

Improvement of the Nutritional Quality of Black Bean (*Phaseolus Vulgaris* L.) Powder During Fermentation: Use of Lactic Starters

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Abstract: The objective of this study was to investigate lactic acid starters able to improve the nutritional quality of black bean powder (BBP) during fermentation. For this purpose, raw and cooked BBP was fermented with *Lactobacillus acidophilus* and BLHN7 isolate. After 120 hours of fermentation, the pH of medium was measured by pH meter, tannins and phytates by spectrophotometry, total proteins by the Kjeldhal method. The minerals by atomic absorption spectrophotometry and the total sugars by the phenol method. From these analyses, it was found that the pH decrease was not significantly different in BBP fermented with *Lactobacillus acidophilus* and BLHN7 isolate. This decrease in pH varied between 4.0 and 4.3. Principal Component Analysis (PCA) of the results shows the variability of nutritional parameters depending on the microorganisms used and the type of treatment applied to the BBP. However, a better increase in protein, iron and microbial growth is observed in the raw BBP fermented with *Lactobacillus acidophilus*. We observed an increase of 267, 240 and 118% respectively. It is also in the raw BBP that we observe a reduction of tannins and a small reduction of sugar content by 76 and 20% respectively. On the other hand, it is in the cooked BBP fermented with *Lactobacillus acidophilus* that phytates were reduced by 3.4% and magnesium increased by 180%. Calcium increased by 165% in cooked BBP fermented with BLHN7 isolate. Considering the effect of *Lactobacillus acidophilus* on the majority of the nutritional parameters studied, it would be interesting to use it to improve the nutritional quality of BBP as a dietary supplement for malnourished children.

Keywords: Black Bean, Lactic Acid Bacteria, Nutritional Quality, Fermentation

1. Introduction

With its scientific name "*Phaseolus vulgaris* L.", black bean is still called a whole bean because it contains the essential amino acids essential for the body [1]. It is composed of protein, fat, carbohydrates, fiber, vitamins, flavonoids, minerals and polyphenol [2]. This food is known to be the second largest source of dietary protein (9 to 25 g/100g) after meat (15 and 30 g/100g) and the third largest source of calories in Africa [3]. Black beans are excellent for health because they contain a low glycemic index. It has the potential to lower serum cholesterol concentrations, stabilize blood sugars and relieve constipation

[4]. It also ensures the maintenance of good blood pressure which promotes heart and bone health, decreases the risk of cancer [5]. It is also known for its therapeutic properties in the treatment of eczema, diabetes, burns, acne, heart, bladder, carminatives, dropsy, dysentery, emollients, hiccups, itching and rheumatism, cancer and obesity [6]. It is commonly used to manage sickle cell disease in the western region of Cameroon [7]. However, the optimal use of black bean is often limited by the presence of anti-nutritional compounds such as enzyme inhibitors, lectins, phenolic compounds, resistant starches, tannins, protease inhibitors such as phytates and cyanoglycosides [8]. These compounds have adverse effects on

human and animal nutrition by causing intestinal disorders, decreasing the availability of micronutrients and limiting the digestibility of nutrients. In order to reduce these anti-nutritional compounds and improve the nutritional quality of black beans, several methods are used such as cooking and fermentation. However, fermentation remains one of the most used technologies and controlled fermentation is more and more recommended. It has been recognized by several authors as having the capacity to reduce the content of anti-nutritional compounds while increasing the digestibility and content of certain nutrients such as proteins and minerals [9]. The work of Jimoh *et al.* [10] observed that during the fermentation of beverages (*burukutu*, *pito*, and palm wine) by yeasts such as *Saccharomyces cerevisia*, the content of some anti-nutritional factors such as oxalic acid was reduced to 90%. In addition, the work of Tchikoua *et al.* [11] showed that during the fermentation of *kutukutu* by *Lactobacillus brevis* and *Lactobacillus fermentum*, the protein content (18.9%), magnesium (50.5%) and iron (70.6%) increased while phytates were reduced (95%). The general objective of this work is to evaluate the influence of lactic starters on the nutritional quality of black beans during controlled fermentation.

2. Materials and Methods

2.1. Plant Material

The black bean (*Phaseolus vulgaris* L.) samples used in

2.4. Inoculation of Lactic Starters into BBP

this study for the production of BBP were provided by the Institute of Agricultural Research for Development (IRAD) of Foumbot (West, Cameroon).

2.2. Microbiological Material

The bean was fermented with 02 lactic acid bacteria. One of the lactic acid bacteria is kindly donated by the Microbiology Laboratory of the University of Yaounde 1 (Cameroon) and identified as *Lactobacillus acidophilus*. The other lactic acid bacteria, coded BLHN7 isolate, was obtained from BBP and characterized macroscopically, microscopically and biochemically after its isolation on MRS medium.

2.3. Preparation of the Raw and Cooked BBP

The black bean seeds were first sorted manually, winnowed and washed with tap water. Then, the washed bean was divided into 04 batches of 500 g each. Batche 1 and batche 2 were dried in an oven at 60°C for 12 hours before being ground in a blender (AIFA) and sterilized in an autoclave at 121°C for 15 minutes. Batche 3 and batche 4 were mixed with 2000 mL of tap water in a 5 L batch and boiled at 95 ± 2°C for 120 minutes. The obtained products were also oven dried at 60°C for 12h, crushed and sterilized. This preparation allowed to obtain 02 batches of raw BBP (batche 1 and batche 2) and 02 batche of cooked BBP (batche 3 and batche 4).

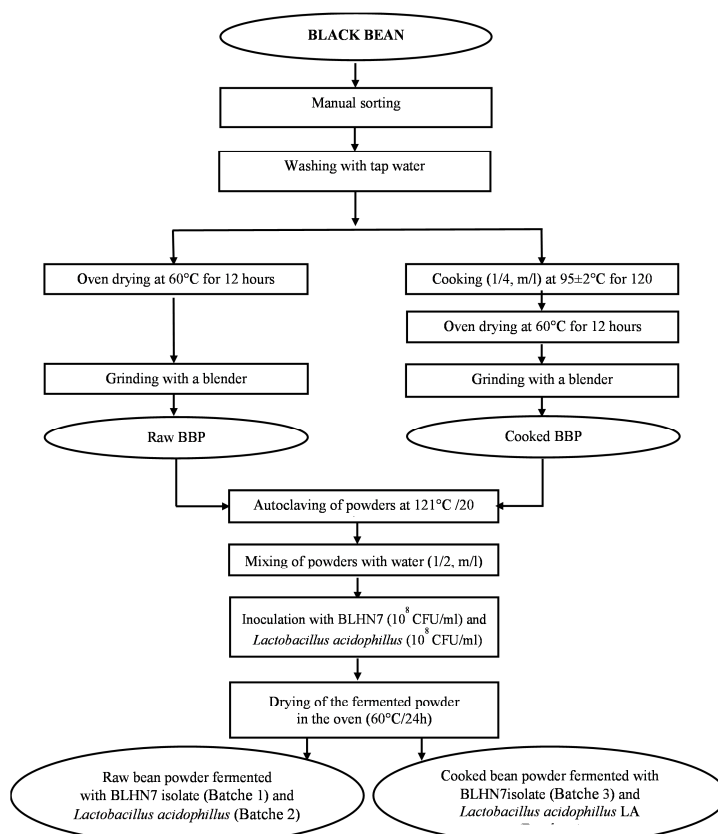


Figure 1. Diagram of production and fermentation of raw and cooked BBP.

A mass of 250 g of BBP of each batch was introduced into a 1 L jar containing 500 mL of sterile distilled water. Then, a concentration of 10^8 CFU/mL of inoculum (*Lactobacillus acidophilus* or BLHN7 isolate) was introduced into the different jars. The whole was fermented at room temperature over 120 hours. To evaluate the bacterial growth, the pH measurement and nutritional compounds, 50 g of each preparation was taken every 24 hours and dried (60°C , 24 hours). The figure 1 shows the production and fermentation diagram of the BBP.

2.5. Evolution of Microbial Growth and pH During Fermentation of Raw and Cooked BBP

The evolution of lactic flora in cooked and raw BBP was determined during fermentation at a frequency of 24 hours on MRS agar. For this purpose, 10 g of samples were taken and induced in 90 mL of sterile physiological water. The prepared solution (10^{-1}) was successively diluted from 10^{-2} to 10^{-8} . A volume of 0.1 mL of each dilution was spread on MRS agar and incubated at 37°C for 48 hours under anaerobic conditions.

The pH was determined by immersing the electrode of the pH meter in solution obtained from a mixture of 10 mL of substrate to 20 mL of distilled water [12].

2.6. Evolution of Tannin Content During Fermentation of Raw and Cooked BBP

Tannin content were determined following the spectrophotometric method using acidified vanillin and (+) catechin as analytical standard [13]. For extraction of total tannins from bean, 2 g of sample was mixed in a 50 mL Erlenmeyer flask with 30 mL of acetone (80%) prepared from 2% acetic acid. After 15 minutes of stirring, the mixture was pressure filtered through a sintered glass using a Buchner. The resulting residue was washed twice with 10 mL of solvent and the acetone was separated from the filtrate using a Rotavapor at 35°C . After extraction, a volume of 0.5 mL of extracts, 3 mL of butanol-HCl reagent (95: 5) and 0.1 mL of ferric reagent (ferric ammonium sulfate 2% in HCl, 2N) were mixed in a 10 mL test tube. The mixture was stirred and incubated in a water bath at 100°C for 1 hours. The absorbance (DO) was read at 550 nm for each sample against a blank made by making a similar mixture without sample. The formula below developed by Porter et al. [14], gives the condensed tannin.

$$\% \text{Tannin} = \frac{\text{DO} \times 78.26}{\text{Dry sample weight}} \times 100$$

2.7. Evolution of Phytates Content During Fermentation of Raw and Cooked BBP

The method of Mohammed et al. [15] described by Olayeye et al. [16] was used for the extraction and determination of phytates content. The extraction was

performed by mixing in a beaker, 2 g of BBP to 100 mL of HCl (2%). The whole was vigorously shaken with a magnetic stirrer and left to stand for 3 hours. The resulting mixture was filtered through filter paper. After extraction, a volume of 25 mL of the filtrate was introduced into a 250 mL flask, then 5 mL of 0.3% ammonium thiocyanate solution (as an indicator) and 53.5 mL of distilled water were added to the mixture to obtain the desired acidity. The resulting solution was titrated with a standard solution of iron III chloride (0.00195 g iron per mL) until a persistent brownish yellow color was obtained for 5 minutes. The phytates content was calculated according to the following formula:

$$\% \text{ Phytates} = \text{Titer volume} \times 0.00195 \times 1.19 \times 100$$

2.8. Evolution of Protein Content During Fermentation of Raw and Cooked BBP

The total protein content was evaluated after determination of the total nitrogen content and multiplication of this content by 6.25. Total nitrogen was determined after mineralization of the samples by the Kjeldahl method [17], followed by determination by the method of Devani et al. [18]. In a test tube, 0.2 mL of the solution obtained after mineralization, 1.2 mL of sodium acetate solution and 1.6 mL of reagent solution (15 mL of formaldehyde + 8 mL of acetylacetone in 77 mL of distilled water) were successively introduced. The mixture was incubated in a water bath (97.5°C) for 15 minutes. Once cooled in a stream of cold water, the tube volume was made up to 10 mL by adding 7 mL of distilled water. The absorbance was read at 412 nm against a blank consisting of the sodium acetate solution, reagent solution and distilled water. The calibration range of the nitrogen solution was established from a 0.4 g nitrogen/mL ammonium sulfate solution. The amount of nitrogen was determined from the calibration curve of the ammonium sulfate calibration curve. The calibration line equation that allowed us to calculate the amount of nitrogen is as follows:

$$X = Y \times \frac{V_t \times 100}{V_p \times m \times a}$$

With:

Y: Optical density;

V_t (mL): Total volume of mineralization;

V_p (mL): Volume of mineralized sample;

m (g): Mass of mineralized sample;

a: Calibration coefficient (0.006);

X: Amount of nitrogen.

The following formula was used to determine the total protein content in our samples expressed in g/100g dry matter. The conventional coefficient of conversion of nitrogen to protein used is 6.25 [19].

$$\text{Protein content} = 6.25 \times X \text{ (g/100 g)}$$

2.9. Evolution of Calcium, Magnesium, Iron and Sugar Content During Fermentation of Raw and Cooked BBP

A volume of 0.5 mL of each sample was introduced into 50 mL propylene tube to which 15 mL of Aqua Regia solution was added. The resulting mixture was shaken for 10 minutes with a mechanical shaker and then centrifuged at 3000 rpm for 10 minutes. The supernatant representing the sample solution was recovered and the determination of Ca^{2+} , Mg^{2+} and Fe^{2+} was performed by flame atomic absorption spectrophotometry [20]. During this determination, standard solutions were required. The calcium and magnesium standard solutions were prepared by introducing into a 100 mL volumetric flask, 25 mL volume of Ca^{2+} (1000 ppm) and 5 mL of Mg (1000 ppm). The volume obtained was made up to the mark with strontium chloride solution (5.75 g of $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ dissolved in deionized water and made to 2 L with this water). For the iron standard solution, a volume of 8 mL of Fe^{2+} (1000 ppm) and 2 mL of zinc (1000 ppm) were introduced into a 100 mL volumetric flask and the whole solution was completed to the mark with Aqua Regia solution (400 mL of concentrated HCl and 133 mL of HNO_3 , 70% dissolved in deionized water and made to 2 L with this water).

Total sugars were determined by the phenol method described by Dubois *et al.* [21]. A mass of 0.5 g of BBP was taken and introduced into a test tube to which 10 mL of 1.5 N sulfuric acid was added. The tube was closed with a stopper fitted with a release tube. The solution was heated in a water bath at 100°C for 15 minutes and allowed to cool to room temperature. A volume of 0.5 mL of 2% zinc sulfate and 0.5 mL of concentrated 10.6% potassium ferrocyanide was also added. The mixture was filtered with filter paper. The filtrate was made up to the mark with distilled water. The amount of total sugars was determined

by calibration curve and calculated according to the following formula:

$$Q = 100 * [QVT/m * V] (100 - Hr)]$$

With:

Q: Quantity of sugar in the test sample,

VT: Total volume of the extract,

m: Test sample in g,

V: Volume of the sample analysed,

Hr: Residual water content.

2.10. Statistical Analysis

The results obtained were presented in the form of curves obtained with the sigma plot software. XLSTAT 2007 software was used to perform the principal component analyses (PCA) to compare the efficiency of the bacteria used during fermentation of the raw and cooked BBP. Excel software was used to calculate the mean and standard deviation.

3. Result

3.1. Evolution of Microbial Growth During Fermentation of Raw and Cooked BBP

It appears that the 02 lactic acid bacteria multiply during the fermentation of the bean (Figure 2). The growth of *Lactobacillus acidophilus* increases from 5.5 to 10.3 $\text{Log}_{10}\text{UFC/ml}$ in the cooked BBP and to 12 $\text{Log}_{10}\text{UFC/ml}$ in the raw BBP. The isolate BLHN7, increases from 5.5 to 10.5 $\text{Log}_{10}\text{CFU/ml}$ for cooked BBP and to 11.68 $\text{Log}_{10}\text{CFU/ml}$ for raw BBP. This result shows that the 02 lactic acid bacteria used for fermentation develop well in the BBP. However, this development, being more important in the raw BBP.

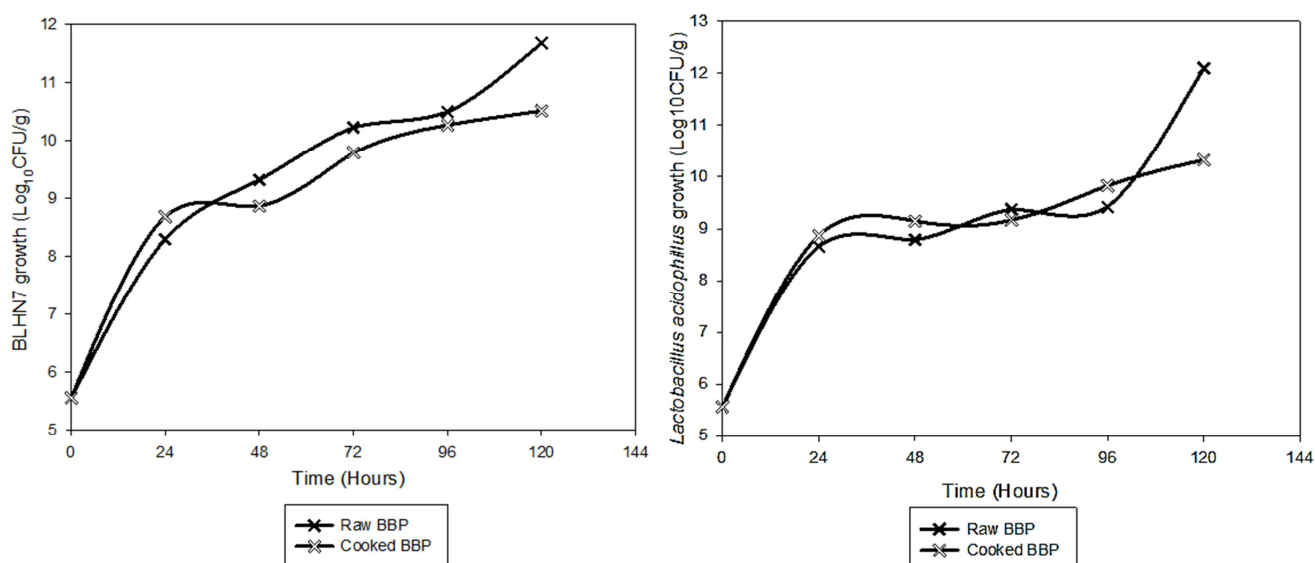


Figure 2. Evolution of the growth of *Lactobacillus acidophilus* and isolate BLHN7 during the fermentation of raw and cooked BBP.

3.2. Evolution of pH During Fermentation of Raw and Cooked BBP

After 120 hours of fermentation, the pH decreased from 6.22 to 4.3 for raw BBP and to 4.46 for cooked BBP fermented with

Lactobacillus acidophillus. Similarly, the pH decreased from 6.22 to 4.07 for raw BBP and from 6.22 to 4.05 for cooked BBP fermented with BLHN7 isolate. The evolution of pH during fermentation of raw and cooked BBP by *Lactobacillus acidophillus* and isolate BLHN7 is shown in figure 3.

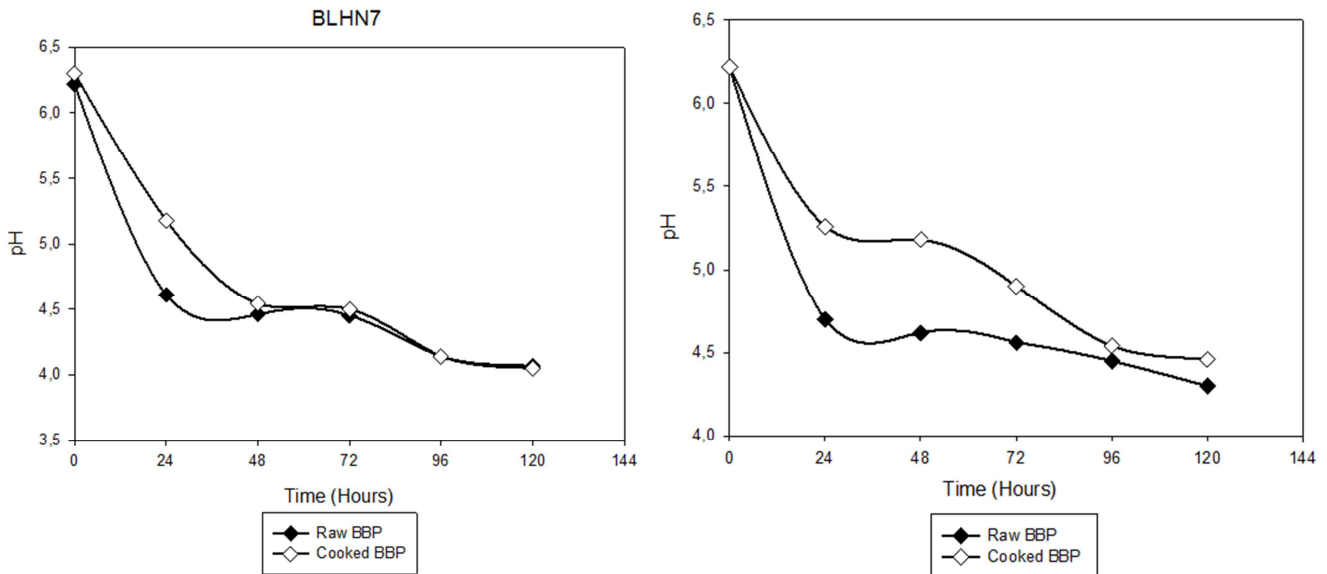


Figure 3. Evolution of pH during fermentation of raw and cooked BBP by *Lactobacillus acidophillus* and isolate BLHN7.

3.3. Evolution of Tannin Content During Fermentation of Raw and Cooked BBP

The evolution of tannin content during fermentation of raw and cooked BBP by *Lactobacillus acidophillus* and BLHN7 isolate is shown in figure 4. It is observed that the lactic acid bacteria reduced the tannin content in the BBP during fermentation. However, the amount of tannin reduced varied

from strain. After 120 hours of fermentation, there was a reduction in tannin content from 0.67 to 0.16% in raw BBP and from 0.41 to 0.08% in cooked BBP when fermented with *Lactobacillus acidophillus*. This reduction was also observed when the BBP were fermented with BLHN7 isolate, this reduction ranging from 0.78 to 0.12% for raw BBP and from 0.45 to 0.12% for cooked BBP after 120 hours.

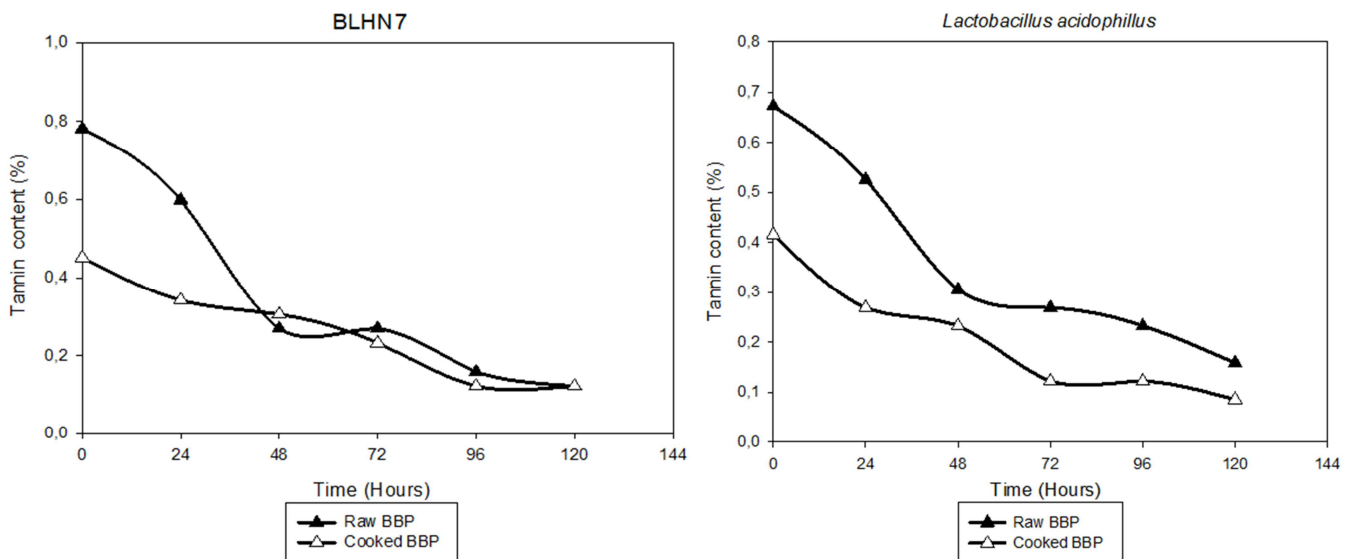


Figure 4. Evolution of tannin content during fermentation of raw and cooked BBP by *Lactobacillus acidophillus* and isolate BLHN7.

3.4. Evolution of Phytate Content During Fermentation Raw and Cooked BBP

A good reduction of phytates is noted after 120 hours of fermentation with the strains (figure 5). This reduction in phytate content went from 3.25 to 3.16% for raw BBP and

from 3.23 to 3.11% for cooked BBP with the *Lactobacillus acidophilus* strain. The BLHN7 isolate showed a reduction in phytates ranging from 3.24 to 3.14% for raw BBP and from 3.25 to 3.20% for cooked BBP.

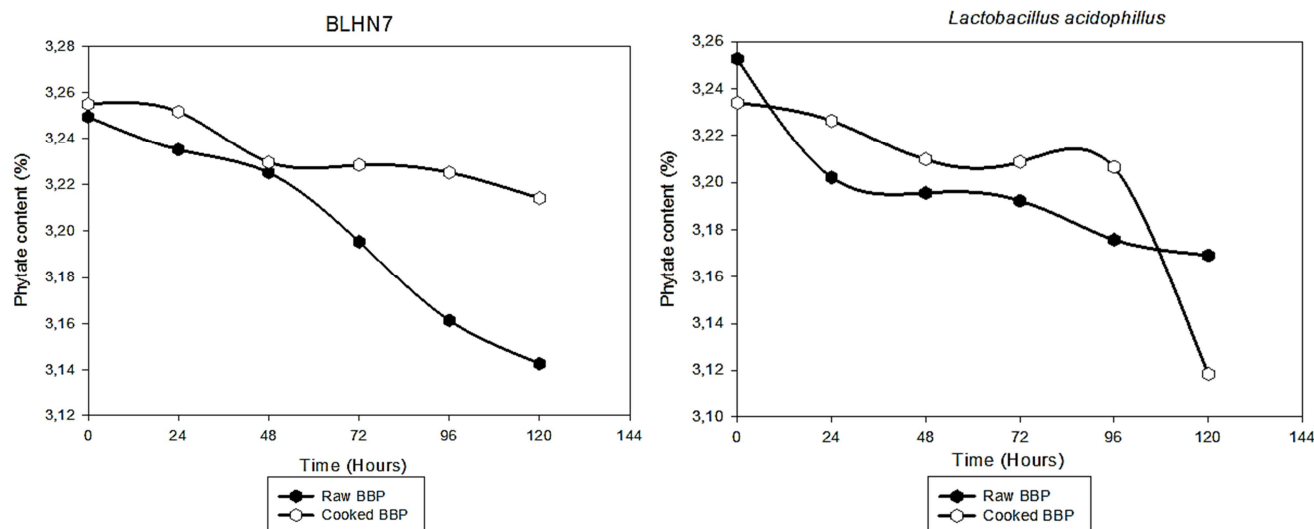


Figure 5. Evolution of phytate content during fermentation raw and cooked BBP by *Lactobacillus acidophilus* and isolate BLHN7.

3.5. Evolution of Protein Content During Fermentation of Raw and Cooked BBP

The evolution of protein content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and isolate BLHN7 is shown in figure 6. From this figure, it appears that

the protein content increases after 120 hours of fermentation. It increases from 13.00 to 47.74 g/100g for the raw BBP and from 10.00 to 30.38 g/100g for the cooked BBP fermented with *Lactobacillus acidophilus*. While it increases from 15.73 to 45.03 g/100g for raw BBP and from 13.56 to 31.68 g/100g for cooked BBP fermented with isolate BLHN7.

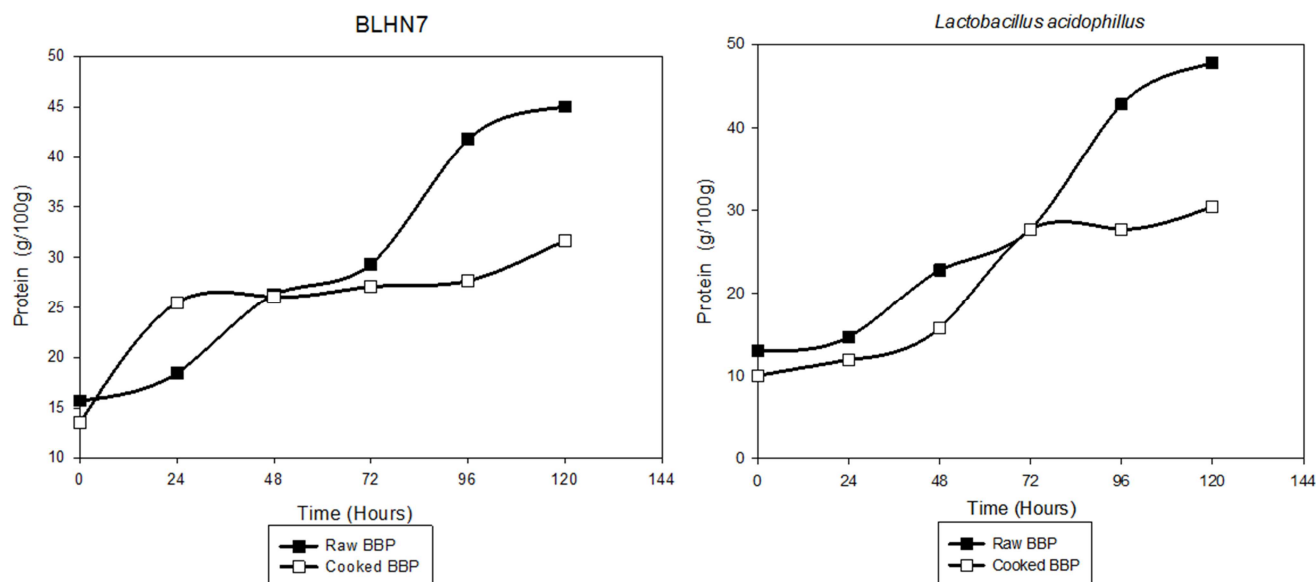


Figure 6. Evolution of protein content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and isolate BLHN7.

3.6. Evolution of Calcium Content During Fermentation of Raw and Cooked BBP

During fermentation, there was an increase in calcium

content in BBP after 120 hours of fermentation. The calcium content varied from 185 to 453 mg/100g and from 195 to 418 mg/100gMS respectively in raw and cooked BBP fermented with *Lactobacillus acidophilus*, which is an increase of 145

and 114%. This increase is also observed in raw and cooked BBP fermented with isolate BLHN7. We observe respectively a variation from 202.5 to 438.78 mg/100g and from 168 to 446 mg/100g, which is an increase of 116 and

114%. The figure 7 shows the evolution of calcium content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and isolate BLHN7.

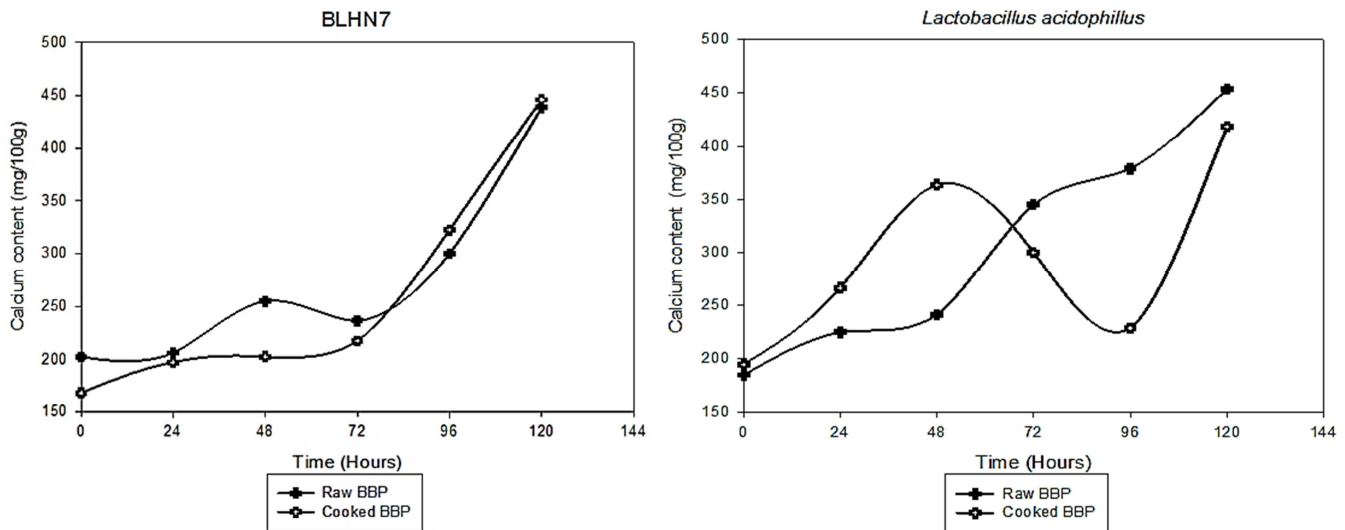


Figure 7. Evolution of calcium content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and isolate BLHN7.

3.7. Evolution of Magnesium Content During Fermentation of Raw and Cooked BBP

The magnesium content increased by 202 and 180% respectively in raw and cooked BBP after 120 hours of fermentation with *Lactobacillus acidophilus*. This increase

in magnesium was also observed during fermentation of raw and cooked BBP with isolate BLHN7. An increase of 175% was recorded in the raw BBP and 143% in the cooked BBP. The figure 8 shows the evolution of magnesium during the fermentation of raw and cooked BBP with *Lactobacillus acidophilus* and isolate BLHN7.

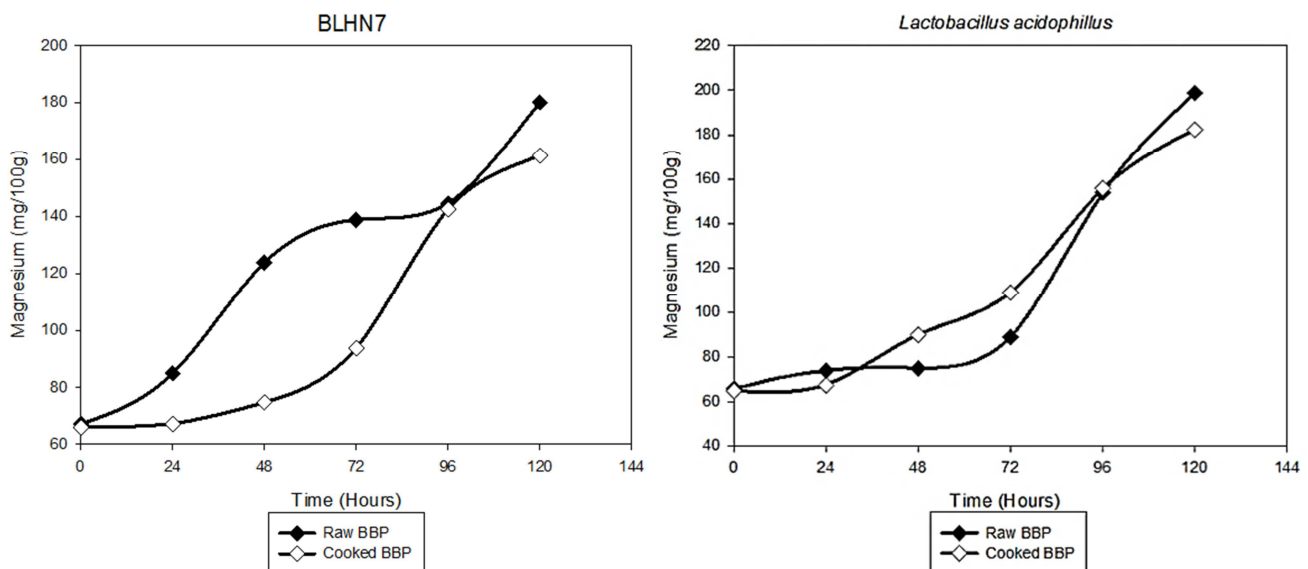


Figure 8. Evolution of magnesium content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and BLHN7 isolate.

3.8. Evolution of Iron Content During Fermentation of Raw and Cooked BBP

The increase in iron content during fermentation is generally observed. After 120 hours of fermentation of BBP with *Lactobacillus acidophilus*, an increase of iron content

from 1.21 to 4.13 mg/100g in raw BBP (241%) and from 1.00 to 2.31 mg/100g for cooked BBP (124%) is noted. The iron content increased from 0.5 to 4.22 mg/100g and from 0.90 to 2.72 mg/100g respectively for raw and cooked BBP fermented with the BLHN7 isolate. This increase was estimated at 730 and 201% respectively. The Figure 9 shows

the evolution of iron content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and BLHN7

isolate.

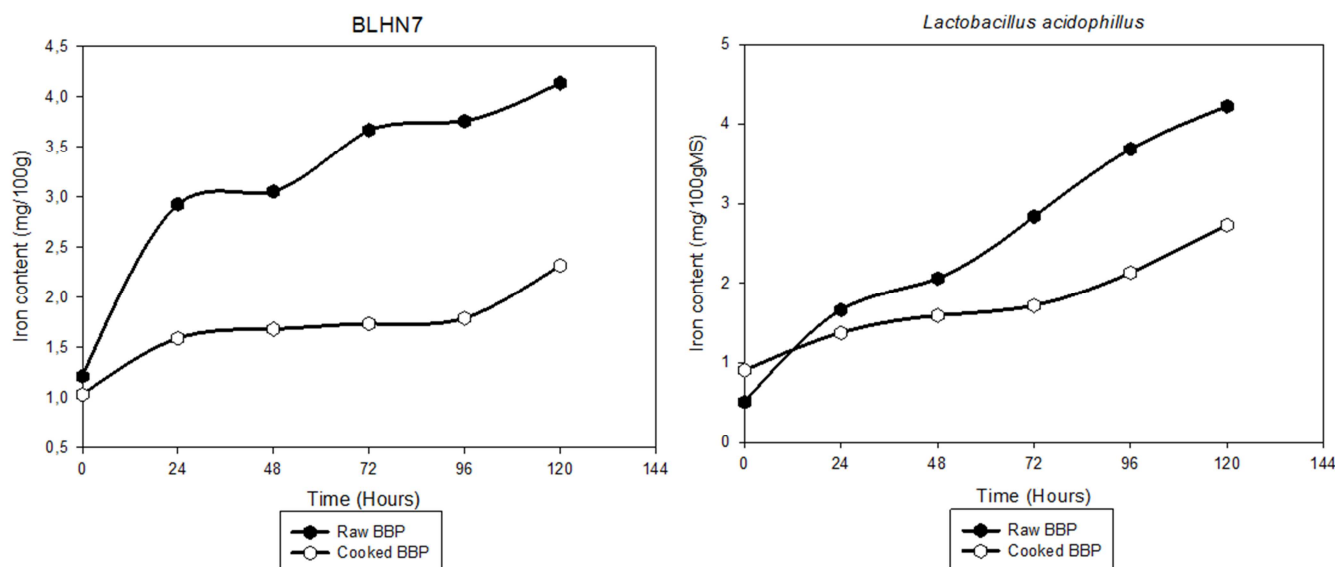


Figure 9. Evolution of iron content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and BLHN7 isolate.

3.9. Evolution of Sugar Content During Fermentation of Raw and Cooked BBP

The figure 10 illustrate the evolution of the total sugar content during the fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and BLHN7 isolate. There is a

reduction of 20 and 35% respectively in raw and cooked BBP fermented with *Lactobacillus acidophilus*. In the case of raw and cooked BBP fermented with isolate BLHN7, a reduction of 25 and 40% of the total sugar content is recorded respectively.

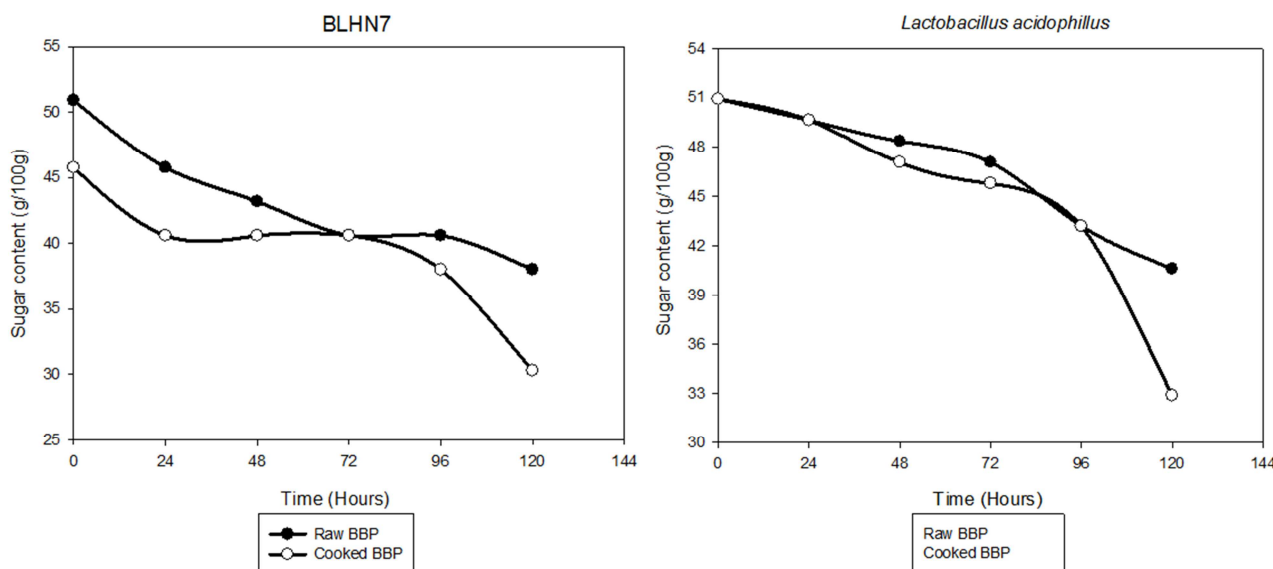


Figure 10. Evolution of total sugar content during fermentation of raw and cooked BBP by *Lactobacillus acidophilus* and isolate BLHN7.

3.10. Principal Component Analysis (PCA)

Given the variability of the results observed when studying the influence of *Lactobacillus acidophilus* and isolate BLHN7 on the nutritional parameters of BBP during fermentation, a PCA was performed to determine which of these lactic acid bacteria had the best influence on these

nutritional parameters. The figure 11 shows the projection of the variables and observations on the F1 (52.29%) and F2 (34.26%) axes. Among the observations studied, cooked and raw BBP fermented with *Lactobacillus acidophilus* contribute the most to the formation of the F1 axis by 36 and 30% respectively. While the cooked BBP fermented with *Lactobacillus acidophilus* and BLHN7 isolate contribute the

majority to the formation of the F2 axis by 37.9 and 60% respectively. Through this projection, we see that the best increase in protein (267%), iron (240%) and microbial growth (118%) was recorded in the raw BBP fermented with *Lactobacillus acidophilus*, with percentages of contribution of 19, 18 and 19% respectively. Under the same conditions, a reduction in tannins (76%) and a slight reduction in sugar content (20%) were also noted, with percentages of contribution to the F1 axis of 16 and 19% respectively. On the other hand, phytates were reduced the most in cooked BBP (3.4%) and magnesium increased by 180%. However, in the cooked BBP fermented with BLHN7 isolate we observe an increase of 165% of calcium with a percentage contribution to the F2 axis of 23%.

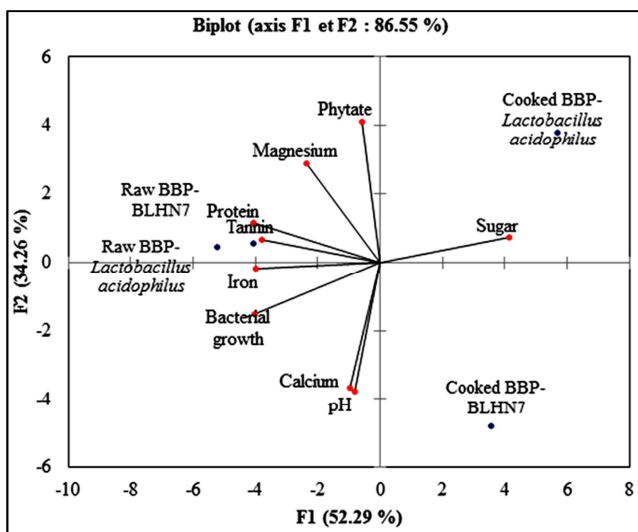


Figure 11. Projection of variables and observations on the factorial plane (F1x F2).

4. Discussion

Fermentation plays a crucial role in food processing and can be used to improve the nutritional quality of food product. During the fermentation of raw and cooked BBP, an increase of *Lactobacillus acidophilus* and isolate BLHN7 was observed. This microbial increase could be explained by the rich composition in nutrient of BBP, which is essential for the growth of lactic acid bacteria [22]. This bacterial multiplication was also accompanied by a decrease in pH in the raw and cooked BBP during fermentation. According to Jabłońska-Ryś et al. [23] the decrease in pH is strongly related to the production of organic acids in the medium. Similarly, the work of Kitum et al. [24] observed that the pH decreases from 6.06 to 3.9 in the red bean during fermentation.

Regarding anti-nutritional compounds, a decrease of tannins and phytates was observed during the fermentation of BBP by *Lactobacillus acidophilus* and isolate BLHN7. This decrease in phytates would be due to phytases and phosphatases produced by lactic acid bacteria that hydrolyze phytates, seninositol and orthophosphatates during fermentation [25]. The work of Tchikoua et al. [26] noted a

reduction in phytate content from 278.7 to 12.4 mg/100gMS (95.5%) and a reduction in total tannin content from 215.1 to 2.5 mg/100gMS (98.8%) in corn pasta fermented for 120 hours with the *Lactobacillus buchneri* M11 and *Lactobacillus plantarum* A6, respectively. The work of Kitum et al. [24] also noted a reduction in tannin content from 306.8 to 109.5mg/100g and a reduction in phytate content from 387.2 to 242.52 mg/100g during red bean fermentation. Beyond the reduction of tannins and phytates by these 02 lactic starters, it was also found that the reduction of tannin content in the cooked BBP was more pronounced than in the raw BBP. This could be explained, in the case of cooked BBP, by the effect of heat during cooking which could inactivate anti-nutritional factors such as phytates, tannins, oxalates and also increases the availability of some amino acids such as lysine [27].

The results obtained also show an increase in total protein content after 120 hours of fermentation. This finding corroborates with the work of Opere et al. [28] who observed an increase in protein content from 285.2 to 678.3 mg/100g during the production of *Ogi* (fermented cereal porridge) after 72 hours of fermentation by *Lactobacillus acidophilus*. This increase could be explained on the one hand by the increase in the molecular weight of the microorganisms, which in itself constitutes a large source of protein, and on the other hand by the production of extracellular enzymes (protease) by the fermenting microorganisms promoting an increase in nitrogen in the medium [24, 29].

Fermentation shows an increase in iron, magnesium, and calcium in BBP. The increase in these minerals may be due to the reduction of phytic acids and tannic acids which have the ability to complex cations thus increasing mineral availability [30]. The work of Abdalbasit et al. [31] showed an increase from 3.03 to 5.10 mg/100 in calcium and 7.56 to 32.67 mg/100g in iron during millet fermentation.

The decrease of total sugars can be explained by their uses for growth needs, for the production of lactic acid and to perform bioconversions well beyond the cell multiplication phase [22, 32].

5. Conclusion

The objective of this work was to evaluate the influence of *Lactobacillus acidophilus* and isolate BLHN7 on pH, anti-nutritional compounds, total sugars, proteins and mineral content after 120 hours of fermentation. After PCA, it was found that unlike isolate BLHN7, *Lactobacillus acidophilus* slightly reduces the total sugar content, increases the protein content better during the fermentation of raw BBP and reduces anti-nutritional compounds such as phytates in cooked BBP. In view of these results, *Lactobacillus acidophilus* can be used for the fermentation of BBP to increase the protein and mineral value, which are very necessary for the growth of children.

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