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Effect of lactic acid fermentation by *Lactobacillus delbrueckii subsp. bulgaricus* on the chromacity of *Moringaoleifera* leaves

HARIMALALA ANDRIAMBELO N.[1], TSIRINIRINDRAVO H.L.[2,3], RAZANAMPARANY J.L[1].

[1] *Laboratory of Biochemistry Applied to Food Sciences and Nutrition. Faculty of Sciences, University of Antananarivo*
[2] *Laboratory of Biotechnology. Faculty of Sciences, University of Antananarivo*
[3] *Indian Ocean Islands University, Madagascar, Mauritius*

ABSTRACT

The nutritional situation in Madagascar is very precarious and structurally fragile. As an indication, 53% of children under 5 are stunted, 42% of children are underweight, 13% suffer from acute malnutrition, 50% suffer from anemia.

To help fight malnutrition in Madagascar, there is the fortification or fortification of staple foods. The fortifying food highlighted in this study is the leaves of *Moringaoleifera* which showed the strong nutritional potential, including a large amount of proteins (> 18%), the quality of amino acids, as well as a large amount of elements. minerals.

However, its use is blocked by its color. Thus, a study of the impact of fermentation, using *Lactobacillus delbrueckii subsp. bulgaricus*, on the color of dried leaves of *Moringaoleifera* was carried out.

Biomasshave grown in the medim.. The OD varied from 0.08 to 0.17. A decrease in the chromacity of the reaction medium was noted. To check whether the fermentation does not negatively affect the fermentation, the total protein and polyphenol content was determined before and after fermentation. The total protein content increased from 3,240 to 4,980 while that of polyphenols decreased from 21,359 to 8,342.

Keywords:*Moringaoleifera, Lactobacillus delbrueckii subsp. Bulgaricus,* Madagascar, malnutrition, fermentation.

I. INTRODUCTION

In Madagascar, malnutrition is both a public health and socio-economic problem that affects a large part of the population, particularly young children, pregnant and breastfeeding women (Pnan III). There are two forms of malnutrition, acute malnutrition and chronic malnutrition, which respectively affect 9% and 47% of children under 5 (Unicef, 2017). Chronic malnutrition affects approximately 2 million Malagasy children, it is the most serious form of malnutrition; it is more often the consequence

of a lack of quality of food than of a lack of quantity. Thus, 60% of children in the highlands are affected by stunting (World Bank, 2018) which has impacts on the physical growth, cognitive development of the child and it is not without future consequences on development country's economy (Unicef, 2017). One of the causes of chronic malnutrition is "hidden hunger" fostered by food insecurity (Agsanv, 2014).

Thus, strategies are implemented to fight against malnutrition, including fortification or enrichment of staple foods (PNANIII). The fortifying food highlighted in this study is *Moringaoleifera* leaves. Studies have shown the strong nutritional potential of leaves from different regions, the results of which have shown the large amount of proteins (> 18%), the quality of amino acids, as well as a large amount of mineral elements (Harimalala Andriambelo, 2021).

However, it is recommended to use the dried *Moringaoleifera* leaves, reduced to powder and not to cook them to keep the nutritional properties (Saint Sauveur and al., 2005). However, this form of use is hampered by its taste and color (Harimalala Andriambelo, 2015).

Thus, in this study we carried out fermentation techniques to remedy the appearance and color of *Moringaoleifera* leaves.

II. MATERIALS AND METHODS

II.1. Materials

The *Moringaoléifera* leaves were harvested in eastern Madagascar, Antsinanana region, on December 2017.

The microorganism used as biomass is the reference strains: *Lactobacillus delbrueckii subsp. Bulgaricus*ATCC 11842, held in the IOI University strain bank.

II.1. Methods

II.1.1 Identification, Confirmation of the strain

Aliquots of 1 mL from conserved strain were spread plated on presolidified de Man, Rogosa, and Sharpe (MRS) agar and incubated at 30°C for 48-72 h. Representative colonies of lactic acid bacteria were randomly picked from countable MRS agar plates. Each bacterial isolate was purified by repeated streak-plating on MRS agar.

Morphological and macroscopic characteristic

Cultural, morphological, physiological and biochemical characterization including microscopic and macroscopic examinations of the various isolates were carried out to the identification of isolates.

Biochemical characteristics

Isolates were identified phenotypically on the basis of biochemical test such as, Catalase, Oxidase, Methyl red test, Nitrate reduction, VP Test, Starch, Casein, Gelatin hydrolysis.

Carbon auxanogram was carried out. It concerns Fructose, lactose, maltose, galactose, arabinose, mannose, xylose, dulcitol, inositol, Mannitol, Raffinose, tréhalose, Rhamnose.

Bacterial isolates were refreshed on MRS broth, and then the broth was incubated at 37°C for 24 h. Turbidity of bacterial suspension was adjusted to 0.1 to 0.5 McFarland standard using spectrophotometer at the absorbance of 600 nm [13].

Each well of Eliza plates was filled with 990 µl of MRS broth with Bromocresol Green (0.04 gm/1000 ml) with pH range of 3.8-5.4.

The first row of wells of the Eliza plates served as a negative control without inoculated with bacterial isolates.

Finally, the plates were incubated at 37°C for 12 to 48 h. The formation of yellow color on the well indicated a positive result for fermentation or acidification, whereas the absence of color change was considered as a negative result.

II.1.2. Inoculation of Inoculation of *Lactobacillus bulgaricus*strain on *Moringa*leaves

25g of powder was taken in a 250 mL flask and mixed in 100ml of Sterile distilled water. The solution was sterilized at 120°C for 20 min.

For all the strains of *Lactobacillus*, glucose is the most efficient carbon source, so it is used as Substrate.

Thus, glucose was added to the medium. Its initial concentration is 30g / l.The solution was seeded with the isolated *Lactobacillus* strains. The concentration of the biomass is Xo = 1.104 cells per ml. The substrate consumption kinetics and the biomass growth kinetics were followed by measuring the optical density at 600nm until the substrate was exhausted.

The ability of lactobacilli to reduce the color of *Moringa* was monitored by measuring chromacity.

The purpose of this spectrophotometric method is to define the process of measuring andcalculating the chromatic characteristics of *Moringa* leaves.
Spectrophotometer to carry out transmittance measurements at a wavelength of between 300 and 800 nm, with illuminant D65 and observer placed at 10º. Use apparatus with a resolution equal to or higher than 5 nm and, where possible, with scan.
Select the pair of cuvettes for the spectrophotometric reading,ensuring that the upper measurement limit within the linear range of the spectrophotometer is not exceeded.
After obtaining and preparing the sample, measure its transmittance from 380 to 780 nm every 5 nm, using distilled wateras a reference in a cuvette with the same optical thickness, in order to establish the base line or the white line. Choose illuminant D65 and observer 10º.
The spectrophotometer must be connected to a computer programme to facilitate the calculation of the colorimetric coordinates (Clarity L*) andtheir derived magnitudes (chroma C* and tone H*), and chromacity [(a*, b*) or (C*, H*)] using the appropriate mathematical algorithms.

A nutritional analysis of the product after fermentation was carried out. Thus, the content of total proteins and polyphenols were determined.

Indeed, fermentation must not reduce these contents, because *Moringa* leaves are exploited for their high contents.

III. RESULTS

Confirmation test

After confirmation of the morphological, cultural and biochemical characters of the strain used, this is the species: *Lactobacillus delbrueckii subsp. bulgaricus*.

Lactobacillus delbrueckii subsp. bulgaricus (until 2014 known as *Lactobacillus bulgaricus*) is one of over 200 published species in the Lactobacillus genome complex (LGC) and is the main bacterium used for the production of yogurt. It also plays a crucial role in the ripening of some cheeses, as well as in other processes involving naturally fermented products. It is defined as homofermentive lactic acid bacteria due to lactic acid being the single end product of its carbohydrate digestion. It is also considered a probiotic.

That are live microorganisms promoted with claims that they provide health benefits when consumed, generally by improving or restoring the microbiome.

Lactobacillus is rod shaped with rounded ends of Gram-positive, aerotolerant anaerobes or microaerophilic, rod-shaped, non-spore-forming bacteria.

Carbohydrates fermented by *L. bulgaricus* (90% or more strains) are fructose, glucose, and lactose. Lactic acid is the major product of fermentation; however, secondary products, such as acetaldehyde, acetone, acetoin, and diacetyl, also can be produced in very low concentrations.

In lactic acid bacteria that do not possess superoxide dismutase, the dismutation of superoxide normally is catalyzed by internally accumulated manganese. *Lactobacillus bulgaricus*, however, has a low capacity to scavenge O_2^- because it does not have superoxide dismutase or high levels of Mn (II) and it is sensitive to O_2 (the ability to grow aerobically must be distinguished from the ability to survive exposure to O_2).

Fermentation

The following table shows the variation of the DO and the concentration of residual glucose during fermentation.

Table 1: Biomass growth kinetics (Lactobacillus bulgaricus) and substrate consumption kinetics (Glucose).

	T0	T6	T12	T18	T24	T48
DO 600nm	0.08	0.12	0.15	0.19	0.18	0.17
S g/l	30	22.5	10.1	2.04	0.04	0.01

According to this table, the bacterial concentration increases during the experiment. DO increases from 0.08 to 0.18 until T18. However, after 18 hours of culture, a decrease in the concentration of viable cells, shown by the decrease in the OD is noted.

This is explained by the depletion of substrates, and possibly the accumulation of metabolism by-products, toxic to biomass.

A decrease in the chromacity of the reaction medium was marked. Thus, the biomass (strain of *Lactobacillus bulgaricus*) therefore has the capacity to considerably discolor the leaves of *Moringaoleifera.*

Table 2 :L,a,b value at T0 and T48 hours

Time (h)	0	48
L	59	58.83
a	-7.97	-2.9
b	34	33.53

The values of (L, a, b) placed on the chromacity diagram show that the color of the fermented powder falls within the region of (white, green, yellow). A lightening of the product is thus obtained. This

parameter could be exploited to improve its visual appearance. Indeed, a visual comparison of unfermented and fermented *Moringaoleifera* leaf powder shows a difference in color in green.

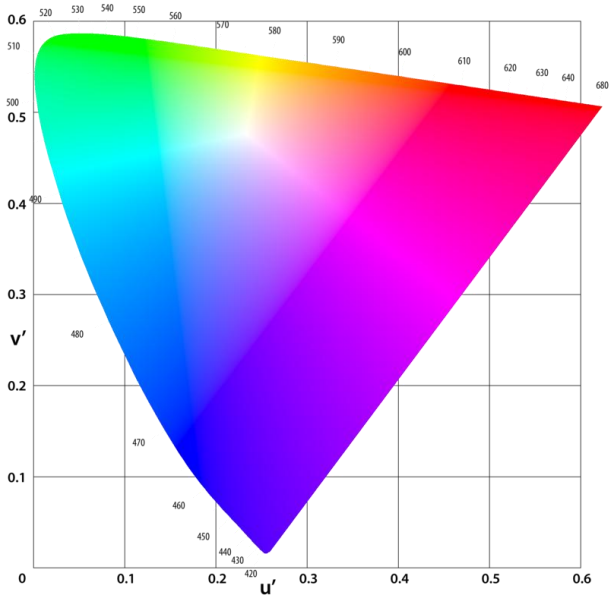

Figure 1: Chromacity diagram

Nutritional analysis

The following table shows the total protein and polyphenol contents before and after fermentation.

Table 3: Total protein and polyphenol content at T0 and T48.

	T0	T48
Proteins %	3.240	4.980
Polyphénols %	21.359	8.342

An increase in protein content was noted. It went from 3.240% to 4.980%. This can be explained by the increase in Biomass in the medium. A large number of microorganisms are an important source of protein. They are exploited industrially during the production of POUCs (Tsirinirindravo and al, 2018).

However, a considerable decrease in the polyphenol concentration was noted: it went from 21.359% to 8.342% in 48 hours. This can be explained by the presence of enzymes such as polyphenol oxidases and peroxidases. These enzymes are produced by bacteria or present in food, and they hydrolyze phenolic compounds (Tsirinirindravo and al, 2018)

REFERENCES :

[1.] Banque mondiale, 2018. Une nouvelle approche évolutive pour mieux lutter contre les retards de croissance.

[2.] HARIMALALA ANDRIAMBELO, 2014. Nutritional quality of fruit pastes enriched with *Moringaoleifera* leaves. In International Journal of Applied Science and Technology. Vol4, N°5, pp163-175.

[3.] HARIMALALA ANDRIAMBELO, 2015. Enrichissement protéique des pâtes de fruits par des feuilles de *Moringaoleifera*. Thèse doctorat. Université d'Antananarivo.

[4.] HARIMALALA ANDRIAMBELO, 2021. Valorization of *Moringaoleifera* leaves by incorporation in tamarin (*Tamarindusindica*) and banana (*Musa sativa*) pastes.

[5.] SAINT SAUVEURA., BROIN M. 2005. *Moringa* news/ Moringa association of Ghana. Produire et transformer les feuilles de *Moringaoleifera*. 36p.

[6.] PNAN III, 2017/2021. La nutrition, garant d'un capital humain pour un développement social et économique durable. 96p.

[7.] PAM, INSTAT, 2014. Analyse globale de la sécurité alimentaire et nutritionnelle et de la vulnérabilité (AGSANV). 96p.

[8.] UNICEF, 2017. Abrégé du plan d'investissement pour la nutrition à Madagascar. 16p.

[9.] TSIRINIRINDRAVO HL, RAFANOMEZANTSOA FM, RANDRIANIERENANA LA, ANDRIANARISOA B, RAHERIMANDIMBY M. Valorisation des graines *d'Hyphaenecoriacea* en boisson alcoolisée. Communication lors des Journées QualiReg 2016. 5eme rencontre de l'Agroalimentaire en Océan Indien « La qualité et l'innovation au service du développement des filières agroalimentaire de l'Océan Indien. Saint Pierre, La Réunion.

[10.] TSIRINIRINDRAVO H.L, MENDES D.S.R., LARROQUE M. Antibacterial effects of probiotics against savage and multi resistant bacteria. International Journal of Food Microbiology 274 (2018) 71-79.

[11.] TSIRINIRINDRAVO H.L**,** RABETSIMIALONA HM, Valorisation de la paille de riz (*Orizasativa)* en vue de la production d'éthanol. Terre d'Espoir. N° 7, 3ème trimestre : juin 2014.