Technical Note

Addition of energy sources and inoculants in the elaboration of cassava-based yogurt

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Abstract

The objective of this work was to evaluate the fermentation of cassava paste with the addition of energy sources of agroindustrial waste and the inoculation of microbial strains available in Cuba. The effect of the addition of molasses, sugarcane juice and whey, as well as the inoculation with the biopreparation IH Plus®, lactic acid bacteria alone and in combination with *Saccharomyces cerevisiae*, was studied. The acidification rate, production of organic acids and buffering capacity were measured at 8, 16 and 24 days of fermentation, for which a trial was conducted in randomized complete blocks for each studied factor with four repetitions. With the addition of whey, sugarcane juice and molasses in a 1:1 ratio (paste: carbon source) cassava-based yogurt was obtained with pH lower than 4,0 and the production of organic acids was significantly improved with the addition of whey at 24 days with regards to the control ($p < 0,05$). The addition of sugarcane juice and whey allowed to maintain a constant increase during 24 days in the production of organic acids and buffering capacity compared with the control. No improvements due to the addition of inoculants were observed, which indicated that the natural flora of cassava was sufficient to ferment the paste. It is concluded that it is possible to improve the traditional method of cassava-based yogurt elaboration with the addition of easily-accessed energy sources without the need of adding inoculants.

Keywords: animal feeding, fermentation, inoculation.

Introduction

Cassava (*Manihot esculenta* Crantz), is considered one of the best sources of carbohydrates in rural areas for human feeding (Oliveira *et al*., 2014).

The cultivation of this species is important in the generation of incomes and employment in the rural sector, particularly among farmers in the tropic, with the social and political implications it has (Beovides-García *et al*., 2014): it constitutes a food security crop for small farms (Guira *et al*., 2016).

In Cuba it has been cultivated through the years and is widely distributed; due to its adaptability to different soil and climate conditions, this root occupies an outstanding place as carbohydrate source, for human as well as animal feeding (INIVIT, 2011).

Hence a method to process and utilize the cassava that is not suitable for human consumption has been disseminated among farmers (Almaguel *et al*., 2011) that consists in making a paste with water and fermenting it, which improves its nutritional quality, confers probiotic properties to it and improves its stability in storage, reporting satisfactory results in pig and poultry feeding (Zacarías, 2012).

On the other hand, Lezcano-Perdigón *et al*. (2014) showed that the silage or fermentation of cassava paste favors its chemical and nutritional properties by decreasing the concentration of cyanhydric acid and increasing the quantity of buffering substances that improve rumen conditions.

The objective of this work was to evaluate the fermentation of cassava paste with the addition of energy sources of agroindustrial waste and the inoculation of microbial strains available in Cuba.

Materials and Methods

The experiments were conducted in the Biochemistry and Biotechnology laboratories of the Pastures and Forages Research Station Indio Hatuey, Matanzas, Cuba.

Cassava paste. Second-class cassava roots were purchased in the local market of Perico, Matanzas. They were washed with tap water, brushed and chopped with the peel in an electric blade chopper. The resulting paste was distributed in 4 one-kilogram lots. To each lot 1 liter of each treatment was added: a) sugarcane juice; b) molasses 5 %; c) whey obtained from the elaboration of cheese from fresh milk; d) and drinking water (control).

Inoculants. They were applied at 10 %. The following were used: a) lactic acid bacteria from natural homemade yogurt (experimental control); b) 50 % mixture of natural yogurt and lyophilized bread production yeast Tradipan® activated 24 hours in 10 % solution of sucrose (3 % s/v); c) biopreparation of microorganisms with native strains IHplus® from the EEPF Indio Hatuey; d) drinking water.

Fermentations: They were made in 48 amber glass flasks of 250 mL with metallic cap (4 energy sources with 4 inoculants in 3 times) under anaerobic conditions.

Variables. The pH and acidity were measured (at the beginning and at 8, 16 and 24 days) by potentiometric titration expressed as lactic acid AOAC (1995), as well as the cation exchange capacity expressed as buffering capacity (BC) in milliequivalents of NaOH mL^{-1} from the titration curves (Levic *et al*., 2005) with a Conductronic PH120 potentiometer.

Experimental design. A randomized complete block design was applied for each study factor (energy sources and inoculants) in quadruplicate.

Statistical analysis. For the variables that did not fulfill the assumptions of normality and variance homogeneity, the non-parametric Kruskal Wallis test was used and in the cases in which at least one of them was not fulfilled (water-sugarcane juice, water-molasses, water-whey, sugarcane juice-molasses, sugarcane juice-whey, molasses-whey), the Mann Whitney test was used to make paired comparisons between each of the treatments. The data processing was made with the statistical package SAS® version 9.0, at a significance level of $p < 0,05$.

Results

For all the treatments a fast decrease of pH was observed, from values close to neutrality to values lower than 4 in the first 8 days. The lowest pH was obtained at 24 days of fermentation with the addition of whey (p=0,026) (table 1).

A higher content of organic acids was obtained (table 2) with the addition of whey, sugarcane juice and molasses on day 16 as well as on day 24 compared with the control (p=0,026).

In addition, the fermented pastes had a higher buffering capacity with the addition of whey and sugarcane juice (p=0,026) (table 3).

As shown in tables 4, 5 and 6, no statistical differences were observed in the physical-chemical characteristics of the obtained products when inoculation was not made or when the inoculation was performed with lactic acid bacteria combined with *S.cerevisiae* (LAB-yeast) or with the multi-inoculant IH plus® compared with the yogurt inoculated with lactic acid bacteria (LAB), as proposed in the method proposed by Rodríguez *et al*. (2008).

Discussion

The similarity in the results among the treatments can be explained by the activity of the natural microflora present in sugarcane juice and cassava.

Table 1. pH of cassava-based yogurt with different energy sources and fermentation.

Treatment	Day			
	0	8	16	24
Water (control)	6,90 ± 0,00^{d}	3,87 ± 0,25	3,89 ± 0,21	3,89 ± 0,09^{b}
Sugarcane juice	6,40 ± 0,00^{a}	3,96 ± 0,13	3,77 ± 0,10	3,69 ± 0,02^{b}
Molasses	6,60 ± 0,00^{b}	3,99 ± 0,34	3,76 ± 0,14	3,84 ± 0,19^{ab}
Whey	6,80 ± 0,00^{c}	3,84 ± 0,03	3,65 ± 0,04	3,63 ± 0,03^{a}

a, b, c, d, Different letters in the same column indicate significant difference (p<0,05).

Table 2. Acidity (gL^{-1}) of cassava-based yogurt with different energy sources.

Treatment	Day			
	0	8	16	24
Water (control)	0,34 ± 0,00^{c}	0,64 ± 0,15^{b}	0,67 ± 0,24^{c}	0,77 ± 0,13^{c}
Sugarcane juice	0,42 ± 0,00^{b}	0,88 ± 0,20^{ab}	1,28 ± 0,23^{ab}	1,33 ± 0,32^{ab}
Molasses	0,33 ± 0,00^{d}	0,79 ± 0,30^{ab}	0,90 ± 0,24^{bc}	0,83 ± 0,23^{bc}
Whey	0,86 ± 0,00^{a}	1,14 ± 0,06^{a}	1,42 ± 0,15^{a}	1,69 ± 0,33^{a}

a, b, c, d, Different letters in the same column indicate significant difference (p<0,05).

Table 3. Buffering capacity (meq. of NaOH mL^{-1}) of cassava-based yogurt with different energy sources at different fermentation times.

Treatment	Day			
	0	8	16	24
Water (control)	0,10 ± 0,00^{b}	0,16 ± 0,04^{b}	0,14 ± 0,06^{c}	0,19 ± 0,03^{c}
Sugarcane juice	0,10 ± 0,00^{b}	0,22 ± 0,05^{ab}	0,30 ± 0,06^{ab}	0,32 ± 0,07^{ab}
Molasses	0,10 ± 0,00^{b}	0,20 ± 0,07^{b}	0,22 ± 0,06^{bc}	0,21 ± 0,05^{bc}
Whey	0,23 ± 0,00^{a}	0,29 ± 0,03^{a}	0,34 ± 0,04^{a}	0,41 ± 0,06^{a}

a, b, c, d, Different letters in the same column indicate significant differences (p<0,05).

Table 4. pH of cassava-based yogurt with and without inoculants at different days of fermentation

Treatment	Day			
	0	8	16	24
LAB (control)	6,68 ± 0,22	3,81 ± 0,02	3,65 ± 0,02	3,69 ± 0,11
Without inoculation	6,68 ± 0,22	3,85 ± 0,20	3,75 ± 0,10	3,78 ± 0,16
LAB-yeast	6,68 ± 0,22	4,14 ± 0,27	3,87 ± 0,18	3,81 ± 0,19
IH plus	6,68 ± 0,22	3,85 ± 0,10	3,79 ± 0,18	3,77 ± 0,15

Table 5. Acidity (gL^{-1}) of cassava-based yogurt with and without inoculants at different days of fermentation.

Treatment	Day			
	0	8	16	24
LAB (control)	0,49 ± 0,25	0,97± 0,21	1,06 ± 0,32	1,15 ± 0,19
Without inoculation	0,49 ± 0,25	0,80 ± 0,17	1,13 ± 0,16	1,08 ± 0,41
LAB-yeast	0,49 ± 0,25	0,70 ± 0,37	0,92 ± 0,51	1,05 ± 0,32
IH plus	0,49 ± 0,25	0,98 ± 0,20	1,15 ± 0,47	1,34 ± 0,21

Table 6. Buffering capacity (meq. of NaOH mL^{-1}) of cassava-based yogurt with and without inoculants at different days of fermentation.

Treatment	Day			
	0	8	16	24
LAB (control)	0,13 ± 0,07	0,24 ± 0,06	0,25 ± 0,08	0,28 ± 0,08
Without inoculation	0,13 ± 0,07	0,20 ± 0,04	0,27 ± 0,04	0,27 ± 0,10
LAB-yeast	0,13 ± 0,07	0,19 ± 0,11	0,21 ± 0,12	0,26 ± 0,11
IH plus	0,13 ± 0,07	0,25 ± 0,05	0,27 ± 0,13	0,32 ± 0,16

The pH, of the cassava paste with water as well as with whey, decreased significantly at eight days (p<0,05), and since that day it remained practically without change. The addition of whey allowed that the production of organic acids and metabolites with cation exchange capacity increased constantly during the 24 days in which fermentation was monitored (statistical difference between time periods p<0,05).

The decrease of pH is desirable in fermentation processes to inhibit the growth of pathogen microflora (Cury-Regino *et al*., 2014). In this work the lowest pH was obtained at 24 days with whey (p<0,05), compared with the other treatments with similar values to those reported by Londoño *et al*. (2010) for the fermentation of whey in the elaboration of beverages, which is translated into the inhibition of contamination by pathogens.

The best results of organic acid production (1,69 g L^{-1}) and buffering capacity (0,41 meq NaOH) were obtained using whey. In this regard, Jasaitis *et al*. (1987) observed that the buffering capacity was high in silages with high protein content, due to the presence of aminoacids, which are found in the proteins the whey contains. On the other hand, the improvements in fermentation can be explained due to the fact that the lactose contained in whey is a natural source of energy and nutrients for lactic acid bacteria (Cury-Regino, 2014).

It is concluded that the addition of whey and sugarcane juice significantly improved the chemical characteristics and fermentation process in the elaboration of yogurt from cassava paste. The addition of whey to the cassava paste as additional energy source contributed to prolong its fermentation.

The inoculants did not have incidence on the elaboration of this product as they did not improve its chemical composition. Nevertheless, it is recommended, as long as they are available, to add them in order to guarantee fermentation and favor the inhibition of the undesirable microfauna. In addition, to conduct experimental tests is suggested in which the feeding of pigs and poultry with cassava paste fermented with whey and sugarcane juice is evaluated.

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