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**Title:**

# "Phytochemical composition and antioxidant activity of fermented *Moringa oleifera* leaf powder"

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**ABSTRACT**:

The aim of this study was to determine the effects of lactic fermentation of *Moringa oleifera* leaf powder at young and mature stage of development. This is to improve the organoleptic quality of *Moringa oleifera* leaf powder. The work was performed in the laboratory of high School of Engineering of Reunion. The fermentation was performed by *Lactobacillus plantarum* (DSM 2601) and *Weissella cibaria* (27A) inoculated at $10^6$ CFU / g. The fermentation was performed at 25 ° C over 5 days. An acidification of fermented products (4 < final pH < 5), a high protein content in mature leaves fermented over 120 hours (T120-Ma), or over 48 h (T48-Ma), by *Weissella cibaria*, a high protein content in mature leaves fermented over 120 h (T120-Ma) and in young leaves fermented over 120 h (T120-I) by *Lactobacillus plantarum* was observed. The results of ANOVA on fermentation by *Weissella cibaria* (27A) and *Lactobacillus plantarum* (DMS 2601) on the nutritional leaves of *Moringa oleifera*, showed there was no significant effect on the α=0,05 fermentation times and stage of leaves maturity for contents of reducing sugar and proteins. But there is a significant effect of fermentation and maturation stage leaves on the pH of the product threshold at α=0.05.The fermentation by *Lactobacillus plantarum* (DMS 2601) showed there was an effect of the fermentation time on the content of phenolic compounds. Principal component analysis (PCA) showed that there is a negative correlation between the protein content and the content of phenolic compounds. By the fermentation process, the organoleptic characteristics of the *Moringa oleifera* leaves powders whose color is to be improved, which is an asset in the process of incorporation of *Moringa oleifera*. Lactic fermentation of *Moringa oleifera* leaves is a method of increasing protein intake of the food and the fight against PEM target populations.

**Keywords**: ***Moringa oleifera*** / fermentation / *Lactobacillus plantarum* / *Weissella cibaria* / Chemical composition / Antioxidant activity

## 1 Introduction :

Protein malnutrition affects populations in most of the southern countries, like Madagascar. In this country, in 2012-2013, the proportion of the population living below the national poverty line was 71.5% and in the extreme poverty was 52.7%. This poverty results in a diet consisting of low-cost roots and tubers with intakes of 477g / day / person [1]. Malnutrition is caused by low household income, and by ignorance and lack of exploitation of edible and available natural resources. *Moringa oleifera* is a plant that grows in the wild or that serves hedge for homes in coastal areas and it is relegated to the back by the highlands population. But in the South, *M. oleifera* leaves are a new food resource, an important source of protein, vitamins and minerals which helps to fight against malnutrition [2, 3, 4, 5]. *M. oleifera* is also used in traditional medicine and is known to be rich in antioxidants, especially polyphenols and flavonoids which can prevent degenerative diseases. Among other to use to struggle against malnutrition, *M. oleifera* serves for enriching different food in proteins and minerals [3, 6, 5]. It serves as a nutritional supplement for example in biscuits and in traditional dishes [7, 8, 9].

Technological processes are searched to improve the bioavailability of nutrients and to increase the energy density of food [10]. It's possible to name the case of lactic fermentation improves the organoleptic properties of food and health and conservation [11, 12]. The organisms implicated are the lactic bacteria such as *Lactobacillus plantarum*. It is used for example in the fermentation of corn pastes, sauerkraut lemon and others foods based on wheat [13, 14, 15].

All over beneficial effect nutritional and health benefits of *M. oleifera* leaves, and in order to increase protein intake, we determined the effect of fermentation on powders leaves by *Lb. plantarum* and *Weissella cibaria* on nutritional composition, on antioxidant capacity as well as on the organoleptic properties especially the food color. Indeed, color as well as texture are important factors to determine sensory quality and acceptability of the consumers for food products. These parameters are needed to improve the process of incorporating the powders of *M. oleifera* leaves in food [3].

## 2 Materials and methods :

### 2.1 Plant material :

The leaves of *M. oleifera* were collected at Tamatave, east coast of Madagascar, in March and April 2015. The leaves were collected and separated in three groups: the first group constituted by young leaves (yo), the second group made of mature leaves (Ma) and the third group was leaves without stage distinction (lea). In this work, the difference between the young and the mature leaves was tenderness and color. The young leaves were more tender, light green color compared to older leaves which were

harder and dark green.Leaves were washed and then dried in ventilated room during 3 days and were regularly stirred: twice a day to avoid mold growing. The dried leaves were powdered with a mixer and stored in dark bag and in a dry place.

### 2.2 Microorganisms:

The strains used in this study were *Lb. plantarum* DSM 2601 and *W. cibaria* 27A provided from the Microbiology Laboratory of QualiSud, Université de La Réunion.

### 2.3 Culture of bacteria:

*Lb. plantarum* DSM 2601 and *W. cibaria* 27A were propagated in de Man, Rogosa and Sharpe (MRS) broth (pH 5, 5) at 37°C for 72 h for activation. The cell number was approximately $10^8$ UFC/ml. The cultures obtained were used for the inoculation of powdered *M. oleifera* leaves solution.

### 2.4 Preparation of *M. oleifera* powder solution:

A mass of 50 g of leaf powder, young (Yo), mature (Ma) or leaves without distinction of maturity (Lea), were introduced into an Erlenmeyer flask of 1000 ml and 400 ml of distilled water was added. It was sterilized at 120°C for 20 min. After cooling, the preparation of powders leaves were inoculated with *Lb. plantarum* (DSM 2601) and *W. cibaria* (27A) at $10^6$ UFC/ml.

The mixtures were homogenized at 150 rpm and the fermentation was carried out at 25°C for 120 hours (5 days). Sample without bacteria was made as control.

### 2.5 Chemicals analysis:

The moisture content was determined on the powder of *M. oleifera* leaves: young (Yo), mature (Ma) and leaves without distinction of maturity (Lea).

Over incubation, 50 ml of each sample were removed immediately after inoculation (T0), after 48h (T48) and after 120 h (T120). Counts of DSM 2601 and 27A were estimated by optical density measurement at 660 nm. The pH value was measured with a pH meter.

Then, the samples were centrifuged at 10°C, 15 min, 5000 x *g* and the supernatants were used for the analysis:

- ✓ Reducing sugars, resulting product of hydrolysis, were determined by the dinitrosalicylic acid (DNS) method of Miller (1959) with some modifications: 500µl of diluted supernatant (1/6 or 1/8) was mixed with 750 µl DNS reagent. The reaction mixture was plugged with aluminum paper and boiled in a water bath 100°C for 5 min and cooled to room temperature in a water ice bath. Then, 7.5 ml of distilled water was added. The absorbance was measured at 540 nm using a spectrophotometer. A calibration curve was prepared using a standard solution of glucose 1g/l;
- ✓ Total protein content was determined by the method of Bradford (1976): 100µl of diluted extract (1/2) was mixed with 3 ml of the Coomassie reagent, incubated at room temperature for 15 min and the absorbance was measured at 594 nm. A standard solution was prepared using bovine serum albumin (BSA) 1g/l;

- ✓ The total phenolic compound content was estimated by the method of Singleton and Rossini (1965) using Folin-Ciocalteu regent: 30 µl of the acetone extract and of the aqueous extract in distilled water (1/8 and 1/150) was mixed with 15 µl of Folin –Ciocalteu reagent. After 5 min at room temperature, 60 µl of $Na_2CO_3$ 2% were added. Then, 195 µl distilled water were added and incubated for 1h in the dark, the absorbance was read at 760 nm with a spectrophotometer against blank. The blank was made from distilled water with all the reactives. The total phenolic compound was calculated using a standard solution of 1mg/ml acid Gallic, the results were expressed as mg acid Gallic equivalents / 100 g of extract.
- ✓ The color of *M. oleifera* leaf powder was assessed using a chromameter, using the color space (L * a * b). The instrument was calibrated with standard white reflector plate of the device.

Each extraction assays were done in triplicate.

### 2.6 Statistical analysis:

To characterize the nutritional properties of fermented powder leaves, an analysis of variance (ANOVA) using 2 factors (fermentation and maturity of leaves) multiple comparison test with the Fischer test at 95% confidence level was performed. An Analysis with principal components (ACP) was carried out to highlight the differences between products. Statistical analyzes were carried out using the software XLTSAT 7.0 to identify the difference between the products.

## 3 Results and discussion:

### 3.1 Effects of fermentation on nutritional properties of *M. oleifera* leaves

To study the evolution of leaf powder at the various stages of maturation (young, mature and mixtures) over lactic fermentation (T0, T48, T120), optical density measurements (OD), pH, reducing sugars (RS), proteins (PR), and Phenolic compounds (PC) level determination were performed. Data are presented in tables 1 and 2.

*Table 1: Effects of fermentation by W. cibaria (27A) on the nutritional leaves of M. oleifera*

| **27A** | Fermentation time | OD[1] | pH | RS[2] | PC[3] | PR[4] |
|---|---|---|---|---|---|---|
| Young (Yo) | T0 | $2.4^{A}$ | $5.2^{AB}$ | $21.5 \pm 0.1^{A}$ | $21.5 \pm 0.1^{AB}$ | $3.4 \pm 0.3^{A}$ |
| | T48 | $1.8^{AB}$ | $4.7^{A}$ | $17.8 \pm 0.1^{A}$ | $21.6 \pm 0.1^{AB}$ | $4.6 \pm 0.1^{A}$ |
| | T120 | $1.9^{AB}$ | $4.7^{A}$ | $23.4 \pm 0.1^{A}$ | $10.9 \pm 0.1^{ABA}$ | $10.1 \pm 0.1^{AB}$ |
| Matures (Ma) | T0 | $1.9^{A}$ | $5.2^{B}$ | $5.4 \pm 0.1^{A}$ | $28.3 \pm 0.1^{B}$ | $4.7 \pm 0.1^{A}$ |
| | T48 | $2.2^{AB}$ | $4.9^{AB}$ | $15.2 \pm 0.2^{A}$ | $15.1 \pm 0.1^{B}$ | $7.2 \pm 0.1^{A}$ |
| | T120 | $2.5^{AB}$ | $4.7^{AB}$ | $22.8 \pm 0.1^{A}$ | $7.2 \pm 0.1^{A}$ | $7.5 \pm 0.1^{AB}$ |
| leaves without distinction (Lea) | T0 | $1.5^{A}$ | $5.2^{B}$ | $4.1 \pm 0.1^{A}$ | $20.4 \pm 0.1^{AB}$ | $6.9 \pm 0.1^{A}$ |
| | T48 | $2.8^{AB}$ | $5.2^{AB}$ | $11.0 \pm 0.1^{A}$ | $15.2 \pm 0.1^{AB}$ | $6.9 \pm 0.1^{A}$ |
| | T120 | $2.5^{AB}$ | $5.2^{AB}$ | $14.9 \pm 0.1^{A}$ | $4.8 \pm 0.1^{A}$ | $7.7 \pm 0.1^{AB}$ |

[1] OD: optical density; [2] RS: reducing sugars; [3] PC: Phenolic compounds; [4] PR: proteins

The results of ANOVA showed that there was no significant effect on the threshold $\alpha = 0.05$ fermentation time and stage of plant maturity on the levels of reducing sugars ($p = 0.334$; $p = 0.893$), phenolic compounds ($p = 0.077$; $p = 0.235$) and protein ($p = 0.062$; $p = 0.299$).

*Table 2: Effects of fermentation by Lb. plantarum (DSM 2601) on the nutritional leaves of M. oleifera*

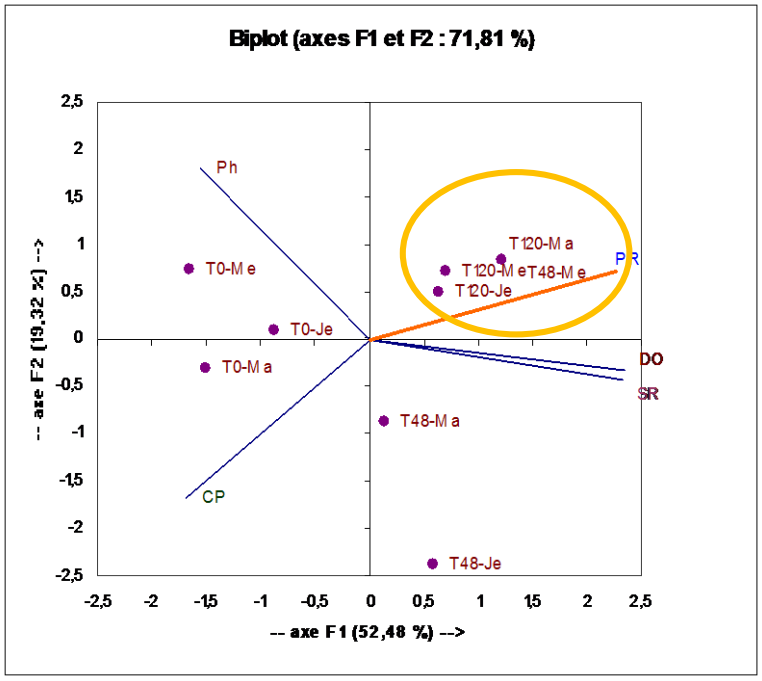
| **DSM** | Age | OD[1] | pH | RS[2] | PC[3] | PR[4] |
|---|---|---|---|---|---|---|
| Jeunes | T0 | $0.7^{A}$ | $5.1^{AB}$ | $18.1 \pm 0.2^{BA}$ | $18.2 \pm 0.1^{AC}$ | $3.4 \pm 0.1^{A}$ |
| | T48 | $2.7^{A}$ | $4.3^{A}$ | $19.3 \pm 0.1^{BA}$ | $24.0 \pm 0.1^{AB}$ | $4.5 \pm 0.1^{AB}$ |
| | T120 | $2.0^{A}$ | $4.6^{A}$ | $13.9 \pm 0.1^{AB}$ | $6.1 \pm 0.1^{A}$ | $7.4 \pm 0.1^{AB}$ |
| Matures | T0 | $0.9^{A}$ | $5.3^{AB}$ | $16.0 \pm 0.1^{A}$ | $30.1 \pm 0.1^{AC}$ | $3.3 \pm 0.3^{A}$ |
| | T48 | $2.1^{A}$ | $4.8^{AB}$ | $20.2 \pm 0.1^{A}$ | $21.5 \pm 0.1^{B}$ | $5.0 \pm 0.1^{AB}$ |
| | T120 | $2.5^{A}$ | $4.9^{AB}$ | $20.5 \pm 0.1^{A}$ | $10.4 \pm 0.1^{A}$ | $9.9 \pm 0.1^{AB}$ |
| leaves without distinction (Lea) | T0 | $0.9^{A}$ | $5.3^{B}$ | $9.1 \pm 0.1^{A}$ | $19.0 \pm 0.1^{AC}$ | $2.9 \pm 0.1^{A}$ |
| | T48 | $2.7^{A}$ | $5.1^{BA}$ | $22.3 \pm 0.1^{A}$ | $7.4 \pm 0.1^{AB}$ | $4.4 \pm 0.1^{AB}$ |
| | T120 | $2.7^{A}$ | $5.1^{BA}$ | $22.3 \pm 0.1^{A}$ | $7.4 \pm 0.1^{A}$ | $4.4 \pm 0.1^{AB}$ |

[1] OD: optical density; [2] RS: reducing sugars; [3] PC: Phenolic compounds; [4] PR: proteins

Statistical analysis showed no significant effect at $\alpha = 0.05$ fermentation time and stage of leaves maturity for contents of reducing sugars ($p = 0.090$; $p = 0.114$) and proteins ($p = 0.062$; $p = 0.299$). But, in the mature stage, the fermentation time influences the content of phenolic compounds ($p = 0.014$).

So generally, statistical analysis showed that there was no significant effect of fermentation and maturation stage leaves on nutritional parameters of the product, but there is a significant effect of fermentation and maturation stage leaves on the pH of the product threshold at $\alpha = 0.05$. To find the difference that may exist between the fermented products, it is made an analysis in principal components where the variables are projected (Figures 1a and 1b).

The analysis in ACP STATE, characterize the products T120-Ma, T120-Me, T48-Me and T120-yo strongly correlated which are rich in protein (figure 1a). In figure 1b, the products T120-Ma and T48-Ma are correlated with the variables proteins and sugars reducers. These products are opposed to T0-Me if considers the variable compounds phenolic.

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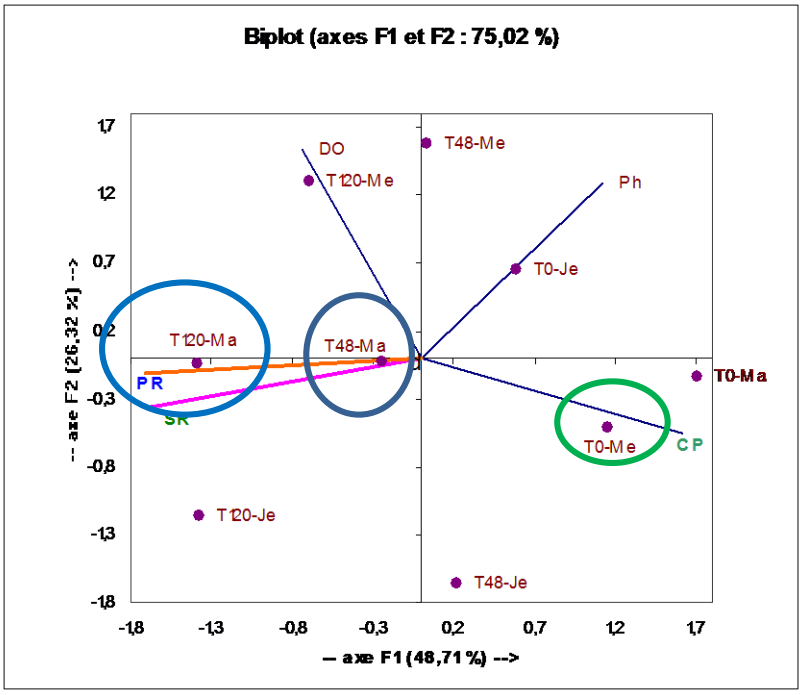
153 Figure 1a: Principal components analysis of fermented leaves by *Lb. plantarum* (DSM 2601) according

154 to the time of fermentation and maturity of the plant.

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156 Figure 1b: Principal components analysis of fermented leaves by *W. cibaria* (27A) according to the time

157 of fermentation and maturity of the plant.

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### 3.2 Effects of fermentation on the chromacity of *M. oleifera* leaves

The values (L, a, b) of fermentation effect are indicated in the tables 3a and 3b. The values of (L, a, b) put on the chromacity diagram shows the color of the fermented powder is situated in the area (white, green, yellow).

Tableau 3a: Colorimetric measurement of young powder leaves fermented by *Lb. plantarum* (DSM2601)

| **Temps (T) en heure** | **0** | **48** |
|---|---|---|
| **L** | 59.0 | 58.8 |
| **a** | -8.0 | -2.9 |
| **b** | 34.0 | 33.5 |

Tableau 3b: Colorimetric measurement of young powder leaves fermented by *W. cibaria* (27A)

| **Temps (T) en heure** | **0** | **48** |
|---|---|---|
| **L** | 59.9 | 59.5 |
| **a** | -7.1 | -1.7 |
| **b** | 35.6 | 35.1 |

There is thus obtained a clarified product. This parameter could be exploited to improve its visual aspect. Indeed, a visual comparison of the powder *M. oleifera* leaves fermented and not fermented shows a difference of coloring in the green (figure 2a and 2b).

The fermentation of *M. oleifera* powder leaves is possible to increase the protein product due to increase of biomass; increasing the reducing sugar content due to the hydrolysis of complex sugars and phenolic compounds. Indeed phenolic compounds are abundant in the young stages of development of plants [16]; they reduce the onset of maturation and then become stable [17].

**Figure 2a** :*M. oleifera* powder leaves unfermented

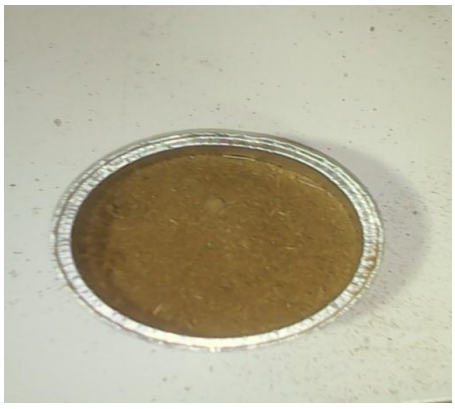

**Figure 2b** :*M. oleifera* powder leaves fermented

## 4 Conclusion

The fermentation powder leaves of *M. oleifera* gives a food of high protein value which will be able to contribute in the fight against malnutrition. The freeze-dried fermented powder can be used to enrich pastes of tamarind or pastes of banana, as we tested it, or of other food support [3].

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