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RESEARCH ARTICLE

ENRICHMENT OF SWEET POTATO LEAVES AND PEELINGS (IPOMOEA BATATAS) IN PROTEIN THROUGH FERMENTATION

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ABSTRACT

In the north of Côte d'Ivoire, sweet potato is a very abundant raw material. The peelings and leaves which constitute by-products are likely to be enriched in proteins. This study proposes to use the fermentation process as a means to increase the nutritional value of sweet potato by-products. Thus, cultivable bacteria and yeasts from sweet potato leaves and peels were counted and isolated following spontaneous fermentation. Subsequently, a controlled fermentation was carried out with twelve (12) microorganisms isolated on a shredded leaf, a shredded peel then on a proportional mixture of shredded leaf and peelings of two varieties of sweet potato. Samples were taken on day 0, on the 5th and 10th day of fermentation in order to determine the pH as well as the protein content of the various substrates. The results showed a diversity of bacteria and yeasts capable of producing protein from sweet potato leaves and peels. Among these microorganisms used, the LevE03 yeast provided a satisfactory result with an increase in the amount of protein ranging from 2.56g/100g to 17g/100g, i.e. an enrichment rate of more than 500% after fermentation of the leaves of sweet potato by this yeast. The LevE03 yeast is of particular interest for the enrichment of the leaf substrate in protein.

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INTRODUCTION

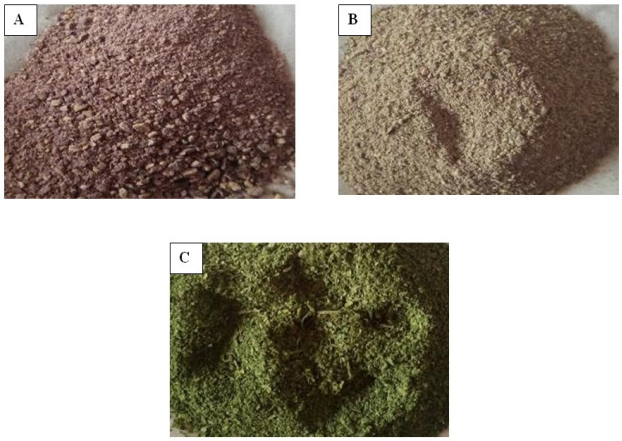
In general, the food budget occupies 40 to 60% of household expenditure and Africa is not left out of this provision (N'Doye, 2012) [1]. The proportion of protein in the human diet is about 15% and its cost can reach 50% of household food expenditure. The main sources of protein in the human diet are generally of animal origin, mostly from livestock (Médale, 2013; FAO, 2014). But livestock farming in sub-Saharan Africa is nowadays confronted with several problems, including animal feed, which remains a factor strongly influencing production yield and producer income.

The increasingly high cost of industrial feed marketed for animal production nowadays pushes breeders to use agricultural by-products such as peanut cake, cotton, wheat, rice, corn as feed (Lambaré, 2015). But the use of these agricultural by-products by breeders induces production yields that are significantly lower than those resulting from the use of marketed industrial feed. One of the causes of this observation is linked to the low levels of growth nutrients contained in these products, in particular protein. To remove this constraint, breeders use fishmeal as a complement to agricultural by-products in suitable proportions. But the availability of fishmeal is no longer sufficient to meet the growing demand (Burel, 2014).

Sweet potato (Ipomoea batatas L Convolvulaceae) is a creeping, perennial plant widely cultivated throughout the territory of Côte d'Ivoire. National sweet potato production is expected to increase and reach 3.5 million tons in 2025 (Kadio, 2015). Sugar-rich sweet potato peels and leaves are currently underutilized. They could be an interesting alternative for developing bioproducts of high nutritional value in animal feed. Hence the interest of adding value to agricultural by-products by increasing the protein content of these products. This study therefore aims to promote the increase in protein levels in sweet potato leaves and peelings by microbial fermentation.

MATERIAL AND METHODS

Plant material: The plant material is composed of tubers and leaves of two varieties of sweet potato (Ipomoea batatas) (white flesh and purple flesh) purchased at the large market in the town of Korhogo. The peelings removed with delicacy as well as the leaves are dried in the shade away from sunlight for 5 days to obtain a dried product. The peelings of each of the sweet potato varieties are then ground into a fine powder (Fig. 1) and then stored in a jar away from light.



A : épluchure de patate douce à chair violette
A : Purple-fleshed sweet potato peel
B : White-fleshed sweet potato peel
C : Leaves of sweet patato

Figure 1. Plant material (crushed sweet potato peelings and leaves)

Microbiological material: The microbiological material consists of twelve (12) microorganisms selected from unidentified isolates from the leaves, peels and the leaf-peel mixture of the two varieties of sweet potato. This set of codified microorganisms is composed of a bacterium from the non-fastidious total flora isolated from the leaves-peelings mixture (FtM01), seven (07) lactic acid bacteria including three (03) isolated from the peelings (BLE01, BLE02, BLE05) and four isolated from the leaves (BLF03, BLF04, BLF06, BLF07) and finally four yeasts including two (02) isolated from the peelings (LevE01 and LevE03), one (01) isolated from the leaves (LevF02) and one (01) isolated from the leaf-peel mixture (LevM04).

Methods

Uncontrolled fermentation: In order to isolate the microorganisms involved in the fermentation of sweet potato leaves and peelings, an uncontrolled ten (10) day fermentation of the latter was carried out. For its production, 4 g of powder of sweet potato peelings, leaves and mixture of leaves and peelings were taken and introduced into sterile jars containing 36 mL of sterile distilled water. The three solutions obtained in triplicate were incubated at 37°C for 10 days in spontaneous fermentation.

Enumeration and isolation of microorganisms involved in fermentation: After the uncontrolled fermentation of the sweet potato by-products (peelings, leaves and leaf-peeling mixture), the products obtained were subjected to microbiological analysis.

- The medium used for counting yeasts and molds is Sabouraud agar supplemented with chloramphenicol (ISO, 2004).
- The medium used for counting lactic acid bacteria is MRS agar (Man Rogosa Sharp) (NF, 1999). nutrient agar medium (GN) is used for the growth of all microorganisms (NF, 1999). The results are expressed as UFC/ml (Colony Forming Unit per milliliter) and given by the following relationship (Mouloudi, 2013; Moroh, 2020):

Équation 1: Enumeration UFC/ml

$$N\ (UFC/ml) = \frac{\sum C}{V\,(n1 + 0.1n2)\,d}$$

∑C: sum of colonies counted on all retained dishes of two successive dilutions
V: Inoculum volume
n1: Number of Petri dishes or tubes inoculated at the 1st dilution
n2: Number of petri dishes or tubes inoculated at the 2nd dilution
d : Smallest dilution retained
N : Number of colonies in UFC/ml
UFC/ml : Colony Forming Unit per milliliter of stock solution
Microbial density was then expressed per gram of sweet potato by-product through the relationship below:

Équation 2 : Enumeration UFC/g

$$N'(UFC/g) = N/C$$

N': Number of colony forming units per gram
C (g/ml): Concentration of stock solution

Macroscopic and microscopic description of isolated microorganisms: Macroscopic observation consists of looking at the different colonies present on the Petri dishes with the naked eye and then describing their color, their shape (round, irregular, star-shaped and invasive), their contour and their surface. Gram staining can differentiate between Gram-positive and Gram-negative bacteria. It also makes it possible to determine their form (Bacillus or Cocci) as well as their mode of association and organization.

Controlled fermentation of sweet potato by-products: The controlled fermentation was carried out with twelve microorganisms chosen from the isolates obtained after the uncontrolled fermentation of the peelings, the leaves and the leaf-peeling mixture of the sweet potato. For its production, in three (03) series of thirteen (13) closing jars each containing three grams (3g) of crushed sweet potato by-products (including peeling powder in the first series, leaf powder in the second series and the powder of the leaf-peeling mixture in the last series), 50 ml of distilled water are added. The jars are then sterilized in an autoclave at 121°C for 15 minutes. After sterilization each of the twelve (12) target isolates is inoculated into twelve jars of each series. The thirteenth jar of each series, not having been inoculated, constitutes the control. The jars were incubated in an oven at 37°C for five (05) and ten (10) days.

Determination of physico-chemical parameters of fermented and unfermented sweet potato peel and leaf

pH determination: For the determination of pH, the potentiometric method of the AOAC was used (AOAC International, 2005).

Determination of protein content: For the determination of the protein content, the biuret method was used. It is a method of colorimetric determination of proteins. The protein reacts with the alkaline cupric reagent (Golnall's reagent). The latterreacts with the peptide bonds leading to the formation of a purple complex whose absorbance is measured at 540 nm (Lowry, 1951).

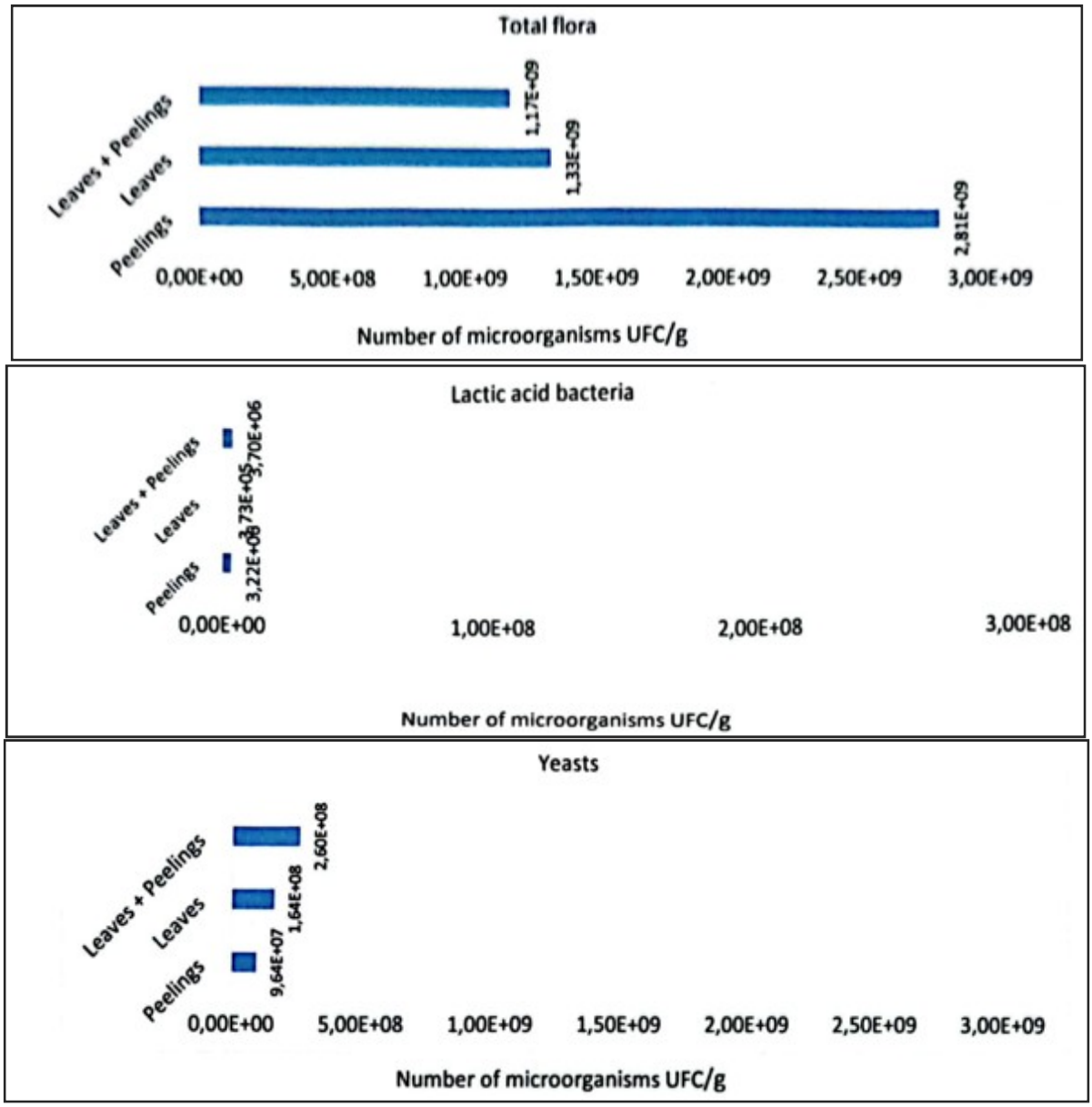
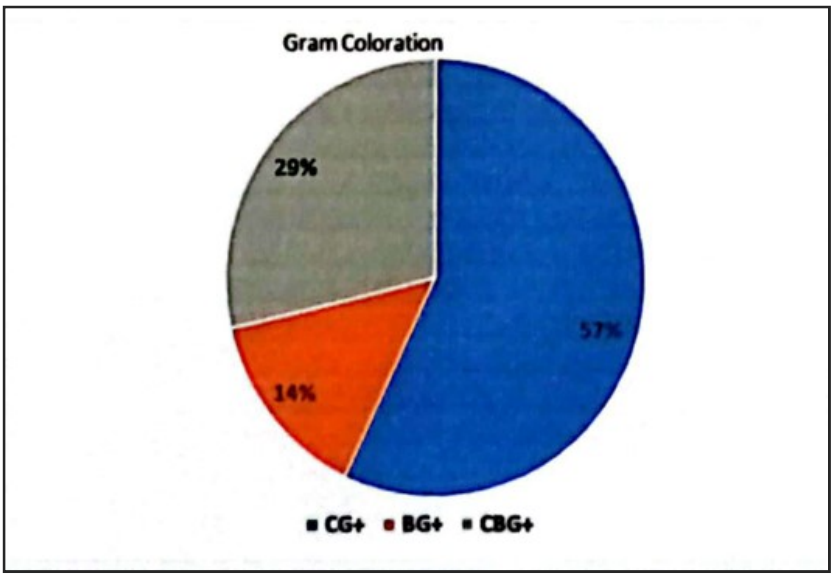


Figure 2. Microbial densities after fermentation of sweet potato peel and leaf



CG+ : Gram positive cocci

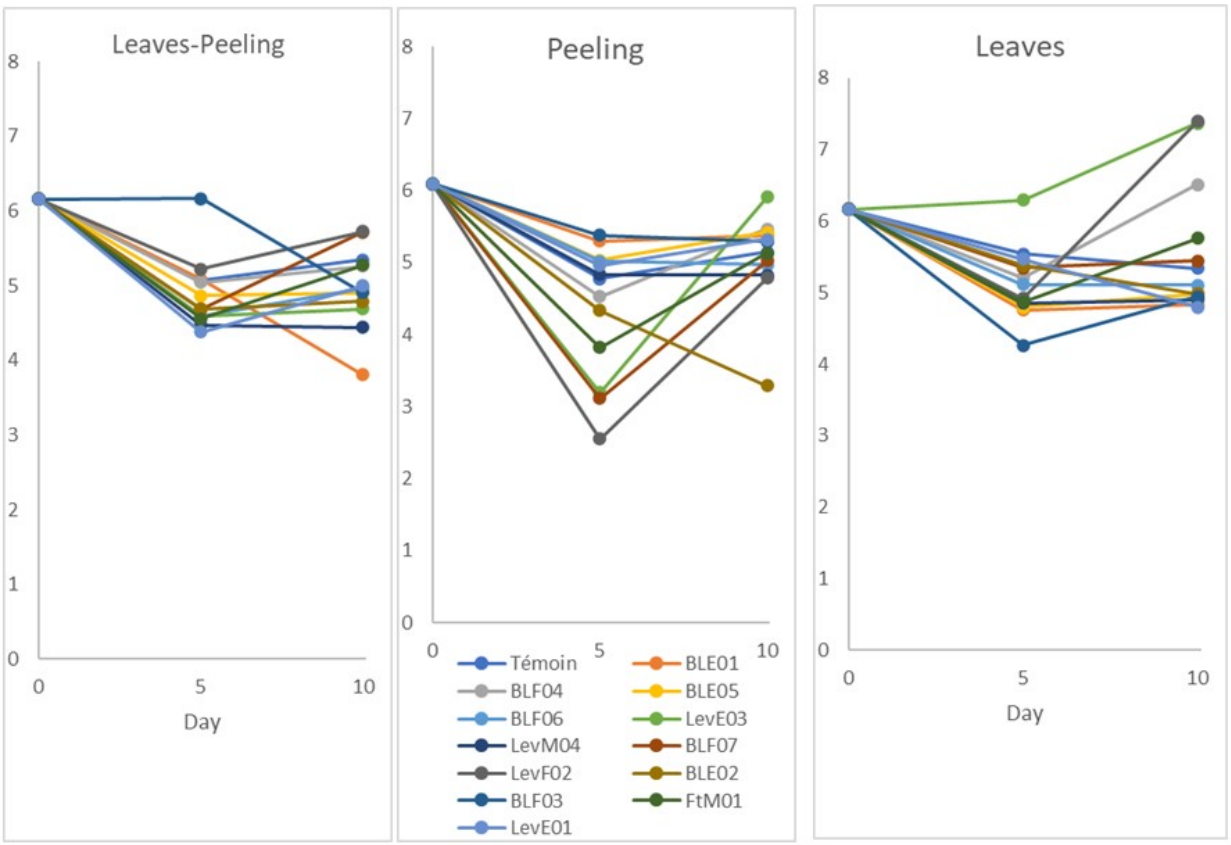


Figure 4. Variation of pH during fermentation

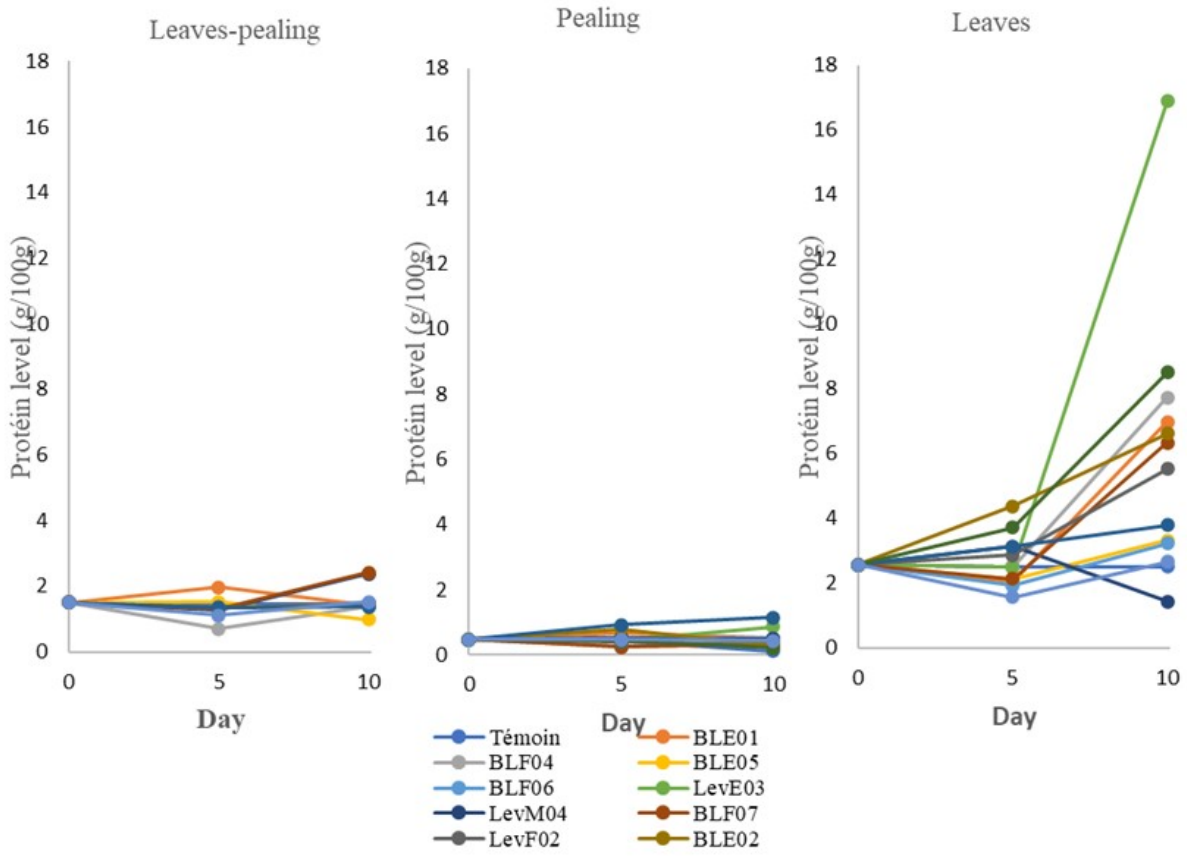


Figure 5. Evolution of protein production during fermentation

DISCUSSION

The reinforcement of protein levels by fermentation was carried out with the microorganisms present naturally on the peelings and leaves of sweet potato. To do this, we first counted and isolated the cultivable microorganisms present on the peelings and the leaves of the sweet potato. The cultivable microbial flora present on sweet potato skin and leaves showed heterogeneity composed of bacteria and yeasts. This microbial observation was also highlighted by Wouters and *al*. [13] and Godard [14]. Almost all of the cultivable bacterial flora isolated on the MRS and GN media in the peelings and the sweet potato leaves are Gram-positive bacteria: Gram-positive Cocci, Gram-positive Bacillus and Gram-positive Coccobacillus). Entation, the protein content of the sweet potato peels, leaves and leaf-peel mixture was 0.46%, 2.56% and 1.51% respectively. These values are close to those obtained by certain authors, in particular Ouédraogo et al., [15] and Sun et al., [16]. Knowing that the formulation of feed for animal production requires protein levels that can vary from 20% to 45%, these protein levels are low. To fill this gap, the fermentation of these agricultural by-products was carried out with each of the isolated microorganisms. After 10 days fermentation with sweet potato leaves results in better protein production. The enrichment rates obtained with the leaves vary between 116.02% and 559.38%. The highest amount of protein has an approximate value of 17g/100g of leaf. This value was obtained by fermentation with the yeast coded LevE03 with an enrichment rate of 559.38%. The protein rate obtained with the LevE03 yeast is close to the proportion of 20g/100g, the value used for the formulation of feed in animal production (poultry, fish farming, rabbit farming). The LevE03 yeast is of particular interest for the enrichment of the leaf substrate in protein. Thechemical composition of the leaves and the general fermentation conditions would induce a greater conversion of carbohydrates into amino acids and then into proteins. Protein production with sweet potato by yeast fermentation was also observed by Ouédraogo and *al*., [15]. The latter managed to ferment sweet potato and yam residues to produce proteins with proportions of 46 g/100g. This fermentation was carried out with Saccharomyces cerevisiae and Candida utilis. Other authors such as Der Bedrosian and *al*. [17] have also succeeded in potentiating the protein level of maize after fermentation during ensilage and have reached levels of 65g/100g.

During fermentation the pH was also measured. From a dynamic point of view, the pH tends to decrease during the first five (05) days of fermentation. This has been demonstrated by Di Cagno and *al*., [18] and Wouters et al., [13]. The first authors showed a reduction in pH from 5.9 to a pH below 4.5 after one day of fermentation of carrots by native layers. The last authors showed a decrease in pH from about 6.5 to 3.6 on average during the first three days by spontaneous fermentation of cabbage, tomato, cucumber and carrots. The decrease in pH limits the growth of undesirable spoilage and pathogenic flora by acidifying the environment by producing lactic acid, acetic acid and ethanol. It also prevents foods poilage.

CONCLUSION

This study aimed to increase the protein level in agricultural by-products by microbial fermentation. After counting, twelve cultivable microorganisms found in sweet potato leaves and peels were isolated. These microorganisms were used to ferment these by-products to assess their ability to produce protein. Among these microorganisms used, the LevE03 yeast gave a satisfactory result with a quantity of protein evaluated at nearly 17g/100g obtained after ten days of fermentation. With the leaves, this yeast promotes an increase in protein with an enrichment rate of 559.38%.

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Glossary of abbreviations

AOAC: Association of Official Agricultural Chemists
BLE: Lactic acid bacteria isolated from peel
BLF: Lactic acid bacteria isolated from leaf
BG+ : Gram-positive bacillus
CG+ : Gram positive cocci
CBG+ : Gram-positive coccobacillusFtM: Total flora isolated Mixture of leaf and peel
Fig.: Figure
g: gram
GN: nutrient agar medium
LevE: yeast Isolated from peel
LevF: yeast isolated from leaf
LevM : Yeast isolated from leaf and peel mixture
ml :millimeter
MRS: ManRogosa Sharp
nm: nanometer
pH: potential hydrogenUFC: Colony Forming Unit per milliliter
°C: Degree celcius
% : percentage

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