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Effect of spontaneous fermentation time on physicochemical, nutrient, anti-nutrient and microbiological composition of Lima Bean (*Phaseolus lunatus*) flour

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ABSTRACT

Objective: Lima bean (*Phaseolus lunatus)* is a protein-rich legume species, which the consumption causes a problem of digestion due to the high content of anti-nutritional factors. Thus, the objective of this study is to improve the nutritional quality of lima bean by means of fermentation.

Methodology and results: The impact of spontaneous fermentation time on lima bean flour proximate composition, antinutrient factors and microbiological composition was evaluated. Lactic acid bacteria counts were the most abundant and their evolution was increased from 0 to 24 hours (3.66 to 10.34 log CFU/mL) and remained stable until 72 hours. A significant increase was observed in yeast and mould counts (2.89 to 6.61 log CFU/mL), while *Bacillus* counts remained stable. Furthermore, there was a significant reduction ($P < 0.05$) of anti-nutritional factors such as tannins, phytate and oxalate during fermentation. Finally, lipids, fibres, ash, proteins and sugar content decreased significantly ($P< 0.05$) unlike carbohydrate content.

Conclusion and Application of results: Spontaneous fermentation improved the nutritional quality of Lima bean flour. Fermented lima bean flour could be used in the formulation of instant infant meal and animal feed.

Keywords: spontaneous fermentation, physicochemical, nutrient, anti-nutrition factors, fermentative microorganisms.

INTRODUCTION

Legumes are flowering plants that produce pods (Schrire *et al*., 2005). They belong to the Fabaceae family. The Fabaceae family is the third most populated family of flowering plants (behind Asteraceae and Orchidaceae) with 670 to 750 genera and 18,000 to 19,000 species (Lewis *et al*., 2005). Legumes play an important role in the nutrition of many people because of their high protein content in the seeds. They are an important source of protein in many developing countries, particularly among the poorest populations, and are rich in essential amino acids such as lysine, which complements the nutritional value of cereal and tuber diets (Graham & Vance, 2003).

The majority of African population is faced with the problem of undernourishment (Yabile, 2011). The consumption of pulses appears to be an alternative to this problem (FAO, 1988). Of these legumes, *Phaseolus lunatus* L. is a bean species that belongs to the family Fabaceae. This bean species is very rich in phytochemicals and proteins. The protein richness of *Phaseolus lunatus* L. may also offset the increased protein deficiency in Africa given the high price of animal protein (Young & Pelett, 1994). However, the consumption of these legumes poses a problem of digestion and bioavailability of certain nutrients due to the high content of anti-nutritional factors (Fadahunsi, 2009). Lima bean seeds contain anti-nutritional factors such as hydrogen cyanide, phytic acids, saponin, oxalate, tannin, trypsin inhibitor activity, haemagglutinin activity (Ezeagu & Ibegbu, 2010).These anti-nutritional factors have been observed to inhibit absorption of nutrients and their subsequent utilization and assimilation by animals. Besides, they cause some level of damages to some organs such as liver, kidney and spleen (Emiola *et al*., 2007). It seems necessary to find ways to reduce this problem in order to make their consumption advantageous.

Fermentation is the traditional means and remains the main and most affordable way of preserving food in many places. It is an interesting process in a context of sustainable development and improvement of food systems since it produces little effluent and requires little energy to be put in place. Several experiments have demonstrated that fermentation of legumes enhances their nutritive value and antioxidant properties; reduces some anti-nutritional endogenous compounds such as phytic acid, and exerts beneficial effects on protein digestibility and biological value of legumes (Oboh *et al*., 2012; Oyewole &Isah, 2012). In addition, many studies have shown that fermentation can be effectively used to improve the nutritional quality of cereal grains by increasing protein content and digestibility (Inyang & Zakari, 2008; Osman, 2011).

The normal fermentation process is carried out with the endogenous flora. However, beans can contain aerobic microorganisms from air, water, soil, containers. These microorganisms may be more numerous than the fermentative species. Fermentation failures can often be attributed both to the preponderance of undesirable organisms and to the retardation of the growth of desirable organisms. However, the microorganisms responsible for the fermentation of any food are usually present. Fermentations can be carried out by spontaneous fermentation or after addition of starter cultures (Hui *et al*., 2004). The microorganisms involved in natural fermentation are most often lactic acid bacteria and yeasts. By their numerous lytic activities (proteolytic, amylolytic, lipolytic, phytasic, etc.), they are responsible for modifying raw materials. Lactic fermentations allow food to be preserved by inhibiting the development of pathogenic bacteria or spoilage through acidification and the production of bacteriocins. However, the recognized importance of lactic acid bacteria and yeasts

should not minimize the role of fungi and species of the genus *Bacillus* in the production of fermented foods or condiments. Fungi are responsible for the fermentation of soybeans to produce *tempeh* and *sufu* (Han *et al*., 2001; Kiers *et al*., 2000), two widely consumed Asian foods, while different *Bacillus* species are responsible for the fermentation of carob and soybean seeds to produce *soumbala* and *kinema*, respectively (Ouoba *et al*., 2004; Sarkar *et al*., 2002). The objective of this study is to improve the nutritional quality of lima bean by means of fermentation.

MATERIAL AND METHODS

Simple collection and flour production: The biological material used for this study consisted of mature seeds of the black red-spotted cultivar of *Phaseolus lunatus* (L.) (Figure 1). A producer in the villages of Assoumoukro (M'batto) and N'guessankro (Bongouanou), the central-eastern park of Côte d'Ivoire, supplied it. The dried bean seed samples were cleaned and sorted according to size and absence of extraneous or abnormal odours and living or dead insects. The bean samples were finally ground in a suitable analytical mill and sieved through a 0.5 mm mesh sieve.

Figure1: Mature seeds of the black red-spotted cultivar of *Phaseolus lunatus* (L.)

Fermentation experiments: Suspensions of bean flour in distilled water were prepared a concentration of 1:12 dilution (w/v). The flour slurry was allowed to ferment naturally at room temperature (26 °C) for 24, 48 and 72 h in 3 containers according to the modified method of Dablado *et al*. (2002). The pH was measured daily. After fermentation, part of the samples was used for microbiological analysis and the rest was dehydrated at 55^{o}C for 24 h to stop microbial growth (Fadahunsi, 2009). The dried fermented samples were ground to pass through a 0.5 mm sieve and stored in glass jars for analyses.

pH and titratable acidity determination: The pH was determined by the AOAC principle (1990), according to the potentiometric method, using the electrode of a pH meter (WTW pH 302). The titratable acidity was also determined according to the method of the AOAC (1990). 10g of each crushed sample was diluted in 100 mL of distilled water and the mixture was filtered. Then, 10 mL of each filtrate was taken and titrated with a solution of NaOH (0.1 N) in the presence of phenolphthalein until the turn pink.

Proximate Analysis: The different samples (unfermented and fermented) were analysed for ash, crude fat, crude protein and crude fibre in proportions of 1 g each, by standard methods recommended by AOAC (1990). Carbohydrates were calculated by the difference based on the total composition of

fermented and unfermented flours (Müller & Tobin, 1980).

The total sugars were determined according to the technique described by Dubois *et al*. (1956) using phenol and concentrated sulfuric acid. The ethanosoluble extract (150 µL) was taken and placed in a test tube. Then, 1 mL of phenol (5%, w / v) and 1 mL of concentrated sulfuric acid (97%) were added. The reaction medium was homogenized and left to cool for 5 min. The optical density was read at 490 nm on a spectrophotometer against a control containing all the products except the ethanosoluble extract. The optical density was converted into the amount of total sugars using a standard curve obtained from a glucose solution (1g / L).

The reducing sugars were determined according to the method of Bernfeld (1955) using 3, 5-dinitrosalycilic acid (DNS). A volume of 150 µL of ethanol-soluble extract was taken and placed in a test tube. To this volume, 300 µl of DNS were added. The mixture was brought to a boiling water bath for 5 min. 2 mL of distilled water were added to the reaction medium after cooling for 5 min on the bench. The optical density was read at 540 nm with a spectrophotometer against a control containing all the products except the ethanol-soluble extract. The optical density was converted into the amount of reducing sugars using a standard curve obtained from a glucose solution (1g / L).

Anti-nutritional factors estimation: Hydrogen cyanide was analysed by the AOAC method (1990). Ten (10) g of flour were homogenized in 200 mL of distilled water. The trapped distillate was left to stand for 3 hours and filtered through whatman paper. The filtrate obtained was distilled from 20 mL of sodium hydroxide (0.1 N) and 2 mL of KI (0.02 N). The distillate was titrated with silver nitrate $AgNO_3$ (0.02 N) until a yellowish haze appears.

The tannin content was estimated spectrophotometrically by the procedure described by Baindridge *et al*. (1996). One millilitre of methanolic extract is introduced into a test tube to which 5 mL of vanillin reagent were added. The tube was left to stand for 20 min in the dark and the absorbance was read with a spectrophotometer at 500 nm against a blank. The blank was prepared for each test by adding 5 mL of distilled water to the test tubes replacing the vanillin reagent. The amount of tannins in the sample was determined using a standard range established from a tannic acid solution (2 mg / mL) under the same conditions as the test.

Phytic acid was determined using the procedure described by Latta & Eskin (1980). One gram of flour was homogenized in 20 mL of HCl (0.65). The mixture obtained is stirred for 12 hours at room temperature. The mixture was centrifuged at 3000 trs / min for 40 minutes. To 0.5 mL of supernatant, 3 mL of Wade's reagent were added. The blank was prepared for each sample with 0.5 mL of distilled water in the test tubes without Wade's reagent. The tubes were left to stand for 20 min in the dark and the optical density was read with a spectrophotometer at 490 nm against a blank. The amount of phytate was determined using a standard range established from a sodium phytate solution (10 mg / mL) under the same conditions as the test.

The oxalate was determined using the method of Day & Underwood (1986). Two grams of flour were homogenized in 75 mL of H_2SO_4 (3M). The mixture was stirred magnetically for 1 hour at room temperature. The whole was filtered through Whatman filter paper. Twenty-five millilitres of filtrate were titrated hot with a solution of potassium permanganate ($KMnO_4$, 0.05 M) until the change to persistent pink.

Mineral content analysis: The following minerals: magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), potassium (K) and sodium (Na) were determined using atomic absorption spectrophotometry as described by AOAC

(1990). Nitric acid and hyperchloric acid (6:1) were used for the digestion of the mixture.

Microbiological analysis: The microorganisms in the samples were enumerated by using the standard plate-count method at every 24 h (up to 3 days) of fermentation. Ten grams of fermented samples were diluted with 100 mL of distilled water followed by homogenization. Hundred microliters of appropriate dilutions were spread on the following media: Man Rogosa and Sharpe agar (MRS; Biokar Diagnoses, France) for the lactic acid bacteria, Sabouraud with Chloramphenicol (Biorad, France) for yeasts and molds. For *Bacillus*, counts samples were treated at 80 °C for 10 minutes in order to kill the vegetative forms and spread on plate count agar (PCA; Conda, Madrid, Spain) supplemented with rice starch (1%). The plates for LAB were incubated anaerobically for 48 h at 30°C; those for yeasts and molds and *Bacillus* were incubated for 72 h at 30°C.

Statistical analysis: The analysis of variance (ANOVA) was used to determine the differences between treatments. When a difference was observed, the multiple range test Duncan at 5% was performed to separate treatment means. Statistical tests were performed using the STATISTICA software version 7

RESULTATS

Evolution of pH and titratable acidity: The pH of lima bean flour decreased significantly ($P< 0.05$) during spontaneous fermentation. The pH was 5.99 ± 0.0 for the unfermented four. Then, it decreased until 72 h of fermentation with a value of 4.07 ± 0.0. Unlike pH, the total titratable acidity increased significantly ($P<0.05$) during spontaneous fermentation. It varied from 0 to 72 h with respective values of 12. 93 ± 0.28 meq / 100 g and 44. 61 ± 0.33 meq / 100g (figure 2).

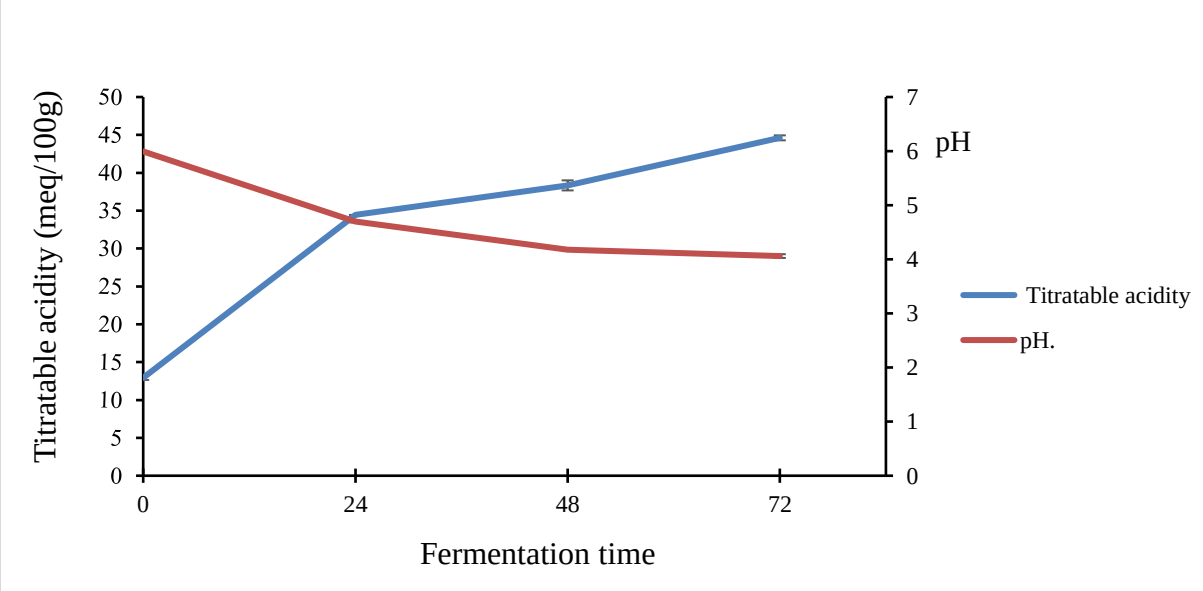

Figure 2: Evolution of pH and titratable acidity of Lima bean four (*Phaseolus lunatus*) during the liquid fermentation

Biochemical composition: The results of this study showed that fermentation had a considerable effect on the nutritional value of *lima bean* four. Table 1 shows the evolution of the protein, lipids, carbohydrate, ash, fibre, reducing sugar and total sugar contents during

the spontaneous fermentation of Lima bean flour. During fermentation, there was no significant reduction in protein contents. Unlike the protein, there was a significant reduction in the lipid contents. Between 0 and 72 hours, the content varied from 1.72 ± 0.04 to 1.25 ± 0.04 mg / 100g respectively. The carbohydrate contents increased gradually and significantly during fermentation. Between 0 and 72 hours, the contents varied respectively from 61.68 ± 0.67 to 67.94 ± 0.66 mg / 100g. During the first 24 hours of fermentation, the variation in the fibre crude content was not significant ($P < 0.05$). After 48 hours, it decreased significantly (4.17 ± 0.29 mg / 100g). The ash content decreased significantly during 72 hours of fermentation with values varying from 4.1 ± 0.10 to 1.88 ± 0.11 mg / 100g. In addition, a significant increase in the content of reducing sugars was observed during the first 24 hours of fermentation. Then, this content decreased significantly until 72 hours. A significant decrease in total sugars was observed during the spontaneous fermentation of lima bean flour.

Table 1: Evolution of biochemical composition of Lima bean flour during the spontaneous fermentation

Fermentation time (h)	**Protein (%)**	**Fat (%)**	**Carbohydrate (%)**	**Fibber crude (%)**	**Ash (%)**	**Reducing sugars (mg/ 100g)**	**Total sugar (g/ 100g)**
0	26.67 ± 0.18^{a}	1.72 ± 0.04^{a}	61.68 ± 0.67^{a}	5.83± 0.58^{a}	4.1 ± 0.10^{a}	33.19 ± 0.27^{a}	4.22 ± 0.06^{a}
24	26.13 ± 0.10^{b}	1.47 ± 0.02^{b}	63.67 ± 0.19^{b}	6.33 ± 0.29^{a}	2.4 ± 0.00^{b}	89.48 ± 0.49^{b}	1.84 ± 0.04^{b}
48	25.93 ± 0.06^{b}	1.34 ± 0.01^{c}	66.46 ± 0.33^{c}	4.17± 0.29^{b}	2.1 ± 0.10^{c}	67.15 ± 0.20^{c}	1.07 ± 0.04^{c}
72	25.43 ± 0.08^{c}	1.25 ± 0.04^{d}	67.94 ± 0.66^{d}	3.5 ± 0.50^{b}	1.88 ± 0.11^{d}	48.10 ± 0.11^{d}	0.89 ± 0.02^{d}

The values are mean ± standard deviation, n = 3; in the columns, the means with different letters indicate a significant difference (P <0.05)

Anti-nutritional factors: The anti-nutritional factors measured during this study were tannin, phytate, oxalate and hydrocyanic acid. Table 2 shows the changes in the levels of these anti-nutritional factors during fermentation. Analysis of the results showed a significant decrease ($P < 0.05$) in all of these factors. Between 0 to 72 hours of fermentation, the phytate content varied from 66.10 ± 0.91 to 51.10 ± 1.50 mg / 100g. The tannin content varied from 71.82 ± 0.56 to 30.78 ± 0.10. That of oxalates varied from 227.33 ± 6.35 to 209 ± 0.00 mg / 100g. Finally, the hydrocyanic acid content varied from 10.45 ± 0.25 to 5.1 ± 0.00mg / 100g.

Table 2: Evolution of anti-nutritional factor contents of Lima bean flour during the spontaneous fermentation

Fermentation time (h)	**Anti-nutritional factor contents (mg/ 100g)**			
	Phytates	**Oxalates**	**Tannins**	**cyanhydric acid**
0	66.10 ± 0.91^{a}	227.33 ± 6.35^{a}	71.82 ± 0.56^{a}	10.45 ± 0.25^{a}
24	58.00 ± 1.20^{b}	220.00 ± 0.00^{b}	44.35 ± 0.56^{b}	9.43 ± 0.25^{b}
48	54.37 ± 1.74^{c}	212.67 ± 3.17^{c}	40.82 ± 0.30^{c}	5.11 ± 0.25^{c}
72	51.10 ± 1.50^{d}	209.00 ± 0.00^{c}	30.78 ± 0.10^{d}	5.10 ± 0.00^{c}

The values are mean ± standard deviation, n = 3; in the columns, the means with different letters indicate a significant difference (P <0.05)

Mineral content: Eight minerals were assayed in this study namely magnesium, calcium, sodium, potassium, phosphorus, zinc, iron and copper. Table 3 shows the evolution of these eight minerals as a function of fermentation time. Analysis of the results showed a significant decrease in the content of Mg, Ca, K, P, Na and Zn after 72 hours of fermentation. For Fe and Cu, a significant decrease was observed only at the first 24 hours. After 24 hours, the variation was not significant.

Table 3: Evolution of mineral contents of Lima bean flour during the spontaneous fermentation

Fermentation time (h)	**Mineral contents (mg/ 100g)**							
	Mg	**P**	**K**	**Ca**	**Na**	**Fe**	**Zn**	**Cu**
0	137.13±2.49^{a}	503.97±2.37^{a}	1425.90±6.44^{a}	101.11± 0.82^{a}	14.74± 0.61^{a}	11.75±0.63^{a}	4.97±0.71^{a}	2.78±0.47^{a}
24	87.64±0.48^{b}	363.92±1.10^{b}	912.64±4.54^{b}	88.32±0.24^{b}	10.21 ± 0.87^{b}	8.88±0.24^{b}	3.76±0.42^{b}	1.44±0.14^{b}
48	68.56±0.63^{c}	310.30±0.85^{c}	742.50±0.95^{c}	85.72±0.74^{c}	8.15 ± 0.43^{c}	8.82±0.36^{b}	1.99±0.21^{c}	1.01±0.32^{b}
72	59.89±0.93^{d}	298.68±0.85^{d}	722.58±0.87^{d}	85.67±0.61^{c}	7.36 ± 0.98d	8.25±0.44^{b}	1.15±0.79^{c}	0.88±0.19^{c}

The values are mean ± standard deviation, n = 3; in the columns, the means with different letters indicate a significant difference (P <0.05)

Evolution of microbial counts: The microorganisms enumerated during the fermentation of Lima bean (*Phaseolus lunatus*) in this study are yeasts and molds, lactic acid bacteria as well as *Bacillus*. Figure 3 illustrates the evolution of their charge over time. During the fermentation, there was a significant increase ($P< 0.05$) in the load of yeasts and molds. Between 0 and 72 h, their load varied respectively from 2.89 ± 0.4 to 6.61 ± 0.95 CFU / mL. Concerning lactic acid bacteria, a significant increase ($P< 0.05$) in their load was observed in the first 24 hours with values varying from 3.67 ± 0.49 to 10.34 ± 0.04 CFU / mL. Then, their charge decreased slightly at 72 hours at a charge of 9.62 ± 0.08 CFU / mL. For *Bacillus*, the variation in their load is not significant ($P < 0.05$) during the fermentation.

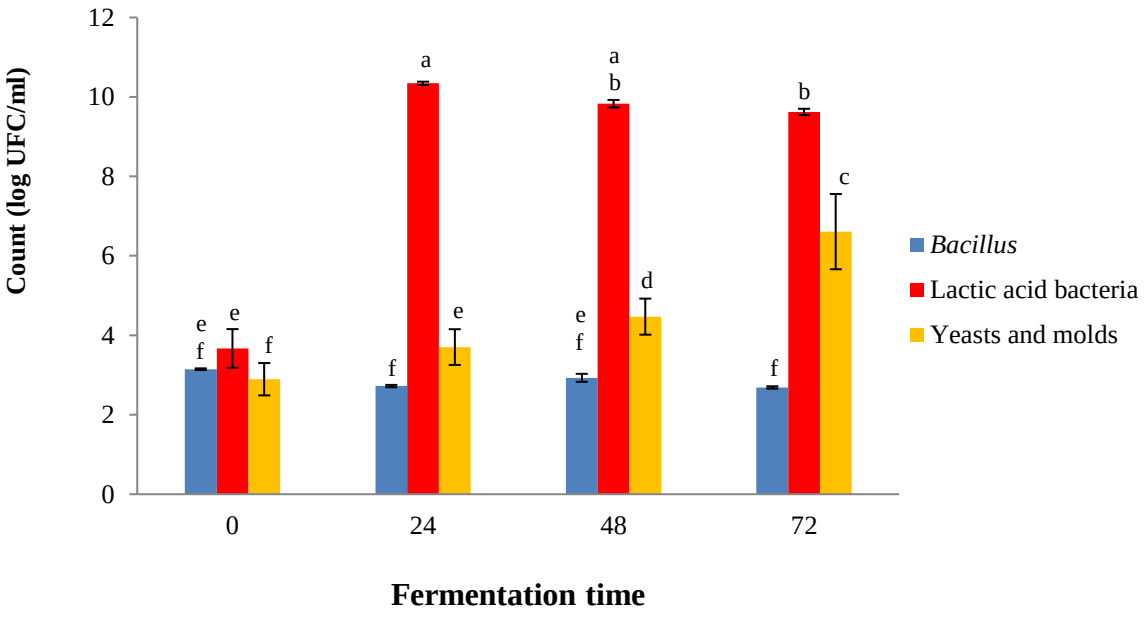

Figure 3: Evolution of *Bacillus*, lactic acid bacteria, yeasts and moulds during the spontaneous fermentation of Lima bean flour

DISCUSSION

The fermentative microorganisms, which were counted in this study, are lactic acid bacteria, yeasts, molds, and Bacillus. During spontaneous or natural fermentation of lima bean, the increase in the yeast and mould loads could be explained by the fact that the environmental conditions were favourable to their growth. The parameters influencing the growth of microorganisms in a food are water activity, pH, temperature and the composition of the food matrix (Blandino *et al.*, 2003). During the fermentation of bean flour, the pH varied from 5.97 ± 0.0 to 4.07 ± 0.0. This variation is included in the optimum pH range for the growth of yeasts and molds which is between 4 to 6.5 for yeasts and 4.5 to 6.8 for molds (Dilbaghi & Sharma, 2007). Yeasts tolerate very wide pH ranges, theoretically 2.4 to 8.6 because their cell envelopes are impermeable to H_3O^+ and OH^- ions (Laouar, 2020). A study had shown that *Candida krusei* could live in the presence of lactic acid because it has a high tolerance to lactic acid (Halm *et al.*, 2004). The increase in the load of lactic acid bacteria is due to the pH of the matrix favourable to their growth. This pH is located in the optimal pH range of lactic acid bacteria, which is between 5.5 to 6.5. As the load of

lactic acid bacteria increases, there is a production of organic acids by them. The production of organic acids leads to a lowering of the pH of the matrix (N'goran-Aw *et al*., 2017). At this pH level, the lactic acid bacteria strains remain viable but their growth is slowed (Zoumpopoulou *et al*., 2008). This would be the cause of the gradual decrease in their load over time. Indeed, the production of organic acid such as lactic acid would be due to the transformation of the carbohydrates contained in the matrix into pyruvate by the phenomenon of glycolysis then into lactic acid by lactase dehydrogenase produced by lactic bacteria (Murphy & Condon, 1984; Piard & Desmazeaud, 1991). At 24 hours of fermentation, the pH was 4.66 ± 0.1. At this pH, the (H +) ions produced by the deprotonation of organic acids block permeases; which changes the permeability of the bacteria membrane. On the other hand, at acidic pH, it is the dissociated form of lactic acid, which enters the cytoplasm of microorganisms. This causes a drop in the cytoplasmic pH of the cells, thereby slowing down their enzymatic metabolism (Cotter & Hill, 2003). The slowing down of the enzyme metabolism leads to a slowing down of the growth of bacteria. The variation in *Bacillus* load during fermentation was not significant ($P < 0.05$). The lactic acid production by bacteria lowered the pH of the matrix and created an unfavourable environment for the growth of Bacilli. Although the pH decreased during fermentation, the number of *Bacillus* was almost constant. In fact, under unfavourable environmental conditions, the Bacilli can take on spore-forming forms in order to resist unfavourable conditions (Nicholson *et al*., 2000). This resistance of spores is due to their particular and compartmentalized structure (Atrih & Foster, 2002). The spore is a particularly poorly permeable structure with low mobility of its structures (Cowan *et al*., 2004; Griffiths & Setlow, 2009). This permeability is also very useful for the spore because it allows it to maintain the dehydrated state of the protoplast and therefore its dormant state. This state of dormancy could be at the base of the weak variation of the load of the bacilli during the fermentation. These results show that lactic acid bacteria, yeasts and molds are responsible for the spontaneous fermentation of lima bean flour. The importance of Bacilli should not be minimized as they are used in the production of fermented foods or condiments with important biological properties (Kiers *et al*., 2000; Tamang *et al*., 2016). Spontaneous fermentation had a significant ($P < 0.05$) effect on the nutritional value of lima bean. The reduction in the protein content of the four could be due to an increase in the catabolism of matrix proteins by microorganisms during fermentation. Indeed, bacteria need nitrogenous substances to synthesize their proteins necessary for their growths. Some microorganisms have the capacity to fix atmospheric nitrogen and others do not have such as lactic bacteria (Loubière *et al*., 1996). During their growths, microorganisms secrete proteases, which will hydrolyse proteins into amino acids. Then, after the amino acid *deamination* reactions by deaminases, the microorganisms will assimilate nitrogen. This could cause the nitrogen level in the matrix to drop. These results are similar to those of Assohoun *et al*. (2013) who observed a reduction in the protein contents in fermented corn, a condiment used in the production of *doklu*. Lima bean is not rich in lipids like most species of the genus *Phaseolus*, unlike oilseeds like soybeans, which happen to be rich in proteins and fats. The reduction in the lipid contents during fermentation could be due to the hydrolysis of lipids by lipases produced by microorganisms during fermentation. During the fermentation of bean seed flour, the fibre content decreased significantly between 24 hours and 48 hours. This could be due to the secretion of extracellular enzymes such as ligninases, cellulases and hemicellulases by fungi (Oboh

& Elusiyan, 2007). The presence of dietary fibre in a food can significantly reduce the availability of mineral elements and proteins because of their chelating effect (Frølich, 1990; Adams *et al.*, 2018). Reducing fibre content could significantly increase the availability of proteins and minerals and improve the nutritional quality of the food. The carbohydrates content increased significantly during fermentation. This increase could be due to the significant decrease in other macronutrients that the microorganisms were able to consume during their growth. Assohoun *et al*. (2013) observed a reduction in the carbohydrates content during the fermentation of corn while Gabriel & Akharaiyi (2007) observed an increase in the carbohydrates content during the spontaneous fermentation of the seeds of *Canavalia ensiformis* (L.) having undergone a heat treatment. The rate of ash decreased in lima bean flour during fermentation. Ojokoh (2009) had shown that during the preparation of ogi, there was a decrease in the ash rate during the spontaneous fermentation of corn, an ingredient in the preparation of ogi. Difo *et al*. (2014) also observed a reduction in the ash rate during the spontaneous fermentation of *Vigna unguiculata* flour. The total sugar content dropped significantly ($P< 0.05$) during fermentation. The reduction in soluble sugar content is believed to be due to their use as an energy source by microorganisms. For their metabolism, microorganisms can secrete enzymes that hydrolyse soluble sugars. During the reduction of soluble sugars, there could be a significant reduction ($P> 0.05$) in flatulence oligosaccharides. These results are similar to those of Assohoun *et al*. (2013) who observed a significant reduction ($P< 0.05$) in the total sugar level during the fermentation of corn for the production of doklu. The increase in the content of reducing sugars during the first 24 hours could be due to the hydrolysis of starch by amylases, oligosaccharides, cellulose by cellulase produced by microorganisms. The reduction in the reducing sugar content after 24h could be due to the use of these as a carbon source by microorganisms during their metabolism. Cuellar-Álvarez *et al*. (2017) observed a significant increase ($P < 0.05$) in the content of reducing sugars during the spontaneous fermentation of the seeds of *Theobroma grandiflorum* (Willd. Ex Spreng.) K.Schum. During the fermentation of lima bean flour, there was a significant reduction in the tannin content. These results are similar to those of Murwan & Ali. (2011). They observed a reduction during spontaneous fermentation in the tannin content of two sorghum cultivars in Sudan. These were the cultivars Dabar and Tabat. Lewis & Starkey (1968) indicated that the reduction in tannin content in sorghum cultivars could be attributed to the activities of microorganisms during fermentation. During the activities of microorganisms, there could be a secretion of tannases, which hydrolyze the tannins. The seeds of lima bean were rich in proteins. The significant reduction in tannins contributes to improving the nutritional quality of flour from lima bean by increasing the bioavailability of proteins. Phytic acid and phytate are widely distributed in plant seeds (including cereals), roots and tubers (Graf, 1986; Lasztity & Lasztity, 1990) and legumes (Wcislo & Szarlej-Wcislo, 2014). The phytate molecule is negatively charged at physiological pH and would bind to nutritionally essential divalent cations such as Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+}, etc., and forms insoluble complexes, rendering thus the minerals unavailable for absorption (Bohn *et al*., 2008; Nielsen *et al*., 2013). It also forms complexes with proteins and starch and inhibits their digestion (Oatway *et al*., 2001). Dephosphorylation of phytate is a prerequisite for improving nutritional value, since the elimination of phosphate groups from the inositol cycle decreases the mineral bonding strength of phytate. Dephosphorylation of phytate is catalyzed by phytase (Cowieson *et al*., 2016). During the fermentation of

Phaseolus lunatus (L.) flour, there was a significant and gradual reduction in the phytate content. These results agreed with those of Sokrab *et al*. (2012). They observed a reduction in the phytate content during the spontaneous fermentation of two genotypes of maize Var-113 (high phytate) and TL98B-6225-9 × TL617 (low phytate). Oxalate is present in many types of edible plants with varying concentrations **(**Noonan & Savage, 1999; Radek & Savage, 2008). In the plant, oxalate is in the soluble and insoluble form which bind the Na^{+} or K^{+} ions and the Ca^{2+} and Mg^{2+} ions respectively, thereby reducing their availability (Savage & Vanhanen, 2018). During the fermentation of *Phaseolus lunatus* (L.) bean flour, the oxalate content decreased gradually and considerably. These results are similar to those of Oke & Bolarinwa (2012) who observed a decrease in the oxalate content of taro flour (*Colocasia esculenta*) during its spontaneous fermentation. The marked reduction observed due to fermentation may be due to the effect of leaching and enzymatic hydrolysis / acid hydrolysis of the starch granule during fermentation. During the fermentation of lima bean flour, there was a significant reduction in the content of cyhanidric acid. Kobawila *et al*. (2005) observed decrease in cyhanidric acid content during spontaneous cassava fermentation. The body uses mineral ions as electrolytes for the regulation, distribution, composition and acidity of its fluids. Magnesium is used as a cofactor in more than 300 enzyme systems and in membrane stabilization. In addition, its major role lies in regulating the transmission of nerve impulses in the nervous system and in all the organs in which it is projected (Al Alawi *et al*., 2018). The spontaneous fermentation of the flour from these seeds caused a significant reduction in the magnesium content. Potassium is the mineral best represented in the cell where it regulates water movements and plays a role in the transmission of nervous current and muscle contraction (Udensi & Tchounwou, 2017). Moreover, spontaneous fermentation of the flour caused a reduction in the calcium content, which passes from 101.11 ± 0.82 mg / 100 g to 85.67 ± 0.61 mg / 100g after 72 h of fermentation. This result is contrary to the conclusion of Oyewole & Odunfa (1990) that fermentation releases bound calcium. Fermentation of the flour caused a reduction in the potassium content which ranging 1425.9 ± 6.44 to 722.58 ± 0.87 after 72 hours of fermentation. However, the bioavailability of potassium make fermented flour a good source of potassium. Phosphorus plays a metabolic role in the production of energy released by sugars and amino acids, and with calcium ensures the rigidity of the skeleton (De Paula & Rosen, 2013). Spontaneous fermentation of the flour from these seeds caused a reduction in the phosphorus content from 503.97 ± 2.37 to 298.68 ± 0.85 after 72 hours of fermentation. However, the bioavailability of phosphorus would make fermented flour from the seeds of lima bean, a good source of phosphorus whose needs are estimated at 460 mg / d from 1 to 3 years and 700 mg / d for adults (Johnson *et al*., 2006). Sodium is essential for the conduction of nerve impulses and an essential factor in hydroelectric balance. Its absorption is stimulated by glucose and amino acids in the digestive tract. A severe restriction causes an impairment of the functions of the nervous system, even growth retardation in children. Muscle exercise can cause significant sodium losses (Soetan *et al*., 2010 ; Sakuyama *et al*., 2016). Despite the reduction of this mineral during fermentation, the significant reduction in tannins and phytate resulted in bioavailability of this. Iron, zinc and copper are essential trace elements **(**Picaud, 2017). Iron, a central component of haemoglobin, it participates among other things, as a redox cofactor, as well in the transport of electrons in the mitochondria as in the metabolism of catecholamines and in the synthesis of DNA (Xu *et al*., 2013; Paul *et al*., 2017). Zinc plays,

in particular, an essential role in all stages of protein synthesis, in the activation of ribonucleic acids (RNA) and DNA polymerase, in the synthesis of prostaglandins and has an antioxidant function by its structural position in superoxide dismutase, independent copper-zinc (Chanmugam, 1984; MacDonald, 2000; Prashanth *et al*., 2015). Copper is involved in bone mineralization, the regulation of neurotransmitters, immunity, iron metabolism, oxidative glucose metabolism, and essential in particular for the functioning of the myocardium, and the elimination of free radicals through the functioning of the superoxide dismutase (Pham-Huy, 2008; Pepa & Brandi, 2016). Fermentation caused a reduction in the iron, zinc and copper content, which went from 11.75 ± 0.63 to 8.25 ± 0.44, 4.97 ± 0.71 to 1.15 ± 0,79, 2.78 ± 0.47 to 0.88 ± 0.19 respectively, after 72 hours of fermentation. Okwu & Aluwuo (2008) reported a reduction in the iron, zinc and copper content of African oil seed beans (*Pentaclethra macrophylla Benth*) during fermentation. Despite the reduction of these minerals, the significant reduction in tannins and phytate resulted in bioavailability of this.

CONCLUSION

Lima bean is a legume rich in protein, iron, copper and magnesium but in anti-nutritional factors. During fermentation, a significant reduction in anti-nutritional factors such as tannins, phytate, cyhanidric acid and oxalate was observed. This reduction has a positive impact on the nutritional quality of lima bean because it leads to the bioavailability of proteins and minerals. Thus, fermented lima bean flour could be used in the formulation of instant infant flour and animal feed.

REFERENCES

Adams S, Sello CT, Qin G-X, Che D, Han R, 2018. Does Dietary Fibber Affect the Levels of Nutritional Components after Feed Formulation? Fibers 6: 29.

Al Alawi AM, Majoni SW, Falhammar H, 2018. Magnesium and Human Health: Perspectives and Research Directions. International Journal of Endocrinology 17p

AOAC, 1990. Official Methods of Analysis Washington, *DC:* Association of Official Analytical Chemists.

Assohoun MCN, Djeni TN, Koussémon-Camara M, Brou K, 2013. Effect of fermentation process on nutritional composition and aflatoxins concentration of *Doklu*, a fermented maize based food. Food and Nutrition Sciences 4:1120 – 1127.

Atrih A et Foster *SJ*, 2002. Bacterial Endospores the Ultimate Survivors. International Dairy Journal 12: 217-223.

Bainbridge Z, Tomlins K, Westby A, 1996. Analysis of condensed tannins using acidified vanillin. Journal Food Science Agriculture 29:77 – 79.

Bernfeld P, 1955. Amylase α and β methods in enzymology 1. S. P. colowick and N.O. K, edition academic press, Inc New-York, pp. 149-154.

Blandino A, Al-Aseeri ME, Pandiella SS, Cantero D, Webb C, 2003. Cereal-based fermented foods and beverages. Food Research International 36: 527–543.

Chanmugam P, Wheeler C, Hwang DH, 1984.The effect of zinc deficiency on prostaglandin synthesis in rat testes. The journal of Nutrition 114 (11): 2066-2072

Cotter PD et Hill C, 2003. Surviving the Acid Test: Responses of Gram-Positive

Bacteria to Low pH. Microbiology and Molecular Biology Reviews 67(3): 429- 453.

Cowieson AJ, Ruckebusch JP, Knap I, Guggenbuhl P, Fru-Nji F, 2016. Phytate-free nutrition: A new paradigm in monogastric animal production. Animal Feed Science and Technology 222: 180–189.

Cowan N, Chen Z, Rouder JN, 2004. Constant capacity in an immediate serial-recall task: a logical sequel to Miller (1956). Psychological Science 15: 634 - 640.

Cuellar-Álvarez L, Cuellar-Álvarez N, Galeano-García P, Suárez-Salazar JC, 2017. Effect of fermentation time on phenolic content and antioxidant potential in Cupuassu (*Theobroma grandiflorum* (Willd. Ex Spreng.) K.Schum.) beans. Agroindustry and Food Science 66(4): 473 – 479.

Day RA et Underwood AL, 1986. Quantitive analysis 5th ed. Prentice. Hall publication. 701p.

De Paula FJA, Rosen CJ, 2013. Bone Remodeling and Energy Metabolism: New Perspectives. Bone Research 1: 72–84.

Difo HV, Onyike E, Ameh DA, Ndidi US, Njoku GC, 2014. Chemical Changes during Open and Controlled Fermentation of Cowpea (*Vigna unguiculata*) Flour. International Journal of Food Nutrition and Safety 5(1): 1-10.

Dilbaghi N et Sharma S, 2007. Food Spoilage, Food Infection and Intoxications Caused by Microorganisms and Methods for their Detection. 1 - 20.

Doblado R, Frias J, Munoz R, Vidal-Valverde C, 2002. Anti-nutritional factors content of dry beans (*Phasealus vulgaris*) as affected by fermentation. Polish Journal of Food and Nutrition Sciences 11(52): 73 - 75.

Dubois M, Gilles KA, Hamilton JD, Rebers RA, Smith M, 1956. Colometric method for determination of sugar and related substance. Analytical Chemistry 28: 350 - 356.

Emiola IA, Ologhobo AD, Gous RM, 2007. Performance and histological responses of internal organs of broiler chickens fed raw, dehulled, and aqueous and dry-heated kidney bean meals. Poultry Science 86:1234 - 1240.

Ezeagu EI et Ibegbu MD, 2010. Biochemical composition and nutritional potential of ukpa: a variety of tropical Lima beans (*Phaseolus lunatus*) from Nigeria. Polish Journal Food and Nutrition Sciences 60(3): 231 - 235.

Fadahunsi IF, 2009. The effect of soaking, boiling and fermentation with *Rhizopus oligosporus* on the water soluble vitamin content of Bambara groundnut. Pakistan Journal Nutrition 8(6):835 - 840.

FAO, 1988. Traditional food plants. Food and Nutrition Paper 42: 1-3

Frølich W, 1990. Chelating Properties of Dietary Fibber and Phytate. The Role for Mineral Availability. New Developments in Dietary Fibber 83–93.

Gabriel RAO et Akharaiyi FC, 2007. Effect of Spontaneous Fermentation on the Chemical Composition of Thermally Treated Jack Beans (*Canavalia ensiformis* L.). International Journal of Biological Chemistry 1(2): 91 – 97.

Graf E, 1989. Phytic Acid–Chemistry and Applications, Pilatus Press, Minneapolis, USA. Cited from: Hidvegi M. and R. Lasztity (2002)**.** Phytic acid content of cereals and legumes and interaction with proteins. Periodica Polytechnica Chemical Engineering 46: 59 - 64.

Graham PH et Vance CP, 2003. Legumes: Importance and Constraints to Greater

Use. Plant Physiology 131(3): 872–877.

Griffiths KK et Setlow P, 2009. Effects of modification of membrane lipid composition on *Bacillus subtilis* sporulation and spore properties. Journal of Applied Microbiology 106(6): 2064 - 2078.

Halm M, Hornbæk T, Arneborg N, Sefa-Dedeh S, Jespersen L, 2004. Lactic acid tolerance determined by measurement of intracellular pH of single cells of *Candida krusei* and *Saccharomyces cerevisiae* isolated from fermented maize dough. International Journal of Food Microbiology 94(1): 97 - 103.

Han BZ, Beumer RR, Rombouts FM, Nout MJR, 2001. Microbiological safety and quality of commercial sufu- a Chinese fermented soybean food. Food Control 12: 541- 547.

Hui YH, Goddik LM, Hansen AS, Josephen J, Nip WK, Stanfield PS, 2004. Handbook of Food and Beverage Fermentation Technology, 1st edn. Marcel Dekker, New York, pp. 23–50.

Inyang CU et Zakari UM, 2008. Effect of Germination and Fermentation of Pearl Millet on Proximate, Chemical and Sensory Properties of Instant "Fura"- A Nigerian Cereal Food. Pakistan Journal of Nutrition **7**(1): 9-12

Johnson LR, Barrett KE, Ghishan FK, Merchant JL, 2006. Physiology of the Gastrointestinal Tract, Volume 1-2, 4th Edition, 2080 p.

Kiers JL, Van laeken AEA, Rombouts FM, Nout MJR, 2000. In vitro digestibility of *Bacillus* fermented soya bean. International Journal of Food Microbiology 60: 163 – 169.

Kobawila SC, Louembe D, Keleke S, Hounhouigan J, Gamba C, 2005. Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. African Journal of Biotechnology 4(7): 689 – 696.

Lasztity R et Lasztity LJ, 1990. Phytic acid in Cereal Technology, In: Advances in Cereal Science and Technology, Pomeranz, Y., Ed., AACC Publ., and St. Paul, MN. USA, Chapter 5. pp. 10-23.

Latta M et Eskin M, 1980. A simple method for phytate determination. Journal Agriculture and Food Chemistry 67: 1313 – 1315.

Lewis AJ et Starkey RL, 1968. Vegetable tannins, their decomposition and effects on decomposition of some organic compounds. Soil ScL 4: 241-247.

Lewis G, Schrire B, Mackinder B, Lock M (Eds.), 2005. Legumes of the world. Royal Botanic Gardens, Kew, Richmond, U.K Expert Review of Hematology 10(1): 65-79.

Laouar A, 2020. Caractérisation physicochimique et microbiologique de deux variétés de dattes" Hmira, Feggous"et production de bioéthanol à partir de rebuts de dattes" Hmira", Mémoire de doctorat, Université Tahri Mohamed Béchar, 213p.

Loubière P et Novak L, Cocaign-Bousquet M, Lindley Nd, 1996. Besoins nutritionnels des bactéries lactiques: interactions entre flux de carbone et d'azote. Le Lait, INRA Editions 76(1-2): 5-12.

MacDonald RS, 2000. The Role of Zinc in Growth and Cell Proliferation. The Journal of Nutrition 130(5): 1500 - 1508.

Müller HG et Tobin G, 1980. Nutrition and Food Processing. London: Croom Helm Ltd.,

Murphy MG et Condon S, 1984. Comparison of aerobic and anaerobic growth of *Lactobacillus plantarum* in a glucose

medium. Archives of Microbiology 138: 49 – 53.

Murwan K, Sabah ELKhier Ali A Abd-ALRaheem, 2011. Effect of fermentation period on the chemical composition, in-vitro protein digestibility and tannin content in two sorghum cultivars (Dabar and Tabat) in Sudan. Journal of Applied Biosciences 39: 2602 – 2606.

N'Goran-AW EBZ, Doudjo S, Sadat A, David AK, Emmanuel AN, 2017. Évaluation des caractéristiques physico-chimiques et microbiologiques d'un beignet traditionnel à base de mil fermenté (*gnomy*) commercialise dans la ville de Yamoussoukro (Côte D'Ivoire). European Scientific Journal 13**(**9).

Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P, 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiology and Molecular Biology Revue 64: 548 – 572.

Nielsen A, Tetens I, Meyer A, 2013. Potential of Phytase-Mediated Iron Release from Cereal-Based Foods: A Quantitative View. Nutrients 5(8): 3074–3098.

Noonan SC et Savage GP, 1999. Oxalate content of foods and its effect on humans. Asia Pacific Journal Clinical Nutrition 8: 64–74.

Oatway L, Vasanthan T, Helm JH, 2001. PHYTIC ACID. Food Reviews International 17(4): 419–431.

Oboh G et Elusiyan CA, 2007. Changes in the nutrient and anti-nutrient content of micro-fungi fermented cassava flour produced from low- and medium-cyanide variety of cassava tubers. African Journal of Biotechnology 6(18): 2150- 2157.

Oboh G, Akinyemi AJ, Ademiluyi AO, 2012. Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. Rubra) and white ginger (*Zingiber officinale* Roscoe) on $Fe2^{+}$ induced lipid peroxidation in rat brain in vitro. Experimental and Toxicologic Pathology 64(1-2): 31 - 36.

Ojokoh AO, 2009. A comparative study on the effect of traditional and improved methods of fermentation in the production of Ogi food. Oriental Journal of Chemistry 25(3): 471-476.

Oke MO et Bolarinwa IF, 2012. Effect of Fermentation on Physicochemical Properties and Oxalate Content of Cocoyam (*Colocasia esculenta*) Flour. ISRN Agronomy 2012: 1–4.

Okwu DE et Aluwuo CJ, 2008**.** Studies on the phytochemical composition and fermentation of the seeds of african oil bean tree (*Pentaclethra macrophylla* benth). International Journal of Chemical Science 6(2): 773-788.

Ouoba LI, Diawara B, Wk AA, Traore A, Moller P, 2004. Genotyping of starter cultures of *Bacillus subtilis* and *Bacillus pumilus* for fermentation of African locust bean (*Parkia biglobosa*) to produce Soumbala. International Journal and Food Microbiology 90: 197 - 205.

Oyewole OB et Odunfa SA, 1990. Co-fermentation of cassava with Legumes for protein rich fermented cassava fufu.wwwoyewole-unaab.net/publications.htm

Oyewole OA et Isah P, 2012. Locally fermented foods in Nigeria and their significance to national economy: a review. Journal Recent of Advances Agriculture 1(4):92 - 102.

Osman MA, 2011. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. Journal of the Saudi Society of Agricultural Sciences 10(1): 1 - 6.

Paul BT, Manz DH, Torti FM, Torti SV, 2017. Mitochondria and Iron: current questions. Expert Review of Hematology 10(1): 65-79.

Pepa GD et Brandi ML, 2016. Microelements for bone boost: the last but not the least. Clinical Cases *in* mineral *and* bone metabolism 3:181 - 185.

Piard J-C et Desmazeaud M, 1991. Inhibiting factors produced by lactic acid bacteria. 1. Oxygen metabolites and catabolism end-products. Lait 71 : 525–541.

Picaud C, 2017. Oligo-éléments : excès, carences et conseil officinal, Thèse d'exercice, Université de Limoge Faculté de Pharmacie, 143p.

Pham-Huy LA, He H, Pham-Huy C, 2008. Free radicals, antioxidants in disease and health. International Journal of Biomedical Science 4(2):89-96.

Prashanth L, Kattapagari KK, Chitturi RT, Baddam VRR, Prasad LK, 2015. A review on role of essential trace elements in health and disease. Journal of Dr. NTR University of Health Sciences 4(2): 75-85.

Radek M et Savage GP, 2008. Oxalates in some Indian green leafy vegetables. *International* Journal of Food Science and Nutrition 59: 246–260.

Sarkar PK, Hasenack B, Nout MJR, 2002. Diversity and functionality of *Bacillus* and related genera isolated from spontaneously fermented soybeans (Indian Kinema) and locust beans (African Soumbala). International Journal and Food Microbiology 77: 175- 186.

Sakuyama H, Katoh M, Wakabayashi H, Zulli A, Kruzliak P, Uehara Y, 2016. Influence of gestational salt restriction in fetal growth and in development of diseases in adulthood. Journal of Biomedical Science 23 (1).

Savage G et Vanhanen L, 2018. Oxalate Contents of Raw, Boiled, Wok-Fried and Pesto and Juice Made from Fat Hen (*Chenopodium album*) Leaves. Foods 8(1): 2.

Schrire BD, Lavin M, Lewis GP, 2005. Global distribution patterns of the Leguminosae: insights from recent phylogenies. Biologiske Skrifter 55:375 - 422.

Soetan KO, Olaiya CO, Oyewole OE, 2010.The Importance of Mineral Elements for Humans, Domestic Animals and Plants- A Review. African Journal of Food Science 4(5): 200 - 222

Sokrab AM, Mohamed Ahmed IA, Babiker EE, 2012. Effect of malting and fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. International Journal of Food Science & Technology 47(5): 1037–1043.

Tamang JP, Shin D-H, Jung S-J, Chae S-W, 2016. Functional Properties of Paramétrique et non paramétrique au Burkina Faso », Rome / FAO 27 p.

Udensi UK et Tchounwou PB, 2017. Potassium Homeostasis, Oxidative Stress, and Human Disease. International Journal of Clinical Experimental Physiology 4 (3): 111 - 122.

Wcislo G et Szarlej-Wcislo K, 2014. Colorectal Cancer Prevention by Wheat Consumption. Wheat and Rice in Disease Prevention and Health, 91–111.

Xu W, Barrientos T, Andrews NC, 2013. Iron and Copper in Mitochondrial Diseases. Cell Metabolism 17(3): 319-328.

Yabile RK, 2011. «Vulnérabilité des populations a l'insécurité alimentaire en côte d'ivoire : cas des régions du Worodougou, de la vallée du Bandama, du N'zi comoe et du Zanzan». Agronomie Africaine 23(2): 179 – 192.

Young V et Pelettp Pl, 1994. Plant proteins in relation to humain protein and amino

acid nutrition. The American Journal of Clinical Nutrition 59 (suppl): 1203S-1212S.

Zoumpopoulou G, Foligne B, Christodoulou K, Grangette C, Pot B, Tsakalidou E, 2008. *Lactobacillus fermentum* ACA-DC 179 displays probiotic potential *in vitro* and protects against trinitrobenzene sulfonic acid (TNBS)-induced colitis and *Salmonella* infection in murine models. International Journal of Food Microbiology 121: 18 – 26.