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Biotechnology as a Tool for the Agroindustrial Exploitation of Residues of the Chain of *Musa* spp.

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Currently, one of the major problems of agricultural and agroindustry production chains worldwide, is generated by the by-products that later become contaminating sources of the environment. It is then, where the use of biotechnology as a tool applied to agroindustry processes manages to transform by-products into products with greater added value. For its part, the cultivation Musa spp., has been a traditional sector of the peasant economy, but at the same time it generates a significant amount of waste (pseudostem and leaves) that remain in the field after harvest. Therefore, it is proposed in this research to identify the potential of the pseudostem of Musa spp., as raw material for the development of nutritional blocks that serve for animal consumption, taking as a reference the characterization, pretreatment and combination of said byproduct with waste from other agroindustry chains, for their nutritional potential such as molasses, glycerin and the cake of palm kernel. In addition, two factors were evaluated: the first with pseudostem from of (San Francisco - Cundinamarca and Tibaná - Boyacá) and the second pre-treatment from (stem in cool and stem in silage); with these were constructed nutritional blocks considering the two research factors and their combinations to establish the behavior and nutritional quality of pretreatments. The results showed a reduction of the lignin level, an increase of the protein content up to 30%, as well as a decrease in the percentage of Neutral Detergent Fiber - NDF, converting of pseudostem blocks into a food supplement with great potential of use in the animal feed and with this in an alternative to mitigate the negative impact of the disposal of these residues in the environment.

1. Introduction

In Colombia, edible Musaceae are grown throughout the country, from sea level up to 2000 m.s.n.m., with average temperatures between 14 ° C and 38 ° C. In 2013, 65% of the area sown in the country was 394,351 hectares (Suárez et al., 2017); demonstrating the wide variety of ecological conditions in which the develops is grown and the importance of the species from an alimentary and economic point of view, since it constitutes a basic product of Colombian society's diet. At present, 80% of the production is in the departments of Quindío, Meta, Antioquia, Tolima, Caldas, Córdoba, Risaralda, Valle, Nariño and Cauca.

However, it is known that its production focuses on the commercialization of the fruit, being that, once this is harvested, the rest of the plant is lost, thus forming a significant amount of residual biomass; hence, when crop is harvested only used 20 to 30 percent of its biomass, being left respectively of a 80 to 70 percent of the plant without used, providing a negative environmental impact since in most cases are incinerated or they deposit outdoors in dumps with low level of technological management or are distributed in the field contributing to the degradation of the ecosystem; Nevertheless, some producers take advantage of planting residues in the form of green manure (Mazzeo et al., 2010) or give them poor use as a food source for animals (De la Cruz & Gutiérrez, 2006).The foregoing indicates that, in the productive chain only the fruit is processed; the rest, which are the fruit peels, rachis of the cluster, pseudostem and bract of the plant does not enter the Colombian agroindustry, but it is part of an environmental and economic problem because few alternatives have been found to process them in a way profitable. From the chemical point of view, the components of the different species of *Musa* spp. plants, such as pseudostem, could be used in animal feed; however, these by-products are still undervalued and therefore underutilized (De la Cruz & Gutiérrez, 2006). The pseudostem of these plants are very useful fodder sources in many tropical countries especially in the dry season; and as a traditional alternative, have been used directly (fresh state) or in silage as part of animal feeding (Vidal et al., 2001); besides, of reduce the dependence of inputs external (De la Cruz & Gutiérrez, 2006).

2. Materials and methods

2.1 Location of the study

The research project was carried out at the Fundación Universitaria Agraria de Colombia - UNIAGRARIA, in company of the Universidad Cooperativa de Colombia - UCC, which are located at Calle 170 No 54A -10 and Av Caracas 37-63 respectively, in the city of Bogotá (Colombia); at 2650 m above sea level, with a relative humidity of 94% and an average annual temperature of 14°C (Suárez et al., 2016). The process of adaptation, pretreatment of the raw materials and elaboration of the blocks was carried out in the phytochemical laboratory of UNIAGRARIA.

2.2 Description of the plant material

The samples of pseudostem of Musa spp. were taken from native plants from the municipalities of San Francisco de Sales, Cundinamarca and Tibaná, Boyacá in Colombia, with 20ºC and 16ºC average temperature and an altitude of approximately 1520 and 2115 meters respectively. The cut of the pseudostem, referenced by Suárez et al. (2016), is to distance of 1 m from the base of the stem, the cut type is transverse and is made to a length of approximately 50 cm. These are then transported at room temperature on sheets of paper to the laboratories of UNIAGRARIA. Before being packaged, traces of dead plant material and soil and insects found superficially are removed. Once the samples to be analyzed reach the destination, they are cut into 2 cm wide slices and stored in a refrigerator in plastic bags with a hermetic seal at a temperature of 4° C.

2.3 Evaluation of the behavior of fermented and unfermented raw material in the physicochemical stability of the nutritional blocks - BN

In the experimental process, a pretreatment was developed to work with a homogeneous material, from the reception of the plant material to its adaptation and dehydration. Subsequently a silage process was carried out with a fraction of pseudostem dehydrated from each source and, to stimulate the development of adequate levels of lactic acid, molasses was added as an easily fermentable source of carbohydrate. The fermentation process was carried out by performing four replicates per pseudostem. After the second week of the fermentation process, the pH of the pulp was monitored, considering that when it reaches a range between 4 and 4.2 and kept it stable for more than 4 days, it would indicate that already the pasta could be used to make the nutritional block. The main intention of silage was the fermentation of sugars that would allow a better use of the vegetal material on the part of the animal; as well as the influence of this type of pretreatment on leaf material in the useful life and quality in the construction of nutritional blocks with alternative raw materials.
The chemical composition of the nutritional blocks was determined in the Calderón Assistência Agrícola LTDA Laboratories, considering the respective methodologies of Table 4. Through a bromatological analysis, through Van Soest evaluated crude protein (PB), ethereal abstract, fiber, among others.

2.4 Preparation of nutritional blocks

For each stem from San Francisco and Tibaná, two types of blocks (with four replicates each) were elaborated, whose difference lies mainly in the state of raw material with which the blocks were constituted: pseudostem dehydrated – silage. Additionally, it was established that the total weight of the block was 1kg. Based on the literature, the nutritional ratios of each component were determined, taking into account the raw materials used and their function in the block, to make this product a supplement that would meet the nutritional requirements of the animal. Taking as a reference the above, the proportions of each of the raw materials established within the block were: 55 % pseudostem (550 g), 21.5 % palm kernel cake (215 g), 10 % glycerin (100 g), 5 % urea 50 g), 5% molasses (50 g), 2 % lime (20 g), 1 % cement (10 g) and 0.5 % Magnesium Sulfate (5 g).

2.5 Physicochemical stability of the blocks

For the analysis of the influence of leaf pre - treatments on the nutritional quality of the Nutritional Blocks - BN, it was considered that these were kept indoors at an average temperature of 22 ° C, to avoid the loss or gain of moisture by effect of the rains or the high temperatures. The physical-chemical measurement variables described in Table 1 were chosen for being the ones with the greatest influence on the quality of the product.

Table 1: Techniques and parameters of analysis for the physical stability of the nutritional block

	Properties	Methods
Physical	Weightloss	Gravimetric
	Loss of moisture	Gravimetric
	Firmness	Penetrometer
Chemicals	pH	pH meter

2.6 Context of experimental design and statistical analysis.

A factorial model of 2^2 was used, having as factor "a" the origin of the raw material (**a_1**= municipality 1: San Francisco de Sales (Cundinamarca) and a_2 = municipality 2: Tibaná (Boyacá)) and as factor "b": Pretreatment b_1 = pseudostem dehydrated and b_2 = silage pseudostem). The above is expressed as:

- T1F (a_1 b_1) corresponds to pseudostem dehydrated (b_1) coming from the Municipality San Francisco de Sales (a_1).
- T2F (a_2 b_1) corresponds to pseudostem dehydrated (b_1) coming from the Municipality Tibaná (a_2).
- T1S (a_1 b_2) corresponds to pseudostem silage (b_2) coming from the Municipality San Francisco de Sales (a_1).
- T2S (a_2 b_2) corresponds to pseudostem silage (b_2) coming from the Municipality Tibaná (a_2).

For the analysis of data was taken as a reference by Suárez (2011) who describes for this type of studies the performance of a simple analysis of variance (ANOVA) between the means of the samples by treatment with a level of significance of the 95% (α = 0.05). This is to establish if there are significant differences for the variables under evaluation. As there were no significant differences between the samples, a multiple range test was performed using the Statgraphics Centurion statistical package.

3. Results and discussion

3.1 Silage

To evaluate the influence of the pre-treatment of the raw material on the nutritional contents of the final block, with respect to the implementation dehydrated pseudostem regular, it has been made, a silage pseudostem enriched with molasses as a source of fermentable sugars, because the content of non-structural sugars is relatively low, as determined by in the bromatological analyzes for a similar raw material studied by Suárez et. al., (2017), allowing infer that the fermentation process would require the enrichment of fermentable sugars (Diez-Antolínez et al., 2016 & Raganati et al., 2016). Additionally, Figure 1 shows the relationship between the initial values of %H and pH; where a1 had an initial %H of 39% and a pH of 4.47, while a2 had an initial moisture of 42% and a pH of 5.5. This relationship may be due to a high moisture content that dilutes both the available carbohydrates for acid production and the acid formed (Cárdenas et al., 2004).

At present, the pH is a variable that serves as reference an indicator of the fermentative quality in silages with low MS content (Mier, 2009); additionally, as referenced by Betancourt et al., (2005), the faster the pH drops, the more likely it is to ensure unfavorable habitat for clostridial bacteria and to reduce respiration; thus, avoiding the proteolysis and proliferation of undesirable microorganisms in the process. Therefore, it was considered that as soon as the pH of the silo was below 4.2, they should open to assemble the blocks. Finally, nutritional blocks were assembled on day 11 of the treatment, when the pH was 3.75 for a_1 and 4.25 for a_2; as shown in Figure 1. Likewise, when comparing the fermentation time between the pseudostem from San Francisco (a_1) and the one from Tibaná (a_2), it was observed that the fermentation process speed is higher in a1 than in a_2, this isn't due to only to the % H of each sample, but also is linked to the non-structural (or easily fermentable) carbohydrate content of each pseudostem, since these (carbohydrates) constitute the nutritional substrate on which the action of the fermentative microflora of the forage. When analyzing the pseudostem, the highest concentration of fermentable sugars was found in a1, consequently, the silage process was faster for a1 than for a_2. Table 2 shows summary of the most relevant statistical data in the evaluation: comparative variation of pH both at the beginning and at the end of the silage process.

At the statistical level, the pH averages of the treatments were tested using Fisher's test, demonstrating that there is no homogeneity between them, both at the beginning and at the end of the process; which indicates statistically significant differences between the grouping of the averages. On the other hand, with said result it can be expressed that the pH values basically lie in the difference that the samples present to release water.

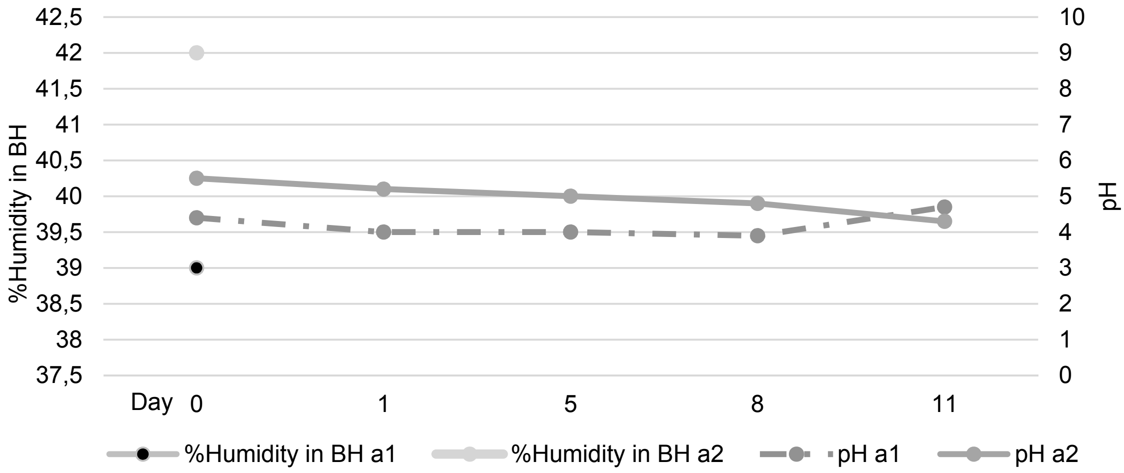

Figure 1. pH variation with respect to the silage time of dehydrated pseudostem enriched with molasses (a_1 and a_2). Initial %H for treatment by assay.

Table 2. Statistical analysis of initial and final silage pH evaluation.

Statistical	pH			
	INITIAL		FINAL	
	T1S	T2S	T1S	T2S
Average	4,375	5,25	3,75	4,5
Standard deviation	0,478	0,288	0,288	0,439
Significance	b	a	b	a

3.2 Physical stability of the nutritional blocks made with pseudostem of *Musa* spp.

Figure 2 shows the variation in weight reflected as %H on wet basis of the blocks over time in the experimental process. The difference in weight loss velocity of the stem (a_1), with respect to the stem (a_2), is remarkable in both the treatment of the block with dehydrated pseudostem, and silage; however, the final moisture content is quite proximal in all treatments. On the other hand, it was possible to identify that the greater weight loss was the T2F treatment, and that at a higher speed than the rest of the treatments; this could be due to both internal and external factors, such as climatic conditions of temperature and humidity in the drying greenhouse, in addition to, differences in moisture contents and compaction quality of the blocks. Also, plant tissue conditions, maturity of the Musa spp. Plant, directly affect the transpiration conditions of the plant material at the time of harvest; together with relative humidity conditions and temperature variations in the greenhouse could significantly influence the representative difference of this treatment compared to the others.

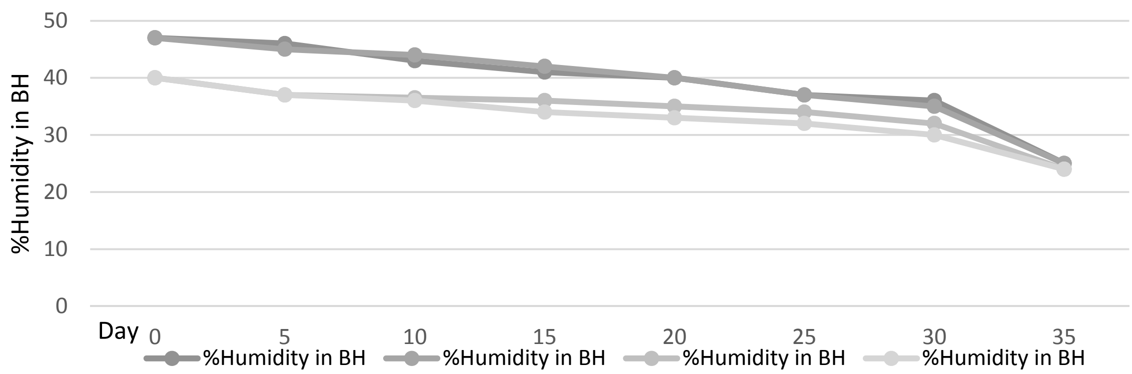

Figure 2. Variation of % H in BH with respect to time.

This allows, visually, to identify the loss of moisture of the blocks progressively; after day 9 it was necessary to change the paper to avoid fungi due to excess humidity. In addition, changes were observed in the physical appearance of the blocks, becoming obsolete during the last days, mainly due to the excessive loss of humidity and quality in the compaction and homogenization of the components. Similar results were obtained by Cigasova et al. (2016), in his study on the use of vegetal residues.

As in the pH evaluation for the pretreatment of silage, the means of the treatments were tested using the Fisher test with 95 % confidence.

Table 3. Statistical analysis of variation evaluation by weight of the blocks.

Statistical	Variation in weight			
	T1F	T2F	T1S	T2S
Average	0,1497	0,1632	0,1599	0,1364
Standard deviation	1,358 $X10^{-3}$	6,722 $X10^{-3}$	5,204 $X10^{-3}$	3,099 $X10^{-3}$
Significance	b	a	a	c

Table 3 shows a summary of the most relevant statistical data in the evaluation of variation in weight of the blocks. During the drying of the blocks, the hardness that was reached, according to Birbe et al. (2006), is a factor that affects the consumption of the same in greater proportion by the animal; additionally, it was inferred that, with greater compaction, the weight of the multinutritional blocks increases and the dry mass increases. Based on this, an analysis was made with the penetrometer for test 2. Table 4 shows a summary of the most relevant statistical data in the evaluation of the penetrometry of the blocks.

Table 4. Bromatological analysis -Van Soest of the blocks of Musa spp.

Analysis		**T1F**	**T1S**	**T2F**	**T2S**
FIBERS (%)	FDN	35,46	46,39	41,96	47,57
	FDA	18,82	25,72	23,63	20,72
	FDK	11,28	9,63	11,12	11,27
PROXIMAL ANALYSIS (%)	Humidity	33,33	38,65	35,96	33,22
	Ethereal Extract	10,97	11,54	10,62	9,71
	Ashes	12,32	12,74	11,4	13,02
	Sílice	0,27	0,29	0,24	0,26
	Digestible Dry Matter (TDN)	74,66	69,84	71,3	73,34
	Crude protein	35,5	32,5	30	30
	Lignin	0	0	0	0
	Cellulose	8,6	6,85	8,65	8,46
	Hemicellulose	3,88	7,82	6,62	12,86
	Non-Structural Carbohydrates	28,73	28,55	32,71	25,95
MINERALS (%) Macroelements	phosphorus	0,2	0,21	0,19	0,15
	Potassium	2,23	1,64	1,58	2,2
	Calcium	3,53	3,1	2,91	2,66
	Nitrogen	5,68	5,2	4,8	4,8
	Magnesium	0,31	0,3	0,28	0,27
	Sulfur	0,46	0,41	0,36	0,38
	Chlorides	0,64	0,52	0,49	0,5
	Sodium (ppm)	163	134	97	129
MINERALS (ppm) Microelements	Iron	154	140	118	119
	Manganese	110	82	95	140
	Copper	12	15	12	14
	Zinc	22	18	13	16
	Boron	17	27	25	21
ENERGIES (kcal/kg)	E. Digestible	3.292	3.079	3.144	3.233
	E. Metabolizable	2.699	2.525	2.578	2.651
	E.N. of Breastfeeding	1.713	1.595	1.631	1.681
	E.N. of maintenance	1.877	1.737	1.779	1.838
	E.N. of Weight Gain	1.157	1.016	1.059	1.118

The multiple range test in the penetrometer hardness evaluation under Fisher's method, did not show a statistically significant difference between the treatments, obtaining a 95 % confidence; which makes it possible to infer that the pretreatment of silage of the pseudostem does not significantly affect the compaction capacity of the pseudostem with the other components in the construction of the nutritional block.

3.3 Chemical composition of the blocks made with pseudostem of *Musa* spp.

The chemical composition of the nutritional blocks was determined in the Laboratorios Calderón Assistencia Agrícola LTDA, considering the respective methodologies of Table 4. By means of a bromatological analysis - Van Soest was evaluated crude protein (PB), ash, ethereal extract, fiber, among between others.
Thus, when comparing the data in the previous Tables it can be said that the energy values, in general of the four types of blocks, are within the normal, which can be determined by the level of lipids present in these which reached up to 11.54 %; however, the protein content of the T1F and T1S blocks was higher (35.5 % and 32.5 %), a similar result was obtained for the percentages of calcium in the four blocks: T1F: 3.53 %, T1S : 3.1 %, T2F: 2.91 % and T2S: 2.66 %. In relation to the phosphorus values, the percentage was 0.2 % for T1F, T1S and T2F blocks, while for T2S it was 0.15 %; that is to say, all treatments had low percentages to those reported by the aforementioned author.

4. Conclusions

Hence, when performing the analysis of VAN SOEST on fresh raw materials it was possible to detect the high nutritional potential of the pseudostem of *Musa* spp. and the difference of composition that can exist for this raw material from if the source is taken into account. The lignin, ash, hemicellulose, non-structural carbohydrates, as well as sodium and manganese contents were highlighted as elements of greater variation in the composition of the pseudostem. A loss of moisture at the beginning of the process with a higher speed in a temperature of 45 °C appeared, regardless of the origin of the pseudostem, only that it stabilized after 18 hours together with the samples that were treated at 35 °C. Although initially, the stabilization process of the nutritional blocks belonging to each of the treatments showed differences in the stabilization process, it was found that the values were kept within the physicochemical ranges established for animal feed, mainly ruminants.

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