Evaluation of the variations of some chemical parameters during the fermentation of the skin of two varieties of mango by their microbial flora

MOROH Jean-Luc Aboya[1], KARAMOKO Detto[1], KONAN Kouakou Sévérin[1], KOSSONOU Kouadio François Baudelaire [1,] KOKORA Aya Philomène[1], COULIBALY Adama[1,2]

1 Biochemistry and Genetics Department, Biological Sciences Training and Research Unit of Peleforo Gon Coulibaly university. BP 1328 Korhogo, Côte d'Ivoire

2 Biochemical Pharmacodynamics Laboratory, Biosciences Biological Sciences Training and Research Unit of Félix Houphouët-Boigny University. 22 BP 582 Abidjan 22 Côte d'Ivoire

Abstract

In the Ivory Coast, more than 30,000 to 40,000 tonnes of mangoes rot in orchards each year. However, in the animal production sector, food remains the only limiting factor, due to the fodder and nutritional deficit, and also to the low valuation of agricultural by-products. These wastes (mango skins) can be used in the manufacture of feed for farm animals. It is with this in mind that this work has been developed, focusing on the transformation of the skin of two varieties of mangoes (Kent and Ameli) through fermentation from their microbiome.

The count of microorganisms in the skin of the mango of these two varieties presents us with a great microbial diversity. The microbial density of the Ameli variety with lactic bacteria flora (5.1 10^5 CFU/g), with total bacteria flora (7.6 10^5 CFU/g) and with fungus flora (3.5 10^4 CFU/g) is clearly higher than that of the Kent variety estimated at 2.10^4 CFU/g (lactic bacteria flora), 2.7 10^5 CFU/g (total bacteria flora) and at 1.2 10^4 CFU/g for fungus flora.

The dry powder of the two varieties of mango fermented with each of the isolated microorganisms allowed us to follow the variation in pH, reducing sugar level, lipids and proteins during 5 and 10 days of fermentation. We notice a drop in pH on the 5^{th} day followed by growth on the 10^{th} day with almost all the microorganisms. Overall, the results tell us a drop in reducing sugar levels on the 10th day that goes through growth on the 5^{th} day with certain microorganisms. We also note during this fermentation a growth of the protein rate reaching up to 47 times the initial rate on the 5^{th} day of fermentation with a lactic acid bacterium.

These microorganisms, if properly chosen in suitable proportions could reduce the amount of inputs for the formulation of animal feed with the skin of the mango.

Keywords: Mangoes, mango skin, agricultural by-products, fermentation, microorganism

Introduction

According to the FAO in 2025, world meat production is expected to increase by more than 16% than in 2015 [1]. Feeding cash crops constitutes a major technical and economic challenge for West African livestock since the food item absorbs more than 50% of production costs, thus remaining the only limiting factor [2].

However, in Sub-Saharan Africa, the fodder and nutritional deficit, the poor management of pastures, the high cost and low availability of agricultural and agro-industrialby-products, the low valuation of agricultural by-products and poor feeding practices constitute constraints on animal production [3] . This is why the reflection on a food strategy that can compensate for the lack of nutrients, through the valorisation of industrial by-products and food waste (constituting pollution by their large quantity) is emerging. These by-products can play an important role in providing the raw material necessary for the feed industry once recovered. Through this food strategy, we could overcome the food shortage once we find waste and by-products at a lower cost and recoverable that can be accessible to all [4, 5]. In the meantime, enormous agricultural by-products such as mango are abandoned in the fields and in nature, thus polluting the soils and rivers and which become the substrate for the development of flies which compromise the microbiological quality of these same products thanks to their bites. Also, several mango-producing countries in tropical regions are unable to market their harvested production because of conservation difficulties and the low level of valuation, leading to post-harvest losses of around 30% of their harvests, i.e. an estimated loss of 30,000 to 40,000 t per year. In addition to these losses, there is a huge amount downgraded by processing plants due to damage and waste (skin, stone and pulp) [3].

The recovery of these wastes or by-products becomes in our case an interesting and more attractive axis for the animal food industries because it can play a role in the production of food intended for animals and contributes to the creation of jobs and the improvement of revenues of these actors (planters and industrialists) [6-8]. Already in Burkina Faso some farmers use these mango by-products as animal feed. Incorporating these feeds into rations for animals, especially pigs, will improve meat production at a lower cost, generating more for producers [3]. It is up to us to recover these wastes and by-products for good conservation and long-term use, given the production of the mango sector once a year. Mango residues represent an important biomass that can be exploited by biotechnology, given its composition and the activity that the microorganisms of interest could exert on them.

In order to corroborate this idea, this present work aims to valorize the residues (skins) of mango in animal feed. He is thus interested in the transformation of the skin of two varieties of mango (Amélie and Kent) by fermentation for use in animal husbandry feed. This fermentation will take place with the natural microorganisms isolated from the skin of these varieties of mange.

II Material and method

II.1 Sampling

Three batches of 10 ripe mangoes each of the Kent and Amélie variety were harvested in 6 different orchards located in the Korhogo area. For the enumeration and isolation of mango skin microorganisms, the mango skins of each of these two varieties were removed and crushed in a sterile blinder.

In addition, the skins of each of the two varieties are also removed and dried separately away from the sun and then powdered. This powder was used to carry out the fermentation tests with the various isolated microorganisms.

II.2 Enumeration and isolation of microorganisms in mango skin

In 9ml of sterile distilled water contained in a test tube, 1 g of mango skin powder is added, then a sufficient amount of sterile distilled water to achieve 10 ml was added. The homogenate obtained constituted the stock solution. It was used to prepare decimal dilutions ranging from 10^{-1} to 10^{-5}.

Then 100 µl of the various dilutions carried out are spread on the surface of the Sabouraud agar with chloramphenicol, nutrient agar and agar (MRS) to enumerate respectively isolate the fungi, the non-demanding total flora and the lactic flora.

The dishes are then incubated for 24 to 48 hours and at the end of this time the counts are carried out. The counting of the colonies is done by taking into account the dishes having colonies of between 30 and 300 colonies.

The results are expressed CFU / ml (Colony Forming Unit per milliliter) and given by the following relationship [9]:

Equation 1: Enumeration CFU / ml

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

With

∑C: sum of colonies of the counted dishes

V: volume of the inoculum (0.1 ml)

d: dilution retained

n1: number of boxes corresponding to the first dilution selected

n2: number of dishes corresponding to the second dilution selected

N: number of colonies in (CFU/ml)

The final count was reduced to the number of colony forming units per gram (CFU/g) according to the following formula:

Equation 2: Enumeration in CFU/g of organ

$$N' = \frac{N}{C}$$

N': number of colony forming units per gram of organ

C : concentration (g/ml) of the comminuted organ (digestive tract or gills) in the mother solution.

Subsequently a macroscopic description followed by a Gram stain allowed us to group the colonies.

II.3 Skin fermentation

It consists of evaluating the action of each isolated microorganism on the skin of the two targeted mango varieties. Into a series of Erlenmeyer flasks are introduced 2 grams of mango skin powder with a sufficient quantity of sterile distilled water to reach 20 ml. Everything is sterilized in an autoclave for 15 minutes at 12°C. After cooling, a young colony of each of the isolated microorganisms is added to the contents of the Erlenmeyer flask. After homogenization, the various Erlenmeyer flasks are incubated at 30°C. for 5 and 10 days. At the end of these times, the contents of each Erlenmeyer flask is dried and then used for biochemical tests.

II.4 Determination of the physico-chemical parameters of the skin of fermented and unfermented mango

II.4.1 Mango skin dry matter content

The determination of the dry matter is carried out by steaming at 105 ° C. ± 5 with 1 gram of the mango skin in capsules of known mass. After 3 hours of steaming, weighings are carried out regularly after cooling in a desiccator until a constant mass is obtained. The humidity level is determined by the following formula (Oussar and Addar 2017):

Equation 3: Humidity level

$$\boldsymbol{h}(\%) = \frac{(\boldsymbol{m_i} - \boldsymbol{m_f})}{\boldsymbol{m_e}} \times \mathbf{100}$$

h: humidity of the sample

mi: initial mass of the sample and the capsule before drying

mf: final mass of the sample and the capsule after drying

me: mass of the sample

The dry matter content is given by the following formula:

Equation 4: Dry matter content

$$\boldsymbol{ms}(\%) = \mathbf{100} - \boldsymbol{h}(\%)$$

ms: dry matter

The determination of moisture and dry matter was repeated twice.

II.4.2 Determination of pH

In a beaker containing 50 ml of distilled water were added 5 g of mango skin powder and then, after homogenization, the pH of the solution was determined using a previously calibrated pH meter used. The tests were repeated twice for each of the mango varieties.

II.4.3 Determination of reducing sugars

A range of concentrations from a standard 0.05 mg/ml D-glucose solution was used to establish a standard curve of reducing sugars. Then, a volume of 0.3 ml of DNS reagent (3.5 Dinitrosalycilic Acid) was added to the different glucose concentrations obtained. After 8 min of incubation in a

boiling water bath, 4 ml of distilled water are added and then the absorbance measurement is taken at 546 nm. The assay of the different samples of fermented and non-fermented mango skin was carried out under the same conditions and the reducing sugar concentrations are determined from the calibration curve established with D-Glucose as the reference sugar [10].

II.4.4 Determination of lipids

Ten (10) grams of crushed mango skin (me) are introduced into a cellulose extraction cartridge. The cartridge is capped with cotton and placed in the Soxhet-type extractor. An empty glass flask (m0) is weighed and 300ml of hexane is poured into it. Everything is connected to the extraction device. The extraction is carried out by the flux-reflux system for 7 hours at boiling point.

At the end of this time, the residual hexane is evaporated by means of a rotary evaporator, then the flask containing the fat is brought to an oven at 100°C for 20 min then cooled using a desiccator and weighed. (m). The lipid content is given by the following equation

Equation 5: lipid level

$$\textbf{Lipids}\ (\%) = \frac{(\boldsymbol{m} - \boldsymbol{m0}) \times \mathbf{100}}{\boldsymbol{me}}$$

II.4.5 Protein content

To 1ml of the mango peel solution is added 4ml of Golnall's reagent. The resulting mixture is incubated in the dark at room temperature for 30min. The reading is taken with a spectrophotometer at 540 nm. The standard was done under the same conditions with Bovine Serum Albumin (BSA) at different concentrations [11].

II.4.6 Data processing and statistical analysis

The MS Excel 2016 software was used for the data processing (table and graph) while the XLSTAT software allows us to do the mean comparison tests (Fisher) with a significant rate of 0.05

III Results

III.1 Microorganisms from the skin of two mango varieties

The enumeration of microorganisms in the skin of the mango revealed a great diversity of bacteria and fungi. The microbial density obtained on the skin of the mango after enumeration was evaluated at $7.6.10^5$ CFU / g and $2.7.10^5$ CFU / g with the non-demanding total bacterial flora for the Amélie variety and the Kent variety respectively. The density of the lactic flora is 5.1 10^4 CFU / g and 2.10^4 CFU / g respectively for the Amélie and Kent varieties. As for the mushrooms, we have $3.9.10^3$ CFU / g for the Amélie variety and $1.3 .10^3$ CFU / g for the Kent variety (Figure 1).

The isolated colonies are all Gram-positive bacteria. The Amélie variety contains 63% Gram-positive Cocci (CG +) and 37% Gram-positive bacillus (BG +). The Kent variety, for its part, has a bacterial flora composed of 33% Gram positive Cocci (CG +) and 67% Gram positive bacillus (BG +) (Figure 2)

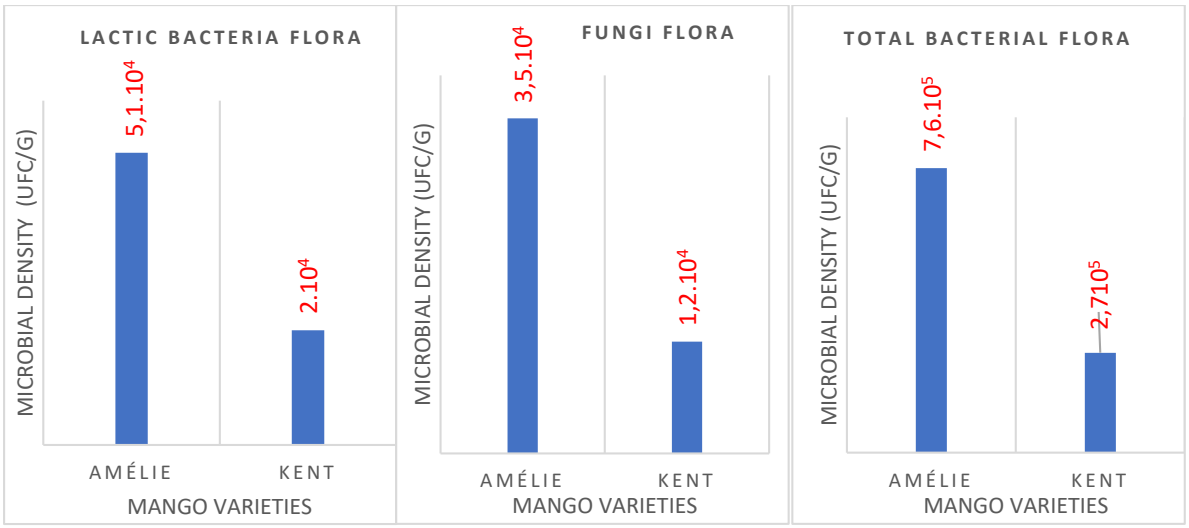

Figure 1: Microbial density

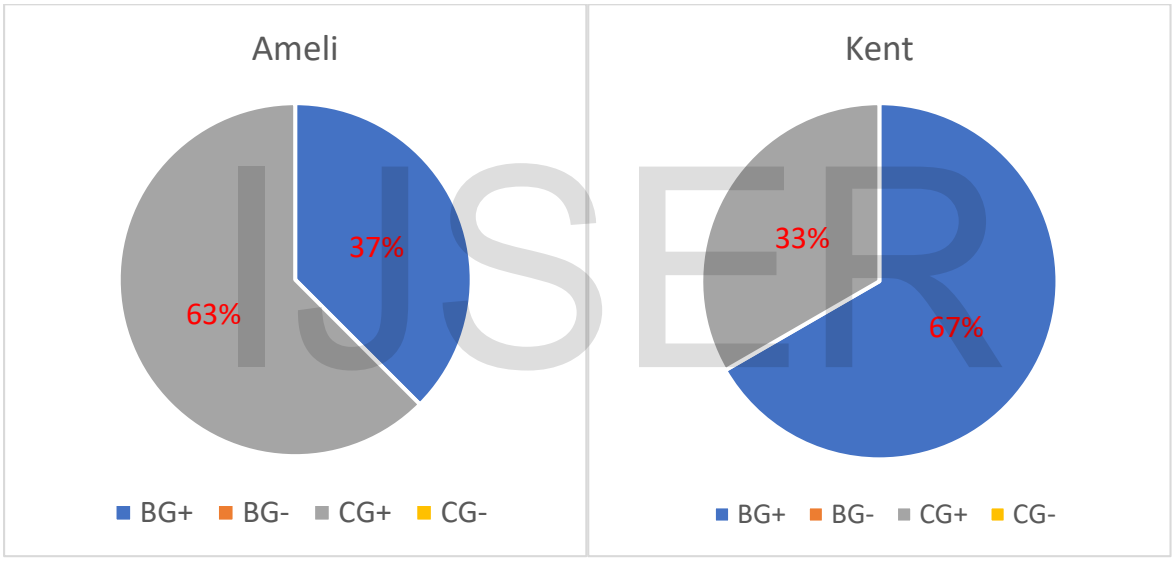

Figure 2: Proportion of type of bacteria on the skin of different varieties of mango

III.2 Dry matter

The humidity level for the Amélie variety is 7.47 ± 0.06% against 7.91 ± 0.02% for the Kent variety which gives respectively a dry matter content of 92.5 ± 0.02% and 92.13 ± 0.03%.

III.3 PH determination

These different measurements were carried out before fermentation, on the 5th and 10th day of fermentation. Before fermentation, the pH of the Kent variety is lower than that of the Amélie variety with respective values of 3.82 ± 0.0 2 and 4.44 ± 0.005

Overall, there is a drop in pH on the 5th day, followed by growth of the latter on the 10th day. This variation is observed in the fermentation by all the microorganisms (bacteria and fungi) used except the AS1 fungus and the AGN3 bacteria as shown in Figure 3.

In fact, with the AS1 fungus, a constant decrease in pH is observed from day 1 to day 10. With the AGN3 bacteria, during the 10 days of fermentation, the pH did not significantly change.

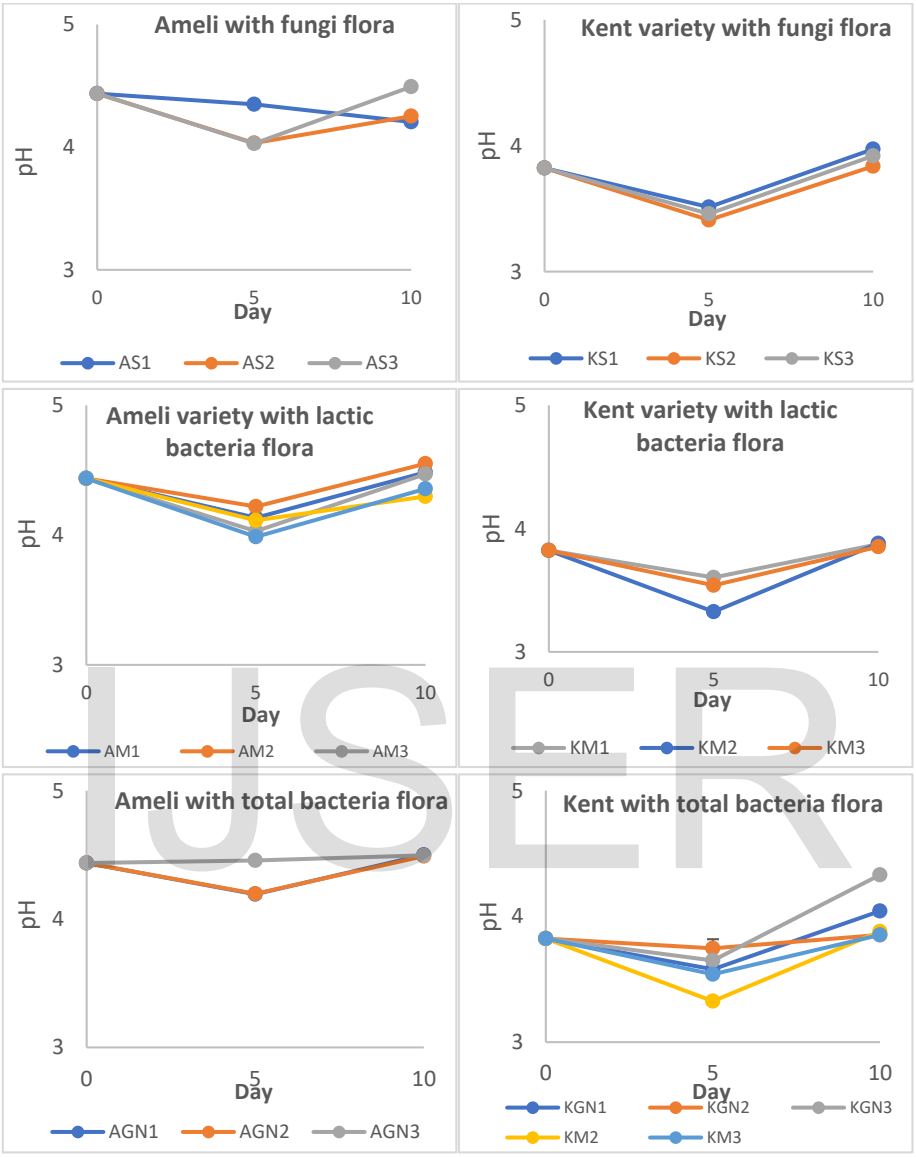

Figure 3: Change in pH during fermentation

III.4 Reducing sugars

Reducing sugars are fermentable sugars indicative of the progress of fermentation. The results on the evolution of reducing sugars in the skin of the two varieties of mango during the 10 days of fermentation by the various isolated microorganisms are presented in figure 4.

This figure shows us in general a decrease in the level of reducing sugar over the 10-day period. In addition, with certain microorganisms, this decrease occurs first through an increase in this rate on the 5th day.

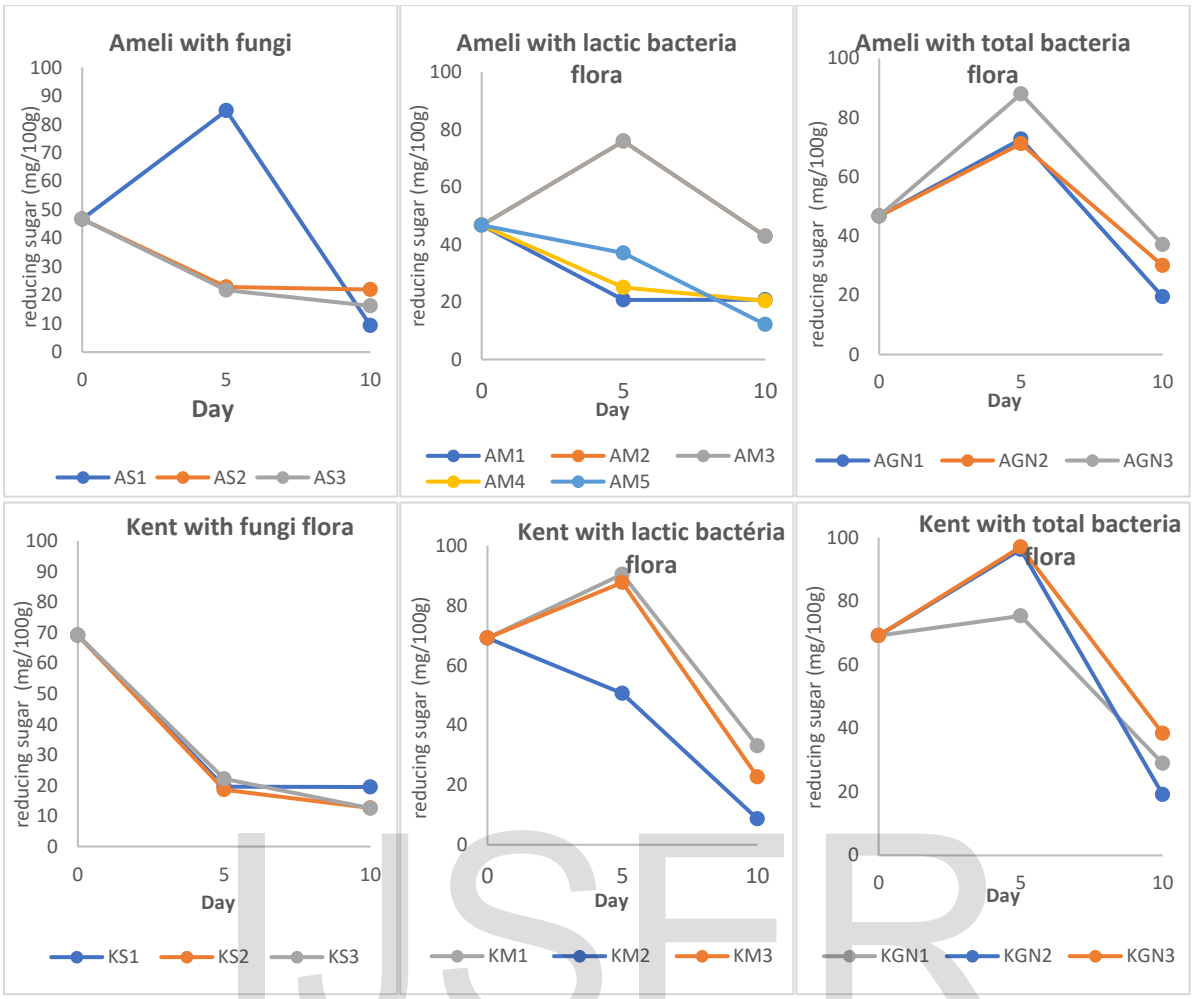

Figure 4: Evolution of the rate of reducing sugars during fermentation

III.5 Lipid level

The lipid level was determined with the skin of the two varieties of mango before and after a fermentation made with all the microorganisms together. The initial lipid level is higher in the Kent variety, ie 0.18 ± 0.015% against 0.11 ± 0.006% for the Amélie variety. After fermentation made with all the microorganisms in the skin of the 2 mango varieties, the lipid level is equal on both sides of the Kent and Amélie varieties, i.e. 0.09% (figure 5)

III.6 Protein content

The study shows that the initial level of protein in the mango skin differs from one variety to another: 0.067 ± 0.003 mg / 100g for the Amélie variety against 0.59 ± 0.032mg / 100g for the Kent variety. As shown in Figure 6, the protein level increases dramatically for most of the microorganisms used. The highest rates are obtained with the Amélie variety with a maximum of 3.018 ± 0.15 on the 5th day with lactic acid bacteria, ie 47% more than the initial concentration. A decrease is observed with all microorganisms from the 5th to the 10th day.

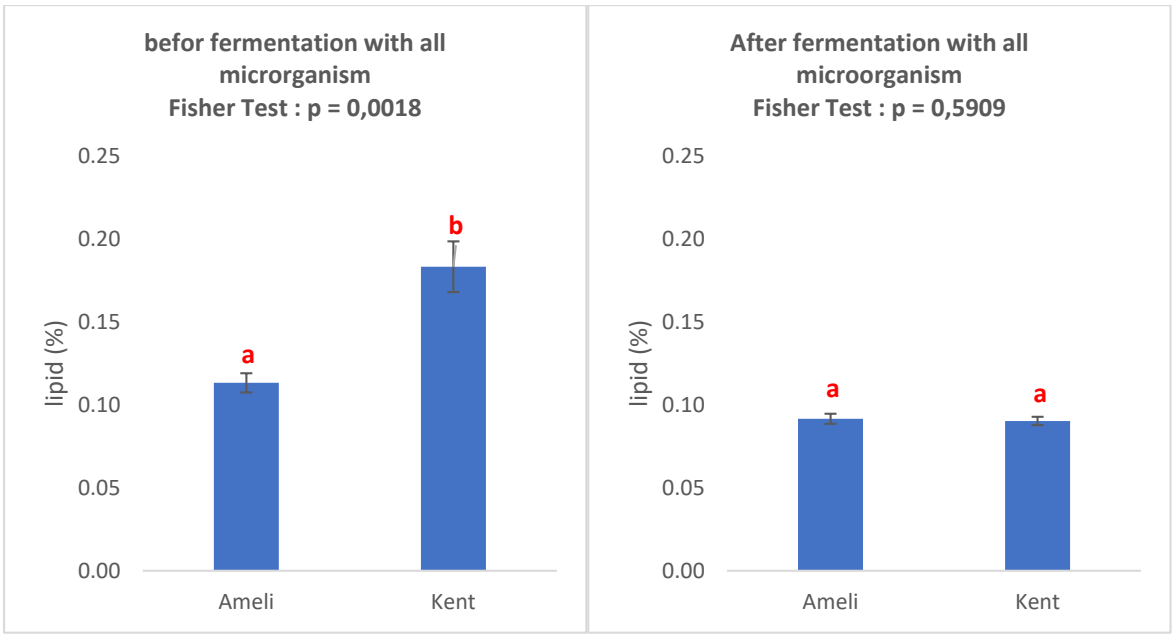

Figure 5: lipid level before and after fermentation

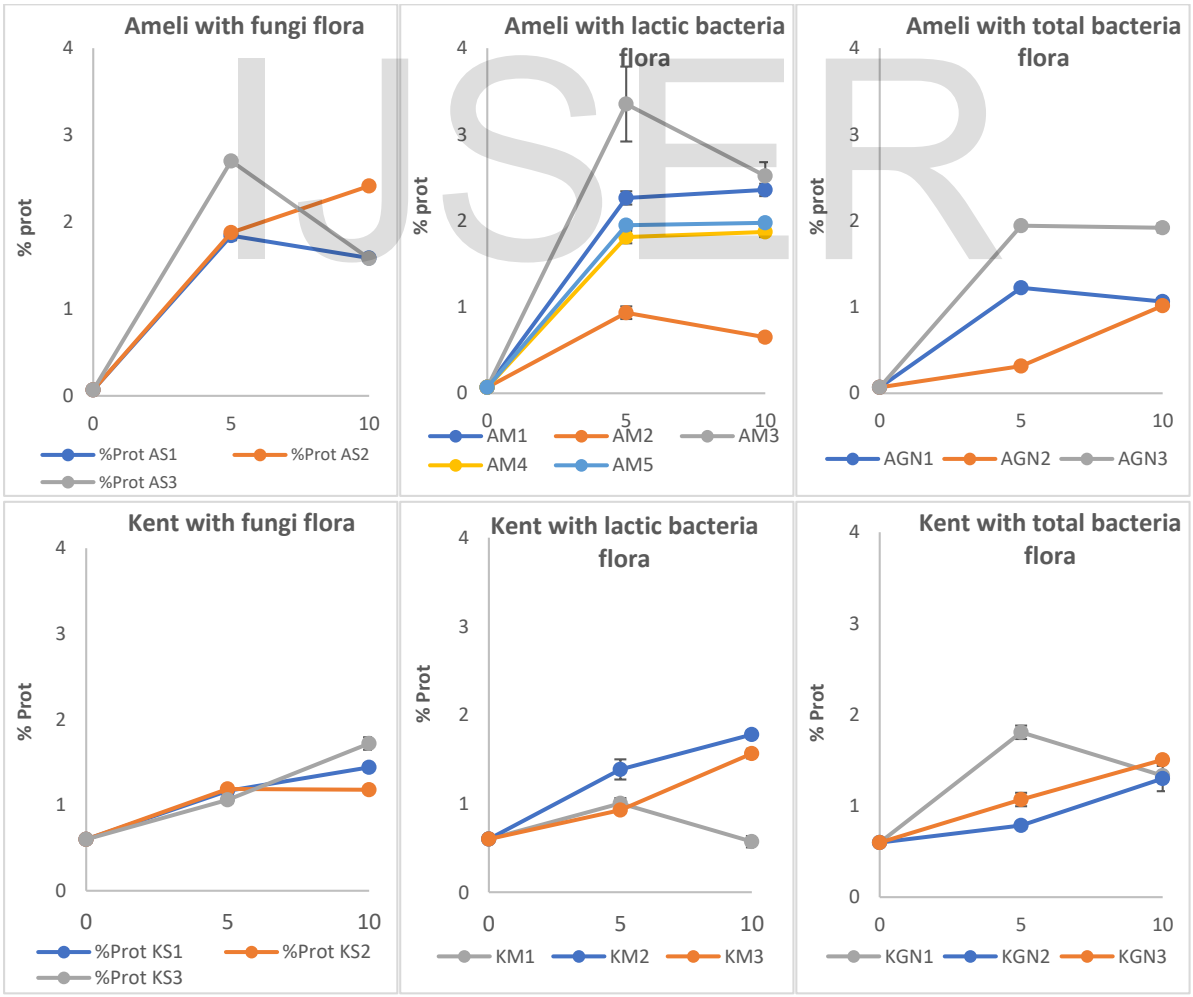

Figure 6: Evolution of protein production during fermentation

IV Discussion

The skin of the mango is an epicarp that protects the pulp and the stone. Its dry matter varies in the order of 92% for the two varieties used. This value found for the two varieties is higher than that reported by Kiendrébeogo [12] or 78.68%. This difference could be due to the fact that the varieties are different or the difference in their degree of maturity.

The literature shows us that the mango skin is made up of sugars (glucose, cellulose, etc.), proteins, lipids, minerals (Calcium, Phosphorus, NaCl) and vitamins, while these represent the various elements including breeders need to feed cash crops. These nutrients contained in the mango peel are of great interest to microorganisms. This microbiological observation composed of bacteria and fungi (yeasts and molds) was also highlighted by Alloue-Boraud [13]. Almost all of the cultivable bacterial flora isolated by the MRS and GN media on the skin of the two varieties are Gram positive bacteria (Gram positive cocci and Gram-positive bacillus).

The microbial load of mesophilic organisms found for the Kent variety is $2.7.10^5$ CFU/g higher than that found by Agassounon [14] on the dried mango slices, i.e. 4.10^4 CFU/g. This load is justified by the fact that the skin is a protective membrane in contact with the environment and therefore a barrier against the passage of these to the pulp. As for the value of the fungal flora $1.9.10^3$ CFU/g higher than ours or $1.3.10^3$ CFU/g could be explained by the development of molds due to poor storage or high humidity.

Each of these bacteria could have different fermentation activities either homofermentative or hetero-fermentative with different types of fermentation either lactic, alcoholic, malolactic ...

The effect of the fermentation of these microorganisms was on reducing sugars. The increase in the rate of reducing sugars observed at the start of fermentation in certain bacteria such as AM2, KM1, KM3, KGN2, KGN3 and AS1 can be explained by the degradation of polyholosides and disaccharides (cellulose, galactose, etc.) which could exist in the medium to give simple oses which are reducing sugars. These oses will then be used by the microorganisms during fermentation. The gradual drop observed in other microorganisms could be explained by an inability of these microorganisms in question to break down polyholosides and disaccharides into reducing sugars. During fermentation, the degradation of reducing sugars leads to the production of organic acids such as lactic acid. These appear to be homofermentative lactic acid bacteria. The drop could be explained by the depletion of reducing sugar in the medium or the production of other compounds in the case of other types of fermentation (alcoholic, Malolactic). This level of lactic acid, indicator of fermentation, also influences the value of the hydrogen potential. These last two evolve in the opposite direction hence this drop in pH during the 5th day of fermentation observed in most. As for the increase in pH over the last five days, it could be explained by the production of alcohol in the fermentation medium. It could also be explained by the synthesis of protein which is an amphoteric compound which in this case behaves like a base [15].

Protein is an essential nutrient in the formulation of feed for animal husbandry (cattle, poultry, fish, etc.). Along with carbohydrates and lipids, it is the most expensive nutrient in the formulation of animal feed. The skin of the two varieties of mango used here has a concentration of 0.067 ± 0.003 mg per 100 g for the Amélie variety and of 0.59 ± 0.032 mg per 100 g of dry mango skin. This value is lower than that found in dried mango according to the work of Mwamba [16]. This very low

value requires a large protein supplement if the mango peel is to be used in animal feed. The fermentation of this skin by the natural microorganisms of the latter allows this protein concentration to be increased to 3.018 ± 0.15 mg per 100g of dry skin. Thus the protein rate has been boosted by 47 times its initial content. The increased protein concentration could be explained by a protein synthesis by the microorganisms of the ferments formed.

The Kent variety contains a high lipid content compared to the Amélie variety, all lower than that reported by Kiendrébeogo [12] or 0.37%. This may be due to the method of extraction or to pedoclimatic variability. On the other hand, after fermentation with all the microorganisms of each variety combined the values decrease and equalize. This decrease suggests that he was consumed by microorganisms during fermentation.

V Conclusion

This work is the start of a much larger work aimed at formulating animal feed from mango by-products (skins). The results obtained after the action of the microorganisms isolated on the skin of the two varieties of mangoes revealed a variation of some nutrients of interest for animal feed. These microorganisms responsible for the production or breakdown of certain useful macronutrients have been identified. It is noted that the microorganisms AS1, KM1, KM3, KGN1 and KGN3 appear to have the capacity to decompose polyholosides into reducing sugars. The microorganisms AM3, AS3 and AGN3 by their fermentation activities have made it possible to boost the protein level up to more than 47% of its initial value with a fermentation made with the microorganism AM3.

All these variations in the level of nutrients in the mango skin after the action of these microorganisms, if the latter are well chosen in suitable proportions could reduce the amount of inputs for the formulation of animal husbandry feeds.

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