molasses, urea, and a yeast inoculum to pineapple silages.

No differences resulting from the inoculum levels or fermentation times were found in TP (Table 2). TP can provide an indirect way to measure microbial growth in SSF processes, because the established microbiota transforms the NPN of urea into protein nitrogen (PN) (Ramos *et al.*, 2006). In average, the TP percentage of the fermented foods of

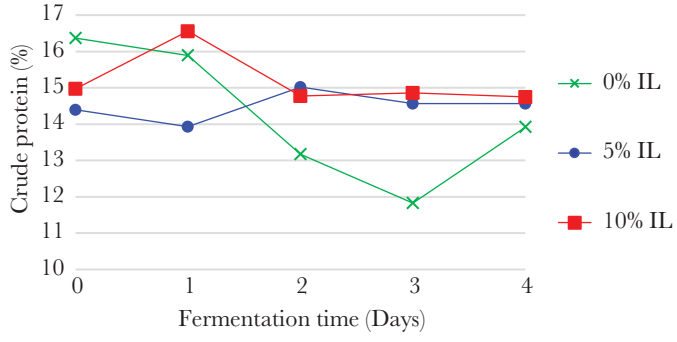

Figure 3. Effect of yeast inoculum (YI) levels and fermentation days on the crude protein content (%) of the fermented food.

this study was 8%: a good amount, based on the protein value of cassava (2.4%). However, these values are lower than those reported by Aruna *et al.* (2017), who enriched yam (*Dioscorea* spp.) peelings using a *Saccharomyces cerevisiae* medium and found a significant increase in TP (4.38 to 13.37%) after a 96 h fermentation.

No differences resulting from the inoculum levels or fermentation times were found in IDDM (Table 2). In average, IDDM was of 80%; these data are slightly higher than those reported by Flores *et al.* (2005), who used *Penicillum roqueforti*- and *Lactobacillus plantarum*-based cultures on coconut paste and found an IDDM of 72 to 75%.

Microbiological analyses

We found a significant interaction of the studied factors regarding yeast growth. No difference between the inoculum levels and fermentation days was found on days 0, 1, 2, and 4; however, on day 3, the addition of 5% YI increased yeast growth (Figure 4).

Table 2. Effect of yeast inoculum levels and fermentation times on the chemical and microbial parameters of the fermented foods.

Factors	Dry matter (%)	True protein (%)	[1]DIMS (%)	[2]BacLac (Log_{10} UFC mL^{-1})
Yeast inoculum levels (%)				
0	34.01a	7.80a	79.64a	6.35a
5	34.32a	7.82a	79.67a	6.36a
10	32.71a	8.28a	81.14a	6.38a
EE±	0.4349	0.2147	0.5828	0.061
Fermentation time (Days)				
0	33.07a	8.19a	79.57a	6.19b
1	33.85a	8.20a	80.10a	6.44ab
2	33.78a	7.85a	81.01a	6.67a
3	33.47a	7.40a	79.46a	6.30b
4	34.22a	8.20a	80.62a	6.21b
EE±	0.5615	0.2772	0.7524	0.061

Means with different letters in the same column are statistically different (Tukey, $p<0.05$).
[1]IDDM (DIMS)=*In situ* degradation of dry matter; [2]LacBac (BacLac)=Lactic bacteria.

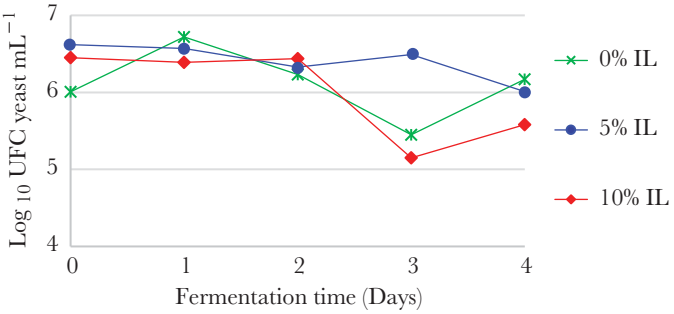

Figure 4. Effect of yeast inoculum levels and fermentation times on the fermented cassava's yeast growth (Log_{10} UFC mL^{-1}).

Yeasts with 0% YI were found on day 0, probably due to the yeasts that are naturally present in sugarcane (Valiño *et al.*, 1994a; 1994b) and to their increase when they are cut 24 h before grinding, mixing, and fermenting the cassava-based feed (Elías *et al.*, 1990).

On the one hand, the lack of growth response in yeast to the addition of YI on days 0, 1, and 2 could be caused by the alkaline pH (Figure 1), which did not allow an optimal yeast growth. On the other hand, the growth observed on day 3 of fermentation after a 5% YI had been added (Figure 4) matches the lower pH found in this treatment (Figure 1).

We previously mentioned that yeast growth is associated with an acid pH. Díaz-Plascencia *et al.* (2011) studied four different substrates with *Saccharomyces cerevisiae* yeast inoculum under submerged solid fermentation conditions and found that yeast concentrations increased with a pH of 3.05 to 5.86. Fonseca-López *et al.* (2018) produced feed for bovines based on SSF-fermented carrot. They observed that a ≥6.05 pH has a negative impact on fungi and yeast growth, as was the case in this study.

No effect was found on lactic bacteria due to the studied inoculum levels. However, there were differences due to fermentation times: the highest growth was found on day 2 (Table 2).

Lactic bacteria growth is associated with a low pH. Fonseca-López *et al.* (2018) found that lactic bacteria did not grow in carrot-based fermented foods with a pH of 6.05; however, they did grow adequately with a pH of 5.

The high pH values found in this study were probably the factor that most affected yeast and lactic bacteria growth. Meanwhile, the CP, TP, and IDDM values found in the cassava-based fermented food support their suitability as part of the bovine diet. We therefore recommend conducting behavior tests.

CONCLUSIONS

The yeast inoculum and fermentation days did not increase true protein contents in the cassava-based fermented food. The data analysis suggests that —given its crude and true protein contents and *in situ* degradation of dry matter— the cassava-based fermented food, with no yeast inoculum and a 24 h fermentation, can be used as an energy-protein supplement in bovine diet that improves production and, possibly, contributes to a reduction of production costs.

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