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# Reduction of bamboo shoot cyanide by lactic acid fermentation

Sanchez-Santos Arlette[1*], Mora-Tello Mauricio[2], Cardoso-Ugarte Gabriel Abraham[3]

*[1]Faculty of Biotechnology, Universidad Popular Autónoma del Estado de Puebla. 21 Sur 1103, Barrio de Santiago, Puebla, México* arlette.sanchez@upaep.edu.mx

*[2]Fundación Produce Puebla, 26 Norte No. 1202, Col, Humboldt, Puebla, Puebla, Mexico*

*[3]Faculty of Gastronomy, Universidad Popular Autónoma del Estado de Puebla. 21 Sur 1103, Barrio de Santiago Puebla, Mexico*

## Abstract

The effect of the type of fermentation (spontaneous, *Lactiplantibacillus plantarum*, *Lactobacillus paracasei* and the combination of the last two) and the concentration of NaCl (1% and 10%) were evaluated on the HCN content, pH, and titratable acidity in heat-treated bamboo shoots (*Dendrocalamus asper*) for 12 days. To quantify the HCN concentration, the eight treatments were analyzed by the spectrophotometric technique of Guignard sodium picrate, which had a linear calibration curve with a correlation of $R^2$=0.99. The spontaneous fermentation showed a difference on the HCN content between the two NaCl concentrations; where the 10% treatment had a higher reduction of this parameter (from 5.94 ppm to 0.6 ppm), with the decrease of pH (from 7.49 to 4.81) and the increase of titratable acidity (from 0.077 to 0.150). In the inoculated fermentation, the NaCl concentration does not influence the cyanide content; however, the different types of fermentations do. At the beginning (day 2 and 4) the combined fermentation showed a greater decrease in cyanide; while in the medium phase (day 6), the treatments with *Lpb. plantarum* decreased drastically below the others. Finally, in the late phase, all the inoculated treatments reach a similar and negligible cyanide content around 0.45 ppm. Besides that, a bromatological analysis was performed on the heat- treated bamboo shoots to determine its moisture (92.61%), ash content (0.27% wet weight), reducing sugar (0.063% ww), protein content (332.66 µg/mL ww) and fat content (0.15% ww and 2.62% dry weight).

**Keywords:** Bamboo shoot, Cyanide, Fermentation, *Lactiplantibacillus plantarum*, *Lactobacillus paracasei.*

**Corresponding Author: Sanchez-Santos Arlette, Faculty of Biotechnology, Universidad Popular Autónoma del Estado de Puebla. 21 Sur 1103, Barrio de Santiago, Puebla, México*

## 1. Introduction

Bamboo is a fast-growing perennial plant, classified in the family Poaceae and the subfamily Bambusoideae. This subfamily encompasses 1400 species that are distributed globally, from Japan at 51°N latitude to southern Argentina at 47°S latitude. Bamboo, due to its geographical distribution, plays an essential role in daily life, with a prominent impact in rural communities and in Asia. (Yeasmin et al. 2015). Every part of bamboo, including leaves, shoots, and culms, is used in both medicinal and food applications. Bamboo shoots are prepared in a variety of traditional ways, such as boiled, dried, roasted, canned, pickled, blanched, fermented, and in flour, especially in Southeast and East Asian regions, such as India and China (Das, 2019; Satya et al. 2010). The nutritional value of bamboo is remarkable due to its low-fat content, richness in vitamins, essential amino acids, minerals, antioxidants, proteins, carbohydrates, and dietary fiber (Satya et al. 2012). This makes it a food with the potential to improve the nutrition of the general population, especially in marginalized or low-income communities, contributing to the mitigation of malnutrition. This is one of the main objectives of international institutions, as it is related to Sustainable Development Goal 2: zero hunger proposed by the United Nations (UN). Despite bamboo's potential, a significant limitation is the natural presence of cyanogenic glycosides, chemical compounds that release hydrogen cyanide (HCN) when chewed or digested. These compounds are found in at least 2000 plant species, some of which are used as food (Bolarinwa et al. 2016). Bamboo shoots contain taxiphyllin, which is harmless by itself. However, when plant cells are damaged, the enzyme β-glucosidase hydrolyzes cyanogenic glycosides, forming a sugar and cyanohydrin, the same which breaks down into an aldehyde or ketone and hydrogen cyanide (HCN), toxic at doses of 0.5 to 3.5 mg/kg (Satya et al. 2010; Bolarinwa et al. 2016). Bamboo shoots require pre-processing to reduce the concentration of hydrogen cyanide. Methods such as boiling, drying, soaking and fermentation are used, the latter being promising due to the benefits of fermented products. Thus, these benefits range from maintaining the balance of gastrointestinal flora and body weight, to reducing the risk of presenting certain diseases such as cardiovascular diseases and type 2 diabetes; this thanks to the transformation and synthesis of compounds, and the arrival of beneficial microorganisms to the gastrointestinal tract (Marco et al. 2017). However, this process is one of the least studied in bamboo shoots, and although there are records of some experiments that confirm its application in the reduction of cyanide, these have been carried out in bamboo species belonging to other regions and mainly with native microorganisms (Singhal et al. 2021). Since the cyanide content in bamboo shoots varies according to species, age, and environmental conditions, it is crucial to conduct trials on the most common species in Mexico. This will make it possible to take advantage of the country's resources in a safe manner and satisfy the nutritional needs of the population with bamboo shoots.

## 2. Material and methods

Bamboo shoots were obtained from the town of Ahuata, in the municipality of Teziutlán¸ located in the northeastern highlands of Puebla, México; at 930 meters above sea level. Bamboos were planted 5 years ago, and shoots were harvested when they are 50-60 cm tall; no fertilizer was used. The shoots were rinsed superficially to remove the soil. The outer sheaths of the shoots were then removed and only the soft white inner parts were used. These were submitted to a heat treatment in boiling water for 20 min, allowed to cool and then frozen for further analysis. Bromatological analysis for heat-treated bamboo shoots was performed using the following methods. Moisture content was determined by the 31.012 AOAC oven drying method at 90°C for 6 hours (AOAC, 1984). For ash determination, 10 g of sample was previously dry by moisture method and calcined in muffle using the 31.012 AOAC method (AOAC, 1984) at 550°C for 5 hours. To determine total reducing sugars the dinitrosalicylic acid (DNS) assay was used Garriga et al. 2017. Lipids were determined by the 920.39C AOAC Soxhlet extraction method (AOAC, 1984) employing 180 g of dried sample, petroleum ether as solvent and an extraction time of 6 hours (36 cycles). Protein content was determined by the spectrophotometric Lowry essay (Waterborg, 2009). To quantify the HCN content, the spectrophotometric technique of Guignard sodium picrate was used (Borja-Zamora et al. 2022) which is described briefly as follows. Strips of filter paper were cut out, impregnated with a 0.05 M picric acid solution, dried and then moistened with a 10% sodium bicarbonate solution. These strips were suspended from the lid of assay tubes, avoiding contact with sample. 2 gr of bamboo shoots samples were mashed by pestle and added to assays tubes along with 1 ml of chloroform. The tubes lids were immediately closed and let at room temperature for 24 hours. The strips were eluted in 10 ml of water and read at 550 nm. A calibration curve for HCN was previously constructed, preparing a stock solution of 0.0024 g of KCN in 25 ml of distilled water, whose final concentration is equivalent to 0.1 mg of HCN per milliliter of solution. Dilutions from 0 to 12 µg/mL HCN were made. For fermentation process heat-treated bamboo shoots were sliced in the sense of their fibers and cut them with an approximate thickness of 0.5 cm. In the case of the spontaneous fermentation 150 g of these strips were added to a glass jar with 270 g of 1% NaCl and to another one with 10% NaCl. For inoculated fermentation the relation of brine and bamboo shoots from spontaneous fermentation was preserved; 225 g of 1% and 10% NaCl were added separately to 125 g of bamboo shoots along with 5% of inoculum (*Lpb. plantarum*, *L. paracasei* and the 1:1 combination of last two). These lactic acid bacteria were grown in MRS Broth for 48 hrs. at 37°C until reaching 2.2 of optical density. 17.5 ml of each broth cultures were centrifugated at 3000 rpm for 10 minutes and the pellet was resuspended in corresponding brine solutions. The spontaneous and inoculated treatments were left at room temperature for 12 days. The pH, titratable acidity (with 0.1 N NaOH) and cyanide content of each treatment were evaluated every 2 days. Antioxidant and prebiotic activity analysis was conducted in treatments with the lowest cyanide content of each type of fermentation (spontaneous and inoculated). DPPH assay was performed according to the methodology of Brand-Williams et al (1995). Briefly, 10 g of fermented bamboo shoot was smashed by pestle, macerated with 20 ml of distillate water (1:3 relation) for 3 hours at 1320 rpm, filtered by vacuum and centrifuged. DPPH reactant and methanol were mixed and diluted until reaching an optical density of 0.7±0.02 at 515 nm. 3.2 ml of this dilution was added to 2 ml of sample extract, incubated in shaker at 37°C for 30 minutes and read at 515 nm. The result was correlated with a previously made calibration curve.

For prebiotic index, the method reported by (Figueroa-González et al. 2019) was used. Briefly, 8 g of 12 days fermented bamboo shoots were mashed by pestle and added to a reconstructed MRS culture medium as carbohydrate source. For control sample normal MRS medium was utilized. The probiotic used was *Lpb. plantarum 299B*. Culture dilutions from 10-1 to 10-8 were made with peptone water, plated it in MRS agar and incubated at 37°C for 48 hours; then UFC were counted.

## 3. Result and discussion

### 3.1 Bromatological parameters of pre-heated bamboo shoots

The nutritional analysis of pre-heated bamboo shoot from *Dendrocalamus asper* showed similar levels of nutrients than this and other species previously reported. In this case, the shoot was characterized by its high moisture content (92.61%) and low-fat content (0.15% ww and 2.62% dry weight), where the first one is similar to the boiled shoot of *D. hamiltonii* with 92.32 % ww (Santosh et al. 2016). Reducing sugar has a value of 0.063% ww and ash content of 0.27% ww, which is consistent with 0.1 % ww and 0.75% ww respectively, reported by (Satya et al. 2010) for *D. asper* boiled bamboo shoots. Finally, the protein content was 332.66 µg/mL ww. The importance of knowing the nutrient content of *D. asper* bamboo shoot in this area of Mexico lies in the fact that nutrient profile of bamboo species can vary according to the region where it was grown, as was described by (Chandramouli and Viswanath 2015). In this sense, this data helps to know the potential of this specie as a valuable source of nutrition for local population.

### 3.2 Construction of HCN calibration curve

The calibration curve used to correlate cyanide content with absorbance was adjust to a linear form, which is described with the following equation y = 0.0093x + 0.0054, where x= HCN concentration (µg/mL) and y= absorbance at 550nm. This equation shows a correlation of $R^2$=0.99. To calculate the HCN content in bamboo shoots an inverse curve was constructed x= (y-0.0054)/0.0093.

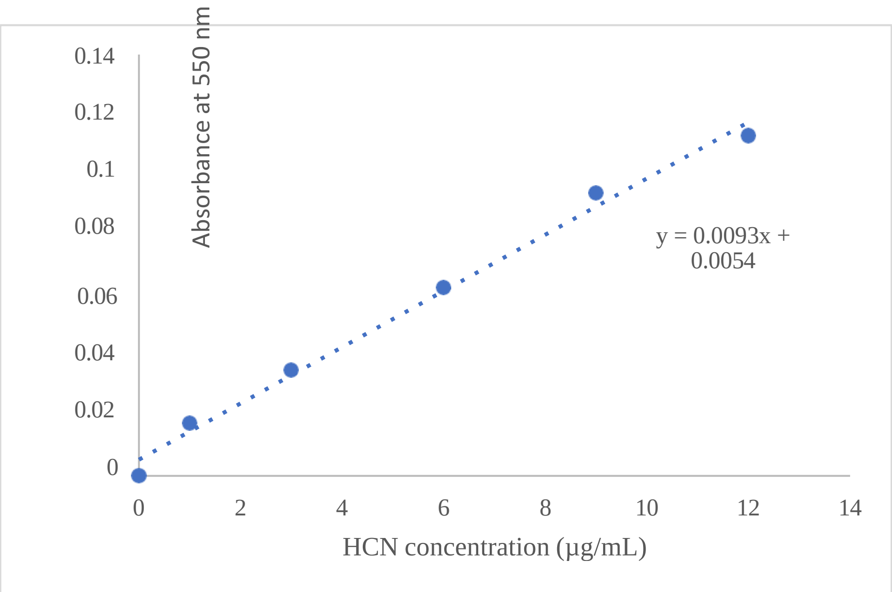


**Figure 1**. HCN calibration curve

### 3.3 Effect of fermentation type in HCN content

After treatments were fermented for 12 days, the HCN content of each one was calculated using the measured absorbances and the respective calibration curve; these results were plotting in Figure 2. To analyze these results, the Tukey test was used. According to the analysis, the 1% and 10% NaCl treatments from spontaneous (1.46 and 0.06 ppm respectively) and Lpb. plantarum (0.53 and 0.70 ppm respectively) fermentations reached their lowest HCN content in day 6; from this day forward, the cyanide concentration did not show a change that is statistically significant, with a confidence interval of 95%. Furthermore, 1% treatment of mix culture and *L. paracasei* stopped their decrease in cyanide in day 8; while 10% of these same microbial cultures did at day 10. Every ferment exhibited a different behavior on each one of the days. In day 0 the levels of cyanide were significantly equals. In day 2, at the beginning of the process, the 10% and 1% mix, and the 10% spontaneous treatment were just the ones that shows a significant difference between the initial value of cyanide and the one in day 2. What means that these microorganisms act in the early phase of fermentation. In day 4, differences in HCN reduction between all the treatments biggins to be present. Where the highest reduction was attributed to 10% mix fermentation and the lowest reduction to 1% and 10 % Lpb. plantarum. Reaching day 6, all treatments significantly get the same level of cyanide, with exception of 1% spontaneous (lowest reduction) and 1% Lpb. plantarum (highest reduction)

Which agrees with the previously reported behavior of Lpb. plantarum, acting in the medium phase of fermentation (Lu et al. 2022). Finally in day 8, 1% spontaneous fermentation continued to be in the same place than day 6, while the other ones become significantly the same, and this continued for the following days (Figure 2).

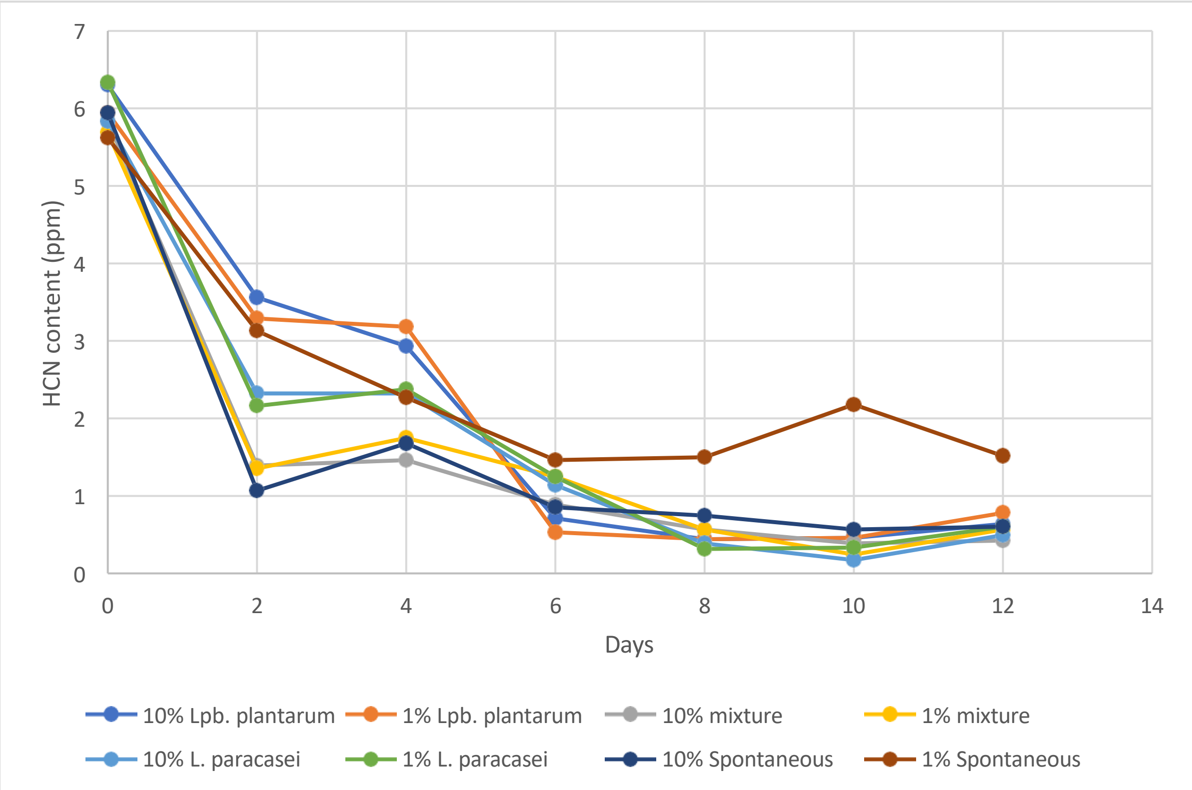


**Figure 2**. HCN content of spontaneous and inoculated bamboo shoot fermentation

### 3.4 Antioxidant activity and prebiotic index of fermented bamboo shoots

Before doing the DPPH essay, the 10% spontaneous fermentation showed an absorbance of 0.4920 and an absorbance of 0.1206 for 1% *L. paracasei*, which is traduced to 30.01 % and 82.84% of inhibition of the DPPH radical respectively. The antioxidant activity of 1% *L. paracasei* is considered high and it is even higher than the reported by (Lu et al. 2022), in which this lactic acid bacteria were used in a fermentation process with soy bean and the results showed a 27% of inhibitions against DPPH. For prebiotic index, after 48 hours of incubation, 1% *L. paracasei* fermentation showed 28x10-7 CFU, the 10% spontaneous fermentation 34x10-7 CFU, and the control 163x10-7 CFU. The prebiotic index for 1% *L. paracasei* is 0.17 and for 10% spontaneous fermentation is 0.20, which are not significantly as they are not higher than 1. In this sense bamboo shoots after fermentation are not a good source of prebiotics; the reason for this could be related to the previous used of the carbohydrate source by the lactic acid bacteria during the fermentation process.

**Conclusions**

After performing the experiments, it was determined that spontaneous fermentation with 10% NaCl and 1% NaCl with L. paracasei were the most optimal for the reduction of HCN content. The 1% L. paracasei treatment showed a better performance in the inhibition of the DPP radical. Besides that, the natural fermentation process displayed variations in HCN levels depending on the NaCl concentrations used. The treatment with 10% NaCl showed a more pronounced decrease in this parameter, reducing it from 5.94 ppm to 0.6 ppm. This was accompanied by a decrease in pH from 7.49 to 4.81 and an increase in titratable acidity from 0.077 to 0.150. Conversely, the NaCl concentration did not affect the cyanide content in the inoculated fermentation, but differences were observed among various fermentation methods. Initially, the combined fermentation resulted in a greater reduction in cyanide levels on days 2 and 4, while treatments involving Lpb. plantarum showed a significant decrease during the middle phase (day 6). In the late phase, all inoculated treatments reached similarly low and insignificant cyanide levels, approximately 0.45 ppm.

**Conflict of interest**

The authors declare there is no conflict of interest

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