BASE LINE TO PROVIDE ADDED VALUE TO FERMENTED HANDCRAFTED RICE BASED PRODUCTS "MASATO"

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ABSTRACT

Fermented artisanal foods increased in popularity and some are considered intangible heritage. "Masato" is a typical drink of Colombia and has lost tradition and even there is ignorance of the product, but it is also the sustenance of many families. There are no reports that correlate natural fermentation, microbiota and the environment with its quality, so we sought to establish a baseline that aims to add value to the product for sustainable development. Samples of "masato" from lots of three volunteer producers who authorized the documentation of processes and the evaluation of microbiological indicators and physicochemical aspects. As a result, it was demonstrated the high prevalence of fermenting microorganisms and others that exceed the criteria allowed for consumption, affecting positively or negatively the quality of the product, this knowledge can be used in training for strategic development and continuous improvement of productivity of microenterprises.

Keywords: natural fermentation, beneficial microorganisms, culinary heritage handcrafted products

1. INTRODUCTION

The popularity of artisanal foods elaborated by generational tradition, together with the culture and customs of their inhabitants, has increased in recent years due to the presence of beneficial microorganisms that could be of interest for the prevention of specific gastrointestinal pathologies or other beneficial effects for health (LÓPEZ, 2010; Dominguez, 2012, VALENCIA *et al*., 2019). Hence the Proinfant-CYTED project to promote cooperation and harmonious development of science and technology in Iberoamerican regions and it was proposed like initiative for promoting strategics on vegetable Functional Foods and at the same time to widen the knowledge on this type of food.
In addition, these traditional foods and culinary knowledge constitute the pillars of the nation's intangible cultural heritage (UNESCO, 2012; MINISTRY OF CULTURE, 2012), therefore the need to document processes and methodologies directly from producers to propose improvements that lead to sustainable developments and add value to their products, especially when many of these products are the livelihoods of families in developing countries.
In this way, the climatic diversity in the different regions has allowed the development of autochthonous native food products (ÁLVAREZ, 2014), elaborated through artisan processes that allow the natural development of the associated microbiota (CHAVES *et al*., 2014; VEGA AND LÓPEZ, 2012). Among some of these fermented foods, those typical of indigenous culture are mainly corn (*Zea mais*) and yucca (*Manihot esculenta*), while those typical of African culture are based on rice (*Oryza sativa*) and banana (*Musa paradisiaca*) (FAO, 2015; ÁLVAREZ, 2014). A typical example in Colombia is the "masato" made from different cereals, whose fermentation and final characteristics depend on the specific preparation of the food, as well as the ingredients with which it is prepared.
The “masato” is a liquid creamy drink, spontaneously fermented for periods ranging from 2 to 5 days for consumption, handmade with uncontrolled processes in the absence of appropriate conservation techniques and are marketed locally. The growing interest in these traditional fermented foods has raised the need for studies focused on the standardization of the manufacturing process (BECERRA, 2014) and physical-chemical and nutritional characteristics (FULA, 2010; BECERRA, 2014). However, only a few studies have reported preliminary microbiological characterization of such raw food materials (LÓPEZ *et al*., 2010).
The development of the natural microbiota depends on many external factors causing bacteria such as lactic acid bacteria (LAB) to be found in this ecosystem, which can modify the fermentable sugars (sucrose, fructose, glucose) present in small quantities in cereals, cost-effectively improving the nutritional value and sensory properties of these foods, and even specific strains of LAB could be found that could have beneficial effects on the health of the consumer (VÄKEVÄINEN *et al*., 2018) special interest has been aroused in those microorganisms that contribute to the intestinal microbial balance of the consumer (some of them called "Probiotics") or those that produce probioactive substances (vitamins, enzymes or antagonistic compounds on specific pathogens (GARCÍA *et al*., 2018; CHAMPAGNE *et al*., 2018).
On the other hand, in these foods there may also be the presence of other microorganisms that may turn the food into a product that is not safe to consume. The presence of all these microorganisms can be correlated with physicochemical analyses that show specific effects of the predominance of some microbial communities. Therefore, this research aimed to establish a baseline to improve the microbiological and physicochemical quality of masatos handmade in Ventaquemada, Boyacá, Colombia (5°21′59″N) as culinary heritage of the country and recovering LAB culture collections like representation of cultivable biodiversity, that will be further characterized for biotechnological traits.

2. MATERIAL AND METHODS

2.1. Collection of "masato" samples

Three artisanal producers of rice masato (P_1, P_2 and P_3) located in the village of Ventaquemada, Boyacá, Colombia (5°21′59″N), who voluntarily accepted to be participants, were selected and signed an informed consent where they accessed the documentation of their processes during the elaboration of three batches of product for each of them and the taking of samples for microbiological and physicochemical analysis. During the elaboration of the batches of "masato", samples were taken at the beginning of fermentation, at an intermediate time of fermentation and at the finished product. The samples were aseptically collected in sterile screw cap bottles, frozen at -18°C±2°C and transported to the laboratory in the shortest possible time for further analysis.

2.2. Microbiological and physicochemical evaluation of "masato" simples

The initial and intermediate samples were evaluated LAB NTC 5034 count from 2002 and moulds and yeasts NTC 5098-2 from 2009, as well as titratable acidity (AOAC method 981.12) expressed in % lactic acid (%p/p) and pH (AOAC method 981.12) and pH curves during fermentation were measured in real time using a device manufactured by the Center for Interfacultative Instrumentation of the University of Antioquia. The electrode was kept in contact with the inoculated mixture for 24 hours and evaluated for 48 - 72 hours.

The finished products were evaluated in addition to the above, the following microbiological criteria: mesophilic counts according to the Colombian technical standard NTC 4519 of 2009, total coliforms and *E. coli* (NTC 4058 of 2018), *Staphylococcus aureus* (NTC 4779 of 2007), *Bacillus cereus* (NTC 4679 of 2006), moulds and yeasts (NTC 5098-2 of 2009) and the test for absence or presence of *Salmonella* spp. (NTC 4574 of 2003). Surface sowing was also performed to count acetic acid bacteria (AAB) using WL agar (Scharlau - Spain) incubating Petri boxes at room temperature for 8 days. And, the LAB count using Man Rogosa Sharpe (MRS- Oxoid-Spain), M17 (Oxoid-Spain) and the yeast agar glucose peptone meat lactose (YGLPB) all supplemented with 100μg/mL of cycloheximide, the BAL boxes were incubated at 30°C for 48 h under anaerobic conditions. (PÉREZ-CATALUÑA *et al*., 2017).

For the microbiological tests, each sample was weighed 10 g and homogenized with 90 mL of 0.1% peptone water. Subsequently, serial dilutions and sowing were made in different culture media. Except for the *Salmonella* spp. test, which had an enrichment process for which 25 g of the feed were weighed in 275 ml of lactose broth. After the incubation period corresponding to each microorganism, the colonies were counted and calculated in Log UFC/ml, for its later analysis.

The physicochemical analyses were: determination of the content of maltose, glucose, fructose, lactic acid, acetic acid, propionic acid and alcohol all by HPLC, for it, the homogenized samples were diluted in 9 mL, filtered and evaluated in a Chromatograph Agilent 1200, Column: HPX87HAminex BIORAD, Column temperature: 35°C, Detector temperature: 35°C, Flow: 0.6ml/min, Mobile Phase: H2SO4 0.008N, Run time: 25 min, Detector: RID, results expressed in g/L. Humidity in the oven (AOAC method 925.45) and rheological behavior of the "masato" in an equipment.

2.3. Statistical analysis of the data

The statistical difference between treatments was determined using the 95% standard deviation limits (LSD). The other response variables were analyzed with the GraphPad Prism 7.0 software using 2-way ANOVA and the Bonferroni test to determine a significant difference between treatments.

3. RESULTS AND DISCUSSION

The average of data obtained from the documentation of the process during its elaboration of the "masato", the standard deviations and the coefficient of variation, for each producer is presented in Table 1.

Table 1. Masato´s Formulations made by producer.

Raw material	Producer 1			Producer 2			Producer 3		
	Quantity (g)	SD	CV	Quantity (g)	SD	CV	Quantity (g)	SD	CV
Water	2667	577	22	3900	173	4	1092	166	15
Rice	500	0	0	500	0	0	527	46	9
Sugar	558	52	9	1167	577	49	250	0	0
Pineapple peel	0	0	0	333	115	0	400	0	0
Bacterial inoculum	0	0	0			0			0
Cinnamon	15	0	0	10	0	0	20	0	0
Clove	6	0	0	3	0	0	0	0	0
Clove water + cinnamon (Rice smoothie)	1500	500	33	0	0	0	1450	87	6
Water to liquefy	3000	1732	58	5667	577	10	503	286	57

The results of the elaborated formulations show a great variation in the content of water, sugar and rice among the same lots of a producer, which indicates the lack of standardization of the process that directly affects the availability of substrate for the microorganisms and variation of the environmental conditions for the growth of the different microbial genera (BECERRA, 2014).

Log average of LAB counts and standard deviations (SD) in samples evaluated during the fermentation process, present at the beginning counts between 7,9 to 8,3 Log CFU/mL, counts that were changing during the process, reaching for P_2 and P_3 the highest values (8,23 and 8,7 Log CFU/mL, respectively), similar values were obtained by Salmerón *et al*. (2015). This behavior is associated with the ability of LABs to tolerate stress conditions such as low pH (BORICHA *et al*., 2019), which is essential for their survival and allows them to metabolize different substrates and be dominant microbiota in vegetable and cereal fermentation processes, similar results were obtained in a rice based fermented chicha (PUERARI *et al*., 2015). The P_1 obtained the lowest LAB Logs with a decrease in the intermediate time, without overcoming the initial number of microorganisms (Fig. 1).

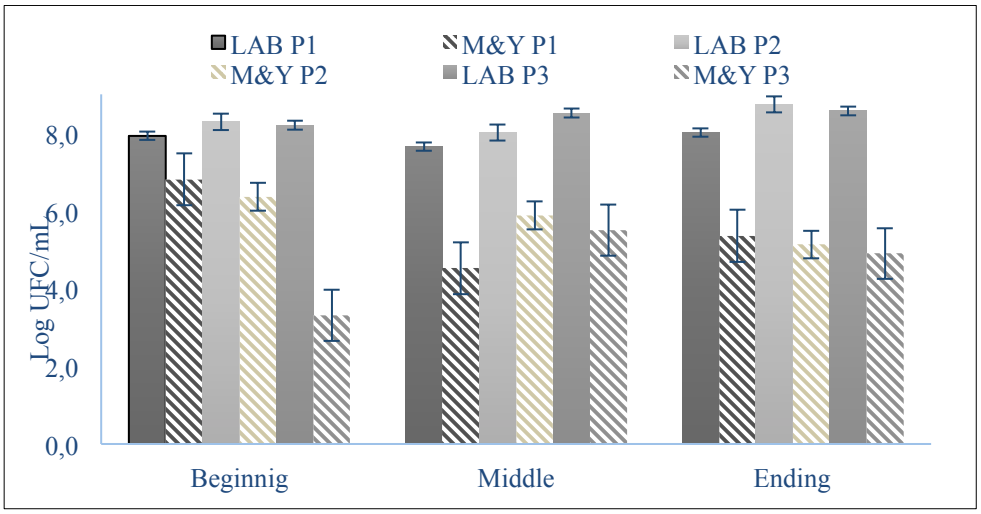

Figure 1. CFU log of lactic acid bacteria and yeasts during the rice masato fermentation process of three different producers.

On the other hand, the diversity and growth dynamics of yeasts reveal that they are important in fermentation, authors such as MENDOZA *et al*. (2017) allude that these microorganisms have been found in several autochthonous fermented products such as Andean Chicha and Colombian Champús (OSORIO *et al*., 2008). In fermented cereals, yeasts make a useful contribution to improving the taste and acceptability of the product. In the case of masato, greater cell concentration is reflected in the initial sample for P1 and P2 with values of 6.8 and 6.36 Log UFC/mL, respectively, which lose viability in the final product, without presenting statistically significant differences in the "masato" of rice for the three producers (5.36 - 5.14 and 4.9 Log CFU/mL). The relationship of LAB and yeasts favor the development of the typical sensory profile, SALMERÓN *et al*. (2014) emphasize that the success of a product is associated with its flavor, which in turn is a critical factor in the acceptance or rejection of a food, for this reason mixtures of yeasts and LAB strains have been proposed, in order to combine their metabolites according to the tastes of consumers (REHMAN *et al*., 2006).

It was also possible to establish that the pH curves were different, with the P_3 curves presenting lower pH, although at the end of the fermentation process, the pH of P_2 did not show significant differences ($P<0.05$) with the pH of P3 (Fig. 2). It should be noted that the fermentations of P_1 rice "masato" presented a pH>5.54, a value that could favor the growth of other microorganisms that generated greater competition for the BALs present there (Fig. 2).

The CFU/mL Log of the counts of microorganisms indicating quality and safety in the finished products of the different elaborated lots are presented in Table 2, where it is observed that the total mesophiles, the AAB and the LAB presented high and similar counts among the producers (less than one logarithmic unit among them), except for the P_1 AAB. The coefficient of variation between the lots of each producer was high for P1, the variability of these microbiological criteria in the same producer could be related to the cross-contamination and the material of the containers used during the elaboration of the masato (Fig. 3), these variations could directly affect the flavor in the finished products and inhibit or favor the presence of undesirable bacteria by means of the production or degradation of different acids (lactic, acetic, propionic). SALMERÓN *et al*. (2015) refers in his research that the variation in the concentrations of lactic acid and acetic acid can change the flavour attributes of a fermented product.

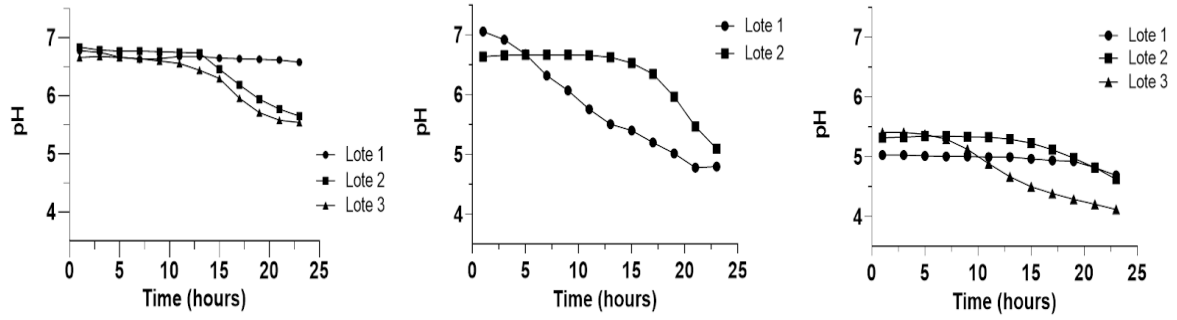

Figure 2. pH during the fermentation process of each producer's batches.

Table 2. Counting of indicator microorganisms in finished product.

INDICATOR	P_1		P_2		P_3	
	X Log CFU/mL	%CV	X Log CFU/mL	%CV	X Log CFU/mL	%CV
Mesophilic	7,8±0,25	3,2	8,7±0,2	2,0	8,5±0,08	0,9
M &Y	5,1±0,71	14,1	5,2±0,2	4,6	3,7±2,32	63,4
TC	4,6±0,15	3,2	4,6±0,6	12,7	1,6±0,87	52,9
E. coli	3,4±2,08	61,2	1,0	0,0	1,0	0,0
S. aureus	2,4±0,32	13,5	4,5±1,1	24,6	1,6±1,00	63,6
B. cereus	1,0	0,0	1,0	0,0	1,0	0,0
AAB	7,6±1,28	16,8	8,3±0,1	1,3	8,4 ±0,10	1,1
LAB	7,9±0,48	6,1	8,7±0,3	3,4	8,6±0,09	1,1



Figure 3. Documented photos during the "masato" production process.

Regarding the prevalence of total coliforms, two of the three producers presented concentrations above 4 Log units and the other, although the count was lower, presented greater variation between the Log units of the batch. In fecal coliforms, only P_1 was above the criteria established for this indicator (3.4±2.08 Log Units), as well as the presence of *Salmonella* spp, in one of the three lots, probably due to deficiencies in hygienic conditions

and the low acidity reached by the lots of this producer. In artisan fermented foods it has been widely reported in the literature values above those allowed for these microbiological criteria (BYAKIKA *et al*., 2019; ILANGO and ANTONY, 2014; NTULI *et al*., 2013; PUERARI *et al*., 2015; VÄKEVÄINEN *et al*., 2018). According to Ilango and Antony (2014), inadequate handling of raw materials and poor hygiene conditions before, during and after the processing of these foods are the main causes.

On the other hand, P_2 showed a growth of 4.5 Log CFU/mL of *Staphylococcus aureus* with a high coefficient of variation between lots, evidencing a lack of good practices. Taking into account the risk that this microorganism represents for human health, because it produces an enterotoxin that causes food poisoning. (CHARLIER *et al*., 2009; RUIZ *et al*., 2017; BYAKIKA *et al*., 2019). *S. aureus* is the third most common cause of confirmed food poisoning in the world, which is why its presence in finished foods is unwanted. In Colombia, the Ministry of Health and Social Protection (2010) has evaluated the risk of this microorganism in non-industrially prepared foods due to the large number of reported outbreaks. However, Villa *et al*., (2012) makes a review highlighting the inhibitory effects of BAL against pathogenic microorganisms such as *S. aureus*, this benefits the industry and public health sectors by increasing the productivity of fermented foods and decreasing the risk of foodborne diseases (TSAS).

Finally, no *Bacillus cereus* counts were evidenced in the evaluated batches of rice "masato". However, authors such as Almeida *et al*. (2007) and Ramos *et al*. (2010) report the presence of *Bacillus cereus* in rice based fermented beverages.

The presence of these indicator microorganisms highlights the importance of implementing good manufacturing practices (GMP) to control the hygiene of the fermentation process by avoiding contamination with unwanted microbiota, without changing the craftsmanship of the process and ensuring that these species do not become dominant and alter the safety, quality and shelf life of the finished product. (RESOLUTION 2674, 2013) This is where LAB plays a crucial role if measures are taken to improve their optimal development. Since, the production of acids and other antimicrobial components during fermentation can promote the safety and stability of the final product by eliminating pathogenic microorganisms. (VÄKEVÄINEN *et al*., 2018; PUERARI *et al*., 2015).

The microbiological counts obtained in the "masato" of rice, allowed to observe the proportion of the evaluated groups of microorganisms and to corroborate the predominance of fermenting microorganisms in the finished product, since, they are in greater quantity LAB, AAB followed by yeasts (Fig. 4); the growth of these microorganisms is favored by the degradation of substrates thanks to the native microbiota that lead to the acidification of the environment and the release of other growth factors such as vitamins and soluble nitrogen compounds, or disadvantaged by the predominance of other different groups that change the characteristics of the finished product.

Thus, the acids produced during fermentation (lactic, acetic and propionic) in the different batches of the producer, present high variability and significant differences between producers, except for the alcohol content (Fig. 5A). These three acids are the most frequent in fermentation processes and are responsible for taste (PUERARI *et al*., 2015). The propionic acid content reflected in P_3 is striking, with no differences with other acids produced in their batches. This acid has a different taste profile than lactic acid (Blandino *et al*. 2003). P_1 presented in the finished products the lowest acid content, data consistent with the pH reached in the batches of this producer. The presence of acid compounds can have inhibitory effects on some bacterial groups, especially those that are demanding and could be affected by pH (VALENCIA *et al*., 2018).

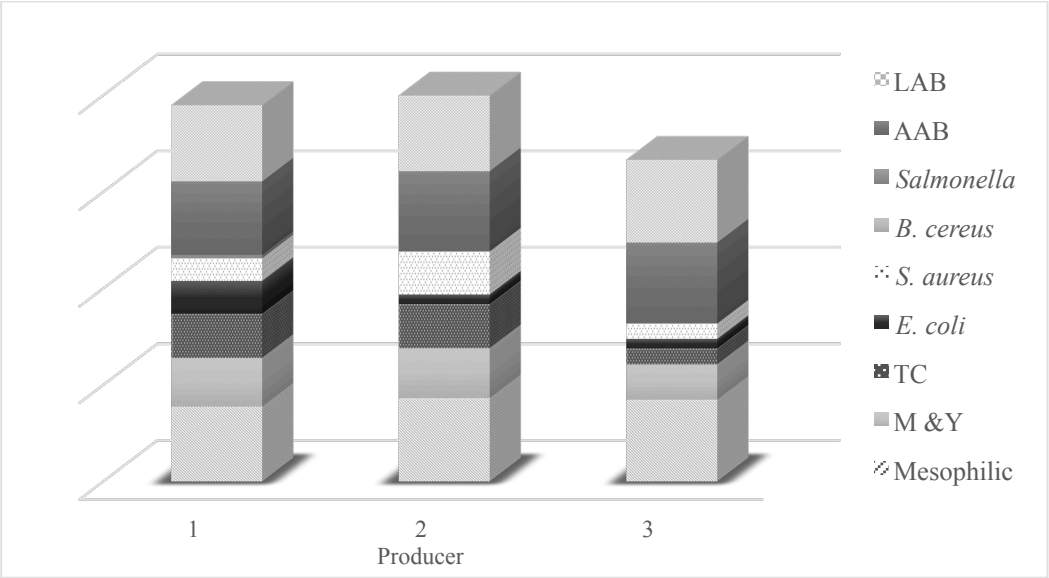

Figure 4. Microbiological predominance in finished product for three producers.

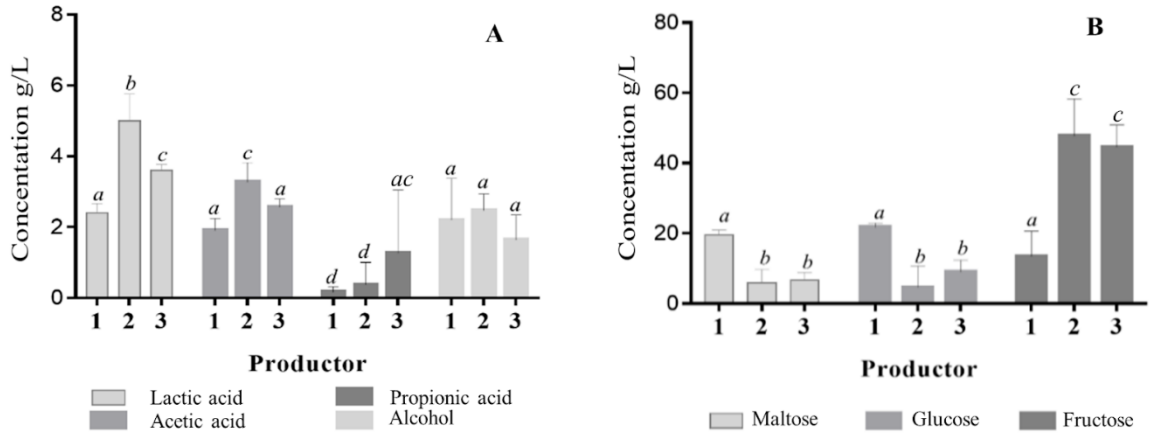

Figure 5. Averages and SD in the finished products of the three masato producers. A: Organic acids (lactic, acetic, propionic) and alcohol. B: Determined sugars. Different letters on the bars show significant difference between treatments (n = 3).

With regard to the quantity of sugars evaluated, the average fructose content of the P2 and P3 lots (47,8 g/L y 44,6 g/L) is striking, which could indicate a low number of microorganisms that degrade fructose, contrary to what is observed in P1 where there are no differences between the average of the quantified sugar content.

In general, the moisture of the finished products did not differ among the "masato" producers (Table 3) despite the differences in the addition of water in the initial formulation, this is probably related to the water retention capacity of starch.

Finally, after the microbiological and physicochemical characterization processes, a total of 80 strains were isolated among the three producers, which met the basic characterization criteria for BAL: Catalase (-), oxidase (-), Gram (+) and hemolysis (-). The highest number of isolates was obtained from P3 with 41%, followed by P2 with 34% and lower amount P1 with 25%

Table 3. Average moisture content of "masato" producer.

"masato" Producer	Moisture (%)	CV (%)
P_1	86,57±1,55	1,8
P_2	85,97±2,11	2,5
P_3	82,53±1,83	2,2

Con n = 3.

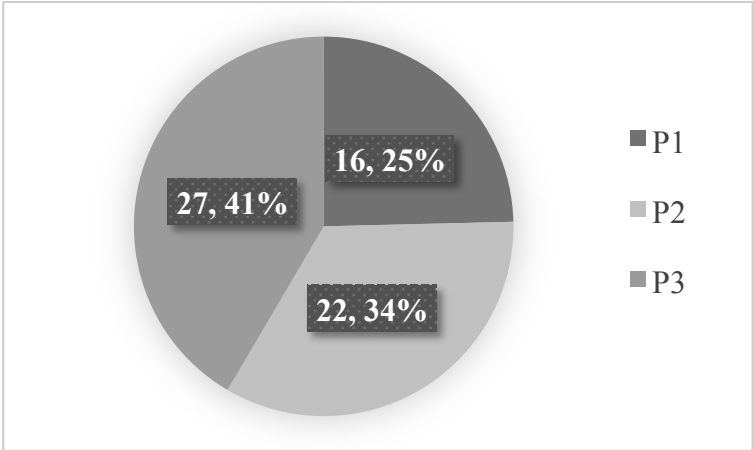

Figure 6. Isolated LAB strains with basic identification characteristics for the three producers.

Thus, the production process of artisan fermented foods, such as "masato", is susceptible to improvement without affecting tradition, since some of these products are made with poorly controlled processes, without adequate technology, these studies provide relevant information, so that the competent authorities can accompany and strengthen these processes, as well as establish specific standards that include adequate criteria for acceptance or rejection ensuring the safety of them. In addition, they provide information that points to the isolation of microorganisms as defined starter cultures that give added value in their contribution to health.

4. CONCLUSIONS

It is exposed that: the variability of the processes of elaboration of the "masato", with the predominance of specific microbial groups that arrive by natural inoculation, and some of these put in risk the sustainability of the producer. In addition, the dependence of this variability on hygienic habits, cross-contamination and production processes to strengthen the number and permanence of beneficial microorganisms for the production of compounds responsible for taste, aroma and preservation of the product. On the other hand, a high number of BAL isolates were achieved, which through the use of molecular techniques may indicate the richness that fermented products of vegetable origin may have.

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