Impact of Lactic Acid Fermentation on the Nutritional Potential and Anti-nutritional factors of Two Widely Consumed Pulses (*Vigna unguiculata* and *Vigna subterranea*) Flours in Côte d'Ivoire

---

.

ABSTRACT

Aims: Impact of *Lactobacillus plantarum*

Place and Duration of Study: *Laboratory of Biotechnology, Agriculture and Valorization of Biological Resources*, between February 2021 and November 2021.

Methodology: Two pulses (*Vigna unguiculata* and *Vigna subterranea*) purchased at the Adjamé market (Abidjan) were subjected, after the unitary operations of sorting, washing drying and grinding of the grains, in fermentation in a sterile flour/water mixture at 37 °C for 48 h by *Lactobacillus plantarum*. The final microbial load was enumerated according to official methods, the fermented flour was dried at 50 °C for 48 h and then the antioxidant and anti-nutritional properties were evaluated.

Results: The sterile conditions of fermentation of the water/flour mixture with the inoculum load of 9.10^7 CFU/g resulted in final microbial loads of $7.8\ 10^9$ CFU/g and $6.7\ 10^9$ CFU/g, in decreased in pH to 3.13 ± 0.06 and 3.27 ± 0.06. This decrease was correlated with an increase in total titratable acidity (4.89 ± 0.02 and 4.26 ± 0.0 %) after 48 h of fermentation for cowpea and voandzou flours, respectively. The fermentative activity of *Lactobacillus plantarum* increased the initial phenolic compound content to 74.28 ± 0.99 mg/100 g (cowpea) and 71.26 ± 0.4 mg/100 g (voandzou) for total polyphenols and 36.52 ± 0.336 mg/100 g (cowpea flour) and 35.33 ± 0.231 mg/100 g (voandzou flour) for total flavonoids after 30 h of fermentation. At the same time, their antioxidant activity doubled from 15 to 30 % while initial phytate and tannin levels were both reduced by 47 % and 50 %, respectively in cowpea and voandzou flours in 30 hours of fermentation.

Conclusion: In view of all these results, cowpea and voandzou flours fermented with *Lactobacillus plantarum* could be used as functional foods.

Keywords: Cowpea, flours, lactic acid fermentation, Lactobacillus plantarum, phytochemical, voandzou

1. INTRODUCTION

Pulses refers to any plant from the *Fabaceae* family, distinguished by fruits called pods that release seeds at maturity in dehiscent slits [1]. These plants are of great agronomic and food interest. Indeed, the whole vegetative part (stem, root and leaves) is used agriculturally as a cover crop, green manure or in livestock feed, while the beans are used in human food [2]. Legumes (beans, peas and lentils) have been consumed for at least 10,000 years and are among the most widely used foods in the world. Their worldwide consumption is believed to be attributed to their interesting biochemical and nutritional composition [3]. Indeed, legumes are an excellent and inexpensive source of protein and micronutrients (vitamins and minerals) [4]. This nutrient richness and diversity has made legumes a staple food in many countries [5]. Legumes also contain a number of bioactive compounds, including phytates, oligosaccharides, enzyme inhibitors, peptides and phenolic compounds, which have been reported to have a positive impact on health [6,7]. Indeed, some phytochemicals such as saponins, phenolic compounds and tannins present in legumes have antioxidant and anticancer effects, which could be used in the treatment of cancer diseases [6]. Similarly, Doué *et al*. [7] have highlighted the dual anti-diabetic and anti-oxidant character of legume peptides. Consumption of pulses also improves the serum lipid profile, which has a positive effect on several other risk factors for cardiovascular disease, such as blood pressure, platelet activity and inflammation [7]. Pulses are rich in fibre and have a low glycaemic index, which makes them particularly beneficial for people with diabetes in helping to maintain healthy blood glucose and insulin levels [8]. Despite the many nutritional benefits mentioned above, there is still the problem of digestive discomfort often associated with the consumption of legumes. These digestibility problems are related to the presence in legumes of anti-nutritional factors (ANFs) such as lectins, trypsin inhibitors and phytic acid that complex essential nutrients [9,10,11] and hinder the acceptability of these foods by the population especially in developing countries such as Côte d'Ivoire.

In Côte d'Ivoire, as in many countries in the sub-region, the preparation of local pulses (soy, common bean, white bean, voandzou) follows a certain number of practices, including soaking, roasting and fermentation [12,13]. The adoption of pulses in African household diet is challenged by the long cooking time required before palatability is achieved, coupled with high fuel energy requirements [14], which constitute serious constraints to their use. These constrains (long preparation time, difficult digestibility, etc.) have led to a sharp drop in their consumption [15]. In this context, nutritional improvement of legumes would require less energy-intensive processing technologies that would significantly reduce anti-nutritional factors such as fermentation.

Fermentation, an ancient food processing and preservation process, to be a sustainable way of improving the nutritional properties of foodstuffs at lower cost by hydrolysis the anti-nutritional factors and reducing the antimicrobial compound. Indeed, food becomes easier to digest and the fibres are less aggressive for the intestines [16]. This process therefore appears to be a way of diversifying the range of foods derived from legumes by increasing their digestibility and even their nutritional value. For legumes, it should be noted that many foods are produced, mainly tempeh in Indonesia and *dawadawa* in Nigeria, two food products resulting from the natural fermentation of soybeans and *néré*, respectively [17,18]. These natural fermentations are the result of spontaneous colonization of foods by local fermentative strains. However, the randomness of this kind of fermentation implies diversity and variability of fermenting microorganisms and does not allow for standardisation of the health, sensory and nutritional quality of the final product [19].

Therefore, using microbial ferments with known technological properties as starter for fermented foods, especially beans, is increasingly becoming the most suitable approach to control and improve the technical properties of processed foods. Recently, starter strains of lactic acid bacteria have been isolated from *magnan* (fermented dought cassava) and characterized at the molecular level [20]. These *Lactobacillus plantarum* strains have shown interesting technological properties that can be exploited in the formulation of functional foods. Given the high fermentation potential of these strains and their interesting technological properties, it seems important to use them to explore their ability to ferment different local legumes in order to control food quality. This is even more important as these Lactobacillus plantarum strains, considered "GRAS" i.e. Generally Recognized As Safe and isolated from food strains are potentially safe for human consumption. The aim of this study was to evaluate the impact of legume flours (*Vigna unguiculata* or cowpea and *Vigna subterranea* or voandzou) fermentation by *Lactobacillus plantarum* on their anti-nutritional and antioxidant properties.

2. MATERIAL AND METHODS

2.1. Biological material

Two legumes (*Vigna unguiculata* or cowpea and *Vigna subterranea* or voandzou) were purchased at the Adjamé market (Abidjan), which is the largest supply area for these seeds in Côte d'Ivoire. Once purchased, they were put in coolers and transported directly to the laboratory. The bacterial material consisted of the *Lactobacillus plantarum* strain previously isolated from "magnan" in Côte d'Ivoire and well-characterised at the molecular level [20]. This strain synthesises an extracellular amylase and β-glucosidase and is capable of degrading starch to glucose. These strains produced a large amount of lactic acid from glucose and has been used for lactic acid fermentation.

2.2. Processing of legume seeds (cowpea and voandzou) into flour

Once in the laboratory, the legume seeds (*Vigna unguiculata* or cowpea and *Vigna subterranea* or voandzou) were sorted, cleaned, dehulled and washed with distilled water. They were dried in an oven (MercK, Germany) at 50 °C for 24 h, ground with a blender (Moulinex, France). The resulted flours were sterilized at 121 °C for 15 min and stored at 4 °C.

2.3. Fermentation of legumes flours

150 g of sterilized flour were mixed with a quantity of sterile distilled water of ration 4/5 (w/v) and inoculated with a suspension of *Lactobacillus plantarum* to obtain a starting bacterial population of approximately 9.10^7 and 8.10^7 CFU/g of fresh product for cowpea and voandzou respectively. Fermentations were carried out in 250 mL Erlenmeyer flasks and incubated at 30 °C for 48 h. During fermentation, pH, total titratable acidity (TTA), growth bacterial of lactic acid flora, nutritional and anti-nutrional factors were measured every 6 h. All fermentations were performed in triplicate.

2.4. Analysis during fermentation by *Lactobacillus plantarum*

2.4.1. Monitoring of pH and Total Titrable Acidity (TTA) during fermentation of two legumes flours

TTA of the fermenting flours was determined according to the AFNOR method, NF V05-101 [21]. TTA was determined by titrimetry with 0.1 N NaOH and the pH was measured with an electronic pH meter (HANNA Instruments, Germany) previously calibrated with standard buffer solutions pH = 4 and 7 [22]. TTA is expressed as percentage of lactic acid (%).

2.4.2. Growth bacterial of lactic acid flora during fermentation of two legumes flours

10 g of sampled every 6 hours was homogenized in 90 mL of peptone water solution followed by decimal serial dilutions. MRS agar medium was used to determine lactic acid bacteria growth as colony forming units (CFU); incubation was at 30 °C for 48 h.

2.4.3. Polyphenols content determination during fermentation of two legumes flours

Polyphenols were extracted and determined using Folin-Ciocalteu's reagent [23]. A quantity (1 g) of flour fermented was mixed in 10 mL of methanol 70 % (w/v) and centrifuged at 1000 rpm for 10 min. An aliquot (1 mL) of supernatant was oxidized with 1 mL of Folin–Ciocalteu's reagent and neutralized by 1 mL of 20 % (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at room temperature and absorbance was measured at 745 nm by using a spectrophotometer (PIOWAY, China). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as a standard.

2.4.4. Flavonoids content evaluation during fermentation of two legumes flours

Flavonoid content of flours fermented was evaluated using the method reported by Arvouet-Grand et al. [24]. 0.5 mL of the methanolic extract (1 g of flour fermented was mixed in 10 mL of methanol 70 %) was mixed with 0.5 mL of $AlCl_3$ (10 %, w/v), 0.5 mL of potassium acetate (1 M) and 2 mL of distilled water. Absorption at 415 nm (PIOWAY, China) was mesured after 10 min against a blank sample. The total flavonoid content was determined using a standard curve with quercetin (Sigma-Aldrich, Germany) (0,50 mg/mL) as the standard.

2.4.5. Antioxidant activity evaluation during fermentation of two legumes flours

Antioxidant activity assay was carried out using the 2,2-diphenyl-1-pycrilhydrazyl (DPPH) spectrophotometric method [25]. To 1 mL of 0.3 mmol/L DPPH solution prepared in ethanol was added 2.5 mL of solution (1 g of flour fermented sample mixed in 10 mL of methanol and filtered through Whatman paper) and was allowed to react for 30 min at room temperature. Absorbance values were measured with a spectrophotometer (Pioway, China) set at 415 nm and the average absorbance values were converted to percentage antioxidant activity using the following formula:

Antioxidant activity (%) = 100 – [(Abs of sample – Abs of blank) x 100/Abs positive control]

2.4.6. Phytate content evaluation during fermentation of two legumes flours

Phytates contents of flours were determined using the Wade's reagent method [26]. To 20 mL of hydrochloric acid (0.65 N) was added 1 g of fermented flour and the mixture was agitated for 12 h using a magnet. The mixture was centrifuged at 12000 rpm for 40 min and 0.5 mL of supernatant was added with 3 mL of Wade's reagent. The reaction mixture was incubated for 15 min and absorbance was measured at 490 nm by using a spectrophotometer (PIOWAY, China). Phytates content of flours was determined using a calibration curve of sodium phytate (10 mg/mL) as standard.

2.4.7. Tannin content evaluation during fermentation of two legumes flours

Tannins content of flours were quantified according to method proposed by Bainbridge et al. [27]. For this, vanillin reagent (5 mL) was mixed of the methanolic extract (1 mL) and the mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was read at 500 nm by using a spectrophotometer (PIOWAY, China). Tannins content of flours was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.

2.4.8. Statistical Analysis

All the analyses were performed in triplicate and data were analyzed using EXCELL and SPSS Statistics 20.0. Differences between means were evaluated by Duncan's test. Statistical significant difference was stated at $p < 0.05$.

3. RESULTS AND DISCUSSION

Fig. 1 shows the changes in biochemeical parametere (pH, total titratable acidity) of the two studied legumes flours during the 48 hour fermentation. It should be noted that the total titratable acidity (TTA) is inversely proportional to the pH during the fermentation of both legume flours. At the beginning of fermentation, the pH of cowpea and voandzou flour was around 6.5, and then decreased to reach 3.13 and 3.27, respectively. There was a sharp drop in the first 24 hours (Fig. 1A). The same trend was obtained by the study made by Menezes et al. [28] on wheat dough fermentation showing the greatest decrease in the pH level in the first few hours of fermentation, until it reached a relatively stable value after several stages. During the same fermentation period, the TTA increased from an initial value of 0.2 % to values of 4.89 ± 0.6 % and 4.26 ± 0.4 % for cowpea and voandzou flours, respectively (Fig. 1B). This increase in the acidity of the fermentation medium is the consequence of the lactic fermentation carried out by these lactic bacteria. This is beneficial for the legume flours studied since it contributes to the enhancement of their stability by inhibiting possible microbial and enzymatic alterations, and therefore favours the extension of the shelf life of these products [29].

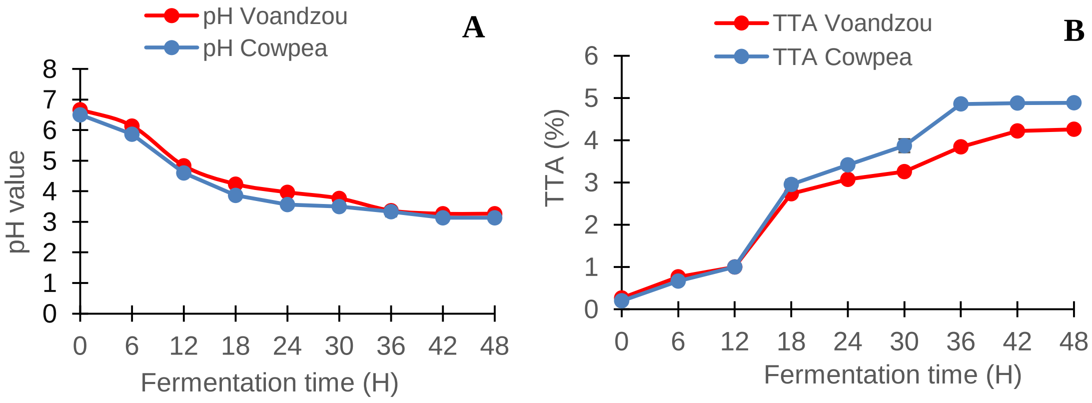

Fig 1. Evolution of pH (A) and total titratable acidity (B) during fermentation of both legumes flours by *Lactobacillus plantarum*

The evolution of the lactic acid bacteria is monitored during the fermentation process as shown in Fig. 2. The microbial population increased slightly in the first 24 hours, then increased rapidly from 42 hours until the end of fermentation (Fig. 2). This rise in the bacterial population is in line with the previously identified increase in the acidity of the medium. Indeed, cowpea and voandzou flours with low microbial loads at the beginning of fermentation show a progressive increase of this load reaching a maximum of microbial load of 8.10^9 CFU/g (cowpea) and 7.10^9 CFU/g (voandzou) after 42 hours of fermentation. The production of various organic acids (with lactic acid as the predominant acid) may have contributed to the low pH [29]. LAB strains produced lactic acid and CO_2 from carbohydrates via the carbohydrate pathway (Embden-Meyerhof, phosphoketolase, 6-phosphate tagatose and Leloir pathways) and carbonic acid produced in this pathway reduces pH. The decrease in pH correlated with increased TTA and cell growth could be explained by the production of lactic acid by *Lactobacillus plantarum*. Indeed, cowpea and voandzou seeds are rich in carbohydrates mainly starch around 50% [30,31]. *Lactobacillus plantarum* strains are able to break down these carbohydrates into simple molecules like glucose since it produces amylolytic enzymes [32,20]. The low pH, high amount of lactic acid and high load of lactic acid bacteria are factors to inhibit the growth of poathogenic bacteria in food. Our fermented flours can be used as an ingredient to develop better quality foods with a longer shelf life.

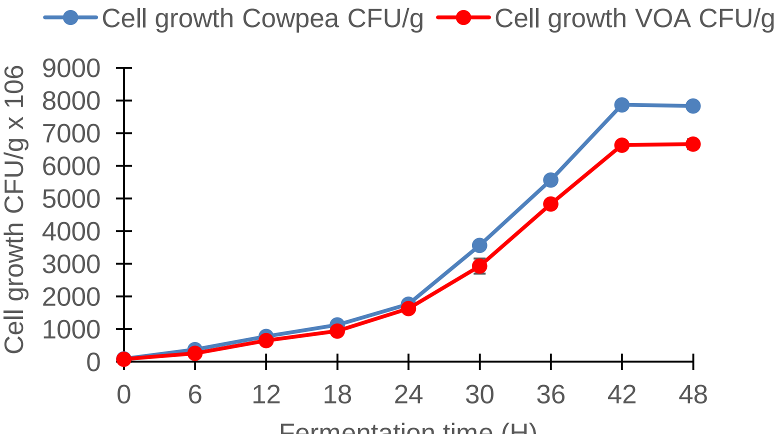

Fig. 2. Microbial growth in cowpea and voandzou pastes during *Lactobacillus plantarum* fermentation

Phenolic compound or polyphenols are secondary metabolites characterised by the presence of an aromatic ring bearing free or carbohydrate-engaged hydroxyl groups. They are present in all parts of higher plants (roots, stems, leaves, flowers, pollens, fruits, seeds and wood). The most represented are flavonoids and tannins [33]. Regardless of the factor studied, an increase in the polyphenol and flavonoid content on the studied legume flours was observed up to 30 h of fermentation. After this peak, these contents decrease until the end of fermentation (Fig. 3A and 3B). The study revealed that the fermentation process impacts on the phenolic compound contents in two steps. First, it induces an increase in the content of these compounds in 30 hours of fermentation reaching a maximum of 74.28 mg/100 g (cowpea) and 71.26 mg/100 g (voandzou) for total polyphenols and 36.52 mg/100 g (cowpea flour) and 35.33 mg/100 g (voandzou flour) for total flavonoids (Fig. 3 and 4). After that, a regular decrease is observed until the end of the fermentation. This same trend was observed in the work of Colosimo et al. [34], who observed a significant increase in polyphenols and flavonoids over a 72 hours sourdough fermentation period.

As previous studies [35,36,7] have highlighted the positive correlation between the presence of phenolic compounds and antioxidant activity, it is appropriate to assess this activity in fermenting legume flours. Figure 4 showed the antioxidant activity values recorded during fermentation in the pastes of the two legume flours studied. It should be noted that a perfect correlation is observed in the evolution kinetics of this parameter during the fermentation It should be noted that a perfect correlation is observed in the kinetics of evolution of this parameter during fermentation with the increase in oxidizing power in the first thirty hours of fermentation reaching the maxumum of 29.88 and 26.52 % for cowpea and voandzou, respectively followed by a progressive decrease (Fig. 4).

These results are consistent with those of other researchers who reported that the antioxidant activity levels of unfermented cowpea and voandzou flour were around 16.2 mg/100 g for cowpea [37] and 7.2 mg/100 g to 20.39 mg/100 g for voandzou [38]. Katina et al. [39] suggested that the fermentation induced structural breakdown of cell walls, leading to the liberation and/or synthesis of various bioactive compounds. During fermentation, enzymes derived (amylases, xylanases and proteases) and LAB contributes to the modification of flour composition, and bound phenolics may released by enzymatic treatment of samples prior to extraction. Moreover, Dordevic et al. [40] indicate that the type of fermentation influence the levels of most bioactive compound. In fact, the acidification of the medium by lactic acid bacteria during fermentation improves the extraction of of TTC. Thus, the feruloyl-esterase and β-glucosidase activities of lactic acid bacteria contribute to the release of more complex-form phenols whichh explains the increase in TTC and flavonoïds [41]. The observed reduction in TTC and flavonoïds in flours fermented with lactic acid bacteria strain was probably due to detoxification and utilization of phenolic acids as a carbon source [42]. As the studied fermented flours displayed high content of polyphenols and flavonoids positively correlated with antioxidant activity, they could be highly recommended in food formulation to contribute to the prevention of certain diseases caused by free radicals desorder such as atherosclerosis, cancer, inflammatory diseases, neurodegenerative diseases and diabetes [43].

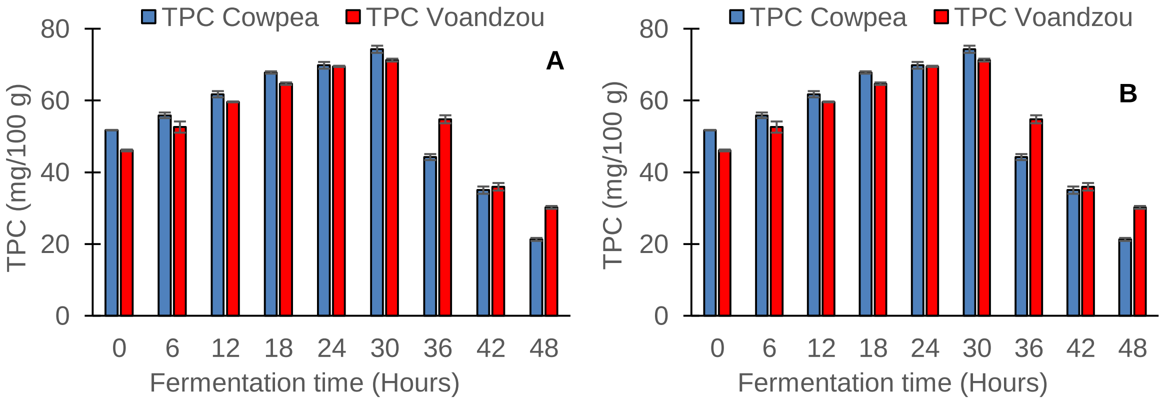

Fig. 3. Effect of *Lactobacillus plantarum* fermentation on total polyphenol (A) and total flavonoids (B) contents of legumes pastes flours

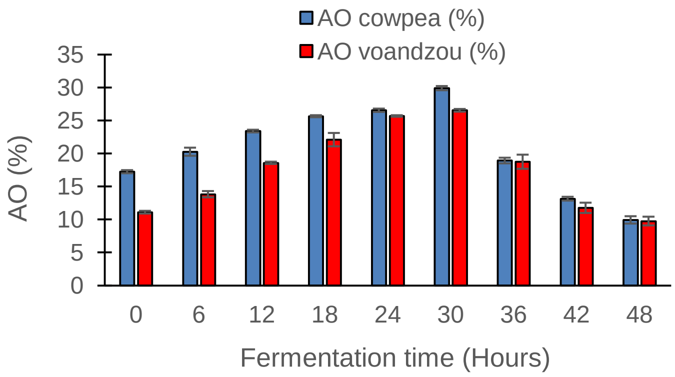

Fig. 4. Effect of *Lactobacillus plantarum* fermentation on antioxidant activity of legumes pastes flours

The occurrence of endogenous anti-nutritional factors such as tannins and phytates in legumes is the most important factor limiting their use and consumption [44]. Table 1 shows the impact of fermentation by *Lactobacillus plantarum* on two main compounds with anti-nutritive effects in the legumes studied. It is noteworthy that the fermentation carried out by the starter strains of *Lactobacillus plantarum* favoured a decrease in tannin and phytate content throughout the fermentation. However, this was only significant ($p \leq 0.05$) during the first 30 and 36 hours of fermentation for tannins and phytates, respectively. Indeed, the tannin and phytate contents drop significantly from 36.83 % to (18.82-18.38%) in the case of cowpea and from 30.75 % to (18.57-18.08 % for voandzou in 30 hours for tannins and from

756.38% to 394.79 % (cowpea) and from 569.02 % to 305.65 % (voandzou) in 36 hours for phytates.

The reduction of tannins and phytates in legume meals during fermentation by *Lactobacillus plantarum* has already been reported by several authors [45,46]. The significant reduction in phytates content observed in this study could be attributed to the production phytases and phosphophytases by *Lactobacillus plantarum* which hydrolysis phytates [47,48]. In fact, during fermentation, lactic acid bacteria ferment carbohydrates into various organic acids such as lactic acid, citric acid and acetic acid, which cause a reduction in pH to levels at which the enzyme phytase (endogenous or from lactic acid bacteria) can dephosphorylate phytate more effectively [49]. With regard to tannin, the observed decrease could be due to the mobilisation of enzymes such as phenyl oxidase, α-galactosidase and other enzymes associated with seeds [16]. Tannins affect the palatability of foods due to their bitter and harsh taste and also reduce digestibility by forming a complex with proteins [50]. Lactic acid bacteria can break down most tannins into small molecules, including catechin, gallic acid and galliccathechins by various enzymatic and/or protein-based mechanisms [51]. Thus, lactic acid fermentation, through its ability to significantly reduce tannin and phytate content, contributes to making legumes more suitable for human consumption

Table 1. Effect of fermentation time on the tannin and phytate content of the legume flours studied

	Tannins (mg/100 g)		**Phytates (mg/100g)**	
Fermentation time (h)	Cowpea	Voandzou	Cowpea	Voandzou
0	36.83 ± 0.84^{a}	30.75 ± 0.66^{a}	756.38 ± 1.62^{a}	569.02 ± 1.24^{a}
6	32.62 ± 1.23^{b}	28.60 ± 0.15^{b}	713.27 ± 1.69^{b}	519.10 ± 1.35^{b}
12	31.17 ± 0.58^{c}	25.62 ± 0.58^{c}	701.75 ± 1.34^{c}	479.28 ± 1.85^{c}
18	27.08 ± 1.23^{d}	23.20 ± 0.50^{d}	674.00 ± 3.12^{d}	417.20 ± 3.12^{d}
24	23.32 ± 0.78^{e}	20.73 ± 0.30^{e}	539.08 ± 1.44^{e}	377.15 ± 5.98^{e}
30	18.82 ± 0.58^{f}	18.57 ± 0.16^{f}	504.35 ± 1.56^{f}	362.27 ± 2.63^{f}
36	18.38 ± 0.19^{f}	18.35 ± 0.09^{f}	463.55 ± 2.86^{g}	322.87 ± 6.47^{g}
42	18.55 ± 0.23^{f}	18.08 ± 0.45^{f}	394.79 ± 0.47^{h}	305.65 ± 0.43^{h}
48	18.70 ± 0.05^{f}	18.25 ± 0.09^{f}	395.17 ± 2.76^{h}	306.00 ± 0.35^{h}

Data are means of three determinations (n = 3) ± SD. Means with different superscripts in each column indicate significant differences at $p \leq 0.05$ based on Duncan multiple range test.

4. CONCLUSION

Controlled fermentation of cowpea and voandzou flours by *Lactobacillus plantarum* induced numerous modifications of nutritional and anti-nutritional factors in an average of 30 hours. The increase in the population of *Lactobacillus plantarum* favoured an acidification of the fermentation medium (TTA and pH) allowing an increase in phenolic compounds (polyphenol and flavonoids) and antioxidant activity inversely correlated with phytates and tannins (anti-nutritional factors) content. This strategy makes it possible to obtain functional foods of better nutritional quality that are useful in the fight against nutritional metabolic diseases.

COMPETING INTEREST

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author GGD designed and supervised the study. Authors MC and WY managed and performed the experimental and statistical analysis. Authors MC and GGD wrote the protocol, and wrote the first draft of the manuscript. 'Author WY and TLZ managed the literature searches. All authors read and approved the final manuscript.

REFERENCES

1. Karlsson H, Ahlgren S, Strid I, Hansson P-A. Faba beans for biorefinery feedstock or feed? Greenhouse gas and energy balances of different applications. Agric Syst. 2015;141:138-148.
2. Kan L, Nie S, Hu J, Wang S, Bai Z, Wang J, Zhou Y, Zeng J, Song K. Comparative study on the chemical composition, anthocyanins, tocopherols and carotenoids of selected legumes. Food Chem. 2018;260:317-326.
3. Tiwari K, Gowen A, McKenna B. Pulses: What are they? In: Tiwari BK, Gowen A, McKenna B, editors. Pulse Foods: Processing, Quality and Nutraceutical Applications. 5th ed. London, UK: Academic Press; 2011.
4. Winham M, Webb D, Barr A. Beans and good health. Nutr today. 2008;43(5):201-209.
5. Wang N, Toews N. Certain physicochemical and functional properties of fibre fractions from pulses. Food Res Inter. 2011;44:2515-2523.
6. Frias J, Penas E, Martinez-Villaluenga C. Fermented pulses in nutrition and health promotion. In: Frias J, Martinez-Villaluenga C, Penas E, editors. Fermented Foods in Health and Disease Prevention. Academic Press; Rome, Italy: Food and Agriculture Organization of the United Nations. 2016. DOI: 10.1016/B978-0-12-802309-9.00016-9.
7. Doue GG, Mégnanou R, Zoué LT. Multifunctional Bioactive Peptides from Germinated Soy (Glycinmax) and Voandzou (Vigna subterranea) Beans: In-vitroAnti-Diabetic Potential through α-amylase α-glucosidase Inhibition, and Antioxidant Ability by DPPH Reducing. European j. nutr. food saf. 2021;13(11):20-32.
8. McCrory A, Hamaker R, Lovejoy C, Eichelsdoerfer E. 2010. Pulse consumption, satiety, and weight management. Adv Nutr Res. 2010;1(1): 17-30. Doi:10.3945/an.110.1006.
9. Matthews RH. Legumes Chemistry, Technology and Human Nutrition. Marcel Dekker, New York, (1989).
10. Granito M, Champ M, David A, Bonnet C, Guerra M. Identification of gas-producing components in different varieties of *Phaseolus vulgaris* by in vitro fermentation. J Sci Food Agric. (2001);81:543-550.
11. Omafuvbe B. O. 2008. Effect of temperature on biochemical changes induced by *Bacillus subtilis* (SDA3) during starter culture fermentation of soybean into condiment (Soy Daddawa). Am J Food Technol. 2008;3:33-41.
12. Jezierny D, Mosenthin R, Bauer E. The use of grain legumes as a protein source in pig nutrition: A review. Anim. Feed Sci. Technol., 2010;157 (3-4):111-128.
13. López-Martínez L, Nayely Leyva-López N, Gutiérrez-Grijalva P, Heredia J. Effect of cooking and germination on bioactive compounds in pulses and their health benefits. J Funct Foods. 2017; 38:624-634.
14. Lokuruka M. Effects of processing on soybean nutrients and potential impact on consumer health: An overview. Afr J Food Agric Nutr Dev. 2011;11:5000-5017.
15. Tamang J, Shin D, Jung S, Chae W. Functional Properties of Microorganisms in Fermented Foods. Front Microbiol. 2016;7:578-585.

16. Adeyemo M, Onilude A. 2013. Enzymatic Reduction of Anti-nutritional Factors in Fermenting Soybeans by *Lactobacillus plantarum* Isolates from Fermenting Cereals. Niger Food J. 2013;31:84-90.
17. Jennessen J, Schnürer J, Olsson J; Samson A; Dijiksterhuis J. Morphological characteristics of sporangiospores of the tempeh fungus Rhizopus oligosporus differentiate it from other taxa of the R. microsporus group. Mycol Res. 2008;112:547-563.
18. Akanni B, Naudé Y, de Kock L, Buys M. Diversity and functionality of bacillus species associated with alkaline fermentation of Bambara groundnut (Vigna subterranean L. Verdc) into dawadawa-type African condiment. Eur Food Res Technol. 2018;244:1147-1158.
19. Yann D, Pauline G. Usefulness of Natural Starters in Food Industry: The Example of Cheeses and Bread. Nutr *Food* Sci. 2014;5:1679-1691.
20. Krabi R, Assamoi A, Ehon F, Brahima D, Niamke S, Thonart P. Isolation and screening of *Lactobacillus Plantarum* strains from attieke fermentation process for a starter culture development. Chiang Mai J. Sci. 2016;43:1-13
21. AFNOR, NF V05 -101. Produits dérivés des fruits et légumes-Détermination de l'acidité titrable.1974.
22. Lopez I, Bautista E, Moreno E, Dentan E. Factors related formation of over fermented coffee beans during the west processing method and storage of coffee. ASIC, XIIIth. International Scientific Colloquim on coffee, Paipa, Colombia, 1989.
23. Singleton L, Orthofer R, Lamuela-Raventos M. Analysis of total phenols and other oxydant substrates and antioxydants by means of Folin-ciocalteu reagent. Methods Enzymol. 1999;299:152-178.
24. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardization of propolis extract and identification of principal constituents. J Pharm Bel.1994;49(6):462-468.
25. Choi W, Kim C, Hwang S, Choi K, Ahn J, Lee Z, Park H, Kim K. Antioxydant activity and free radical scavenging capacity between Korean medicinal plant and flavonoids by assay guided comparaison. Plant Sci. 2002;63:1161-1168.
26. Latta M, Eskin M. A simple method for phytate determination. J Agric Food Chem. 1980;28:1313-1315.
27. Bainbridge Z, Tomlins K, Wellings K, Westby A. Analysis of condensed tannins using acidified vanillin. J Food Sci Agric. 1996;29:77-79.
28. Menezes A, Minervini F, Filannin P, Sardaro S, Gatti M, De Lindner J. Effects of Sourdough on FODMAPs in Bread and Potential Outcomes on Irritable Bowel Syndrome Patients and Healthy Subjects. Front Microbiol. 2018;9:1-7
29. Rodríguez G, Mohamed F, Bleckwedel J, Medina B, De Vuyst L, Hebert M, Mozzi F. Diversity and functional properties of lactic acid bacteria isolated from wild fruits and flowers present in Northern Argentina. Front Microbiol. 2019;10:10-19.
30. Boateng A, Addo K, Okyere H, Adu-Dapaah H, Berchie N, Tetteh A. Physicochemical and functional properties of proteinates of two Bambara groundnut (*Vigna subterranean*) landraces. Afric J Food SciTechnol. 2013;4(4):64-70.
31. Diallo KS, Koné KY, Soro D, Assidjo N, Yao KB, Gnakri D. Caractérisation biochimique et fonctionnelle des graines de sept cultivars de voandzou (*Vigna subterranea* (L.) Verdc. Fabaceae) cultives en côte d'ivoire. Eur Sci J. 2015;11:288-304.
32. Di Cagno R, Coda R, De Angelis M, Gobbetti M. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiol. 2013;33:1-10.
33. Boizot N. Charpentier P. Méthode rapide d’évaluation du contenu en composés phénoliques des organes d’un arbre forestier. Le Cahier des Techniques de l’INRA. 2006.
34. Colosimo R, Gabriele M, Cifelli M, Longo V, Domenici V, Pucci L. The effect of sourdough fermentation on *Triticum dicoccum* from Garfagnana: 1H NMR characterization and analysis of the antioxidant activity. Food Chem. 2020;305:125510
35. Rizzello CG, Lorusso A, Russo V, Pinto D, Marzani B, Gobbetti M. Improving the antioxidant properties of quinoa flour through fermentation with selected autochthonous lactic acid bacteria. Int. J. Food Microbiol. 2017;241:252-261

36. Kouakou AB, Megnanou R-M, Djoman ES, Doue GG, Zoue LT, Akpa LR, Kouassi AT, Dembele T, and Niamke LS. Shea Press Cake, an Organic Resource of Bioactive Molecules: Biochemical and Phytochemical Profiles of Alcoholic Extracts. Asian J Biotechnol Bioresour Technol. 2021;7(2):51-57.
37. Bente LH, Kari H, Mari CWM, Ingrid B, Erlend H, Siv FR, Anne-Brit W, Karin H, Halvard B, Lene F. A, Jan M, David RJ, Rune B. A systematic screening of total antioxidants in dietary plants. J Nutr. 2002;132(3):461-471.
38. Abel M, Adama H, Mahama O, Eloi P, Michel N, Yaya M, Mouhoussine N. Etude comparative des teneurs en polyphénols et en antioxydants totaux d'extraits de graines de 44 variétés de voandzou (*Vigna subterranea* (L.) Verdcourt). Int J Biol Chem Sci. 2013;7:861-871.
39. Katina K, Laitilaa A, Juvonen R, Liukkonen K-H, Kariluoto S, Piironen V, Landberg R, Aman P, Poutanen K. Bran fermentation as a means to enhance technological properties and bioactivity of rye. Food Microbiol. 2007;24(2):175-186
40. Dordevic´ TM, Šiler-Marinkovic´SS, Dimitrijevic´-Brankovic´SI. Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. Food Chem. 2010;119(3):957-963.
41. Rodríguez H, Curiel JA, Landete JM, de las Rivas B, de Felipe FL, Gómez-Cordovés C, Mancheño JM, Rosario Munoz R. Food phenolics and lactic acid bacteria. Int. J. Food Microbiol. 2009;132(2-3):79-90.
42. Filannino P, Cavoski I, Thlien N, Vincentini O, DeAngelis M, Silano M, Gobbetti M, Di Cagno R. Lactic acid fermentation of cactus cladodes (*Opuntia ficus-indica* L.) generates flavonoid derivatives with antioxidant and anti-inflammatory properties. PLoS ONE.2016; 11(3):e0152575.
43. Grosso, G. Dietary Antioxidants and Prevention of Non-Communicable Diseases. Antioxidants. 2018;7(7):94.
44. Samtiya M, Aluko RE, Dhewa T. Plant food anti-nutritional factors and their reduction strategies: an overview. Food Production, Processing and Nutrition. 2020;2(6):1-14. https://doi.org/10.1186/s43014-020-0020-5
45. Fischer MM, Egli IM, Aeberli I, Hurrell RF, Meile L. Phytic acid degrading lactic acid bacteria in tef-injera fermentation. Int J Food Microbiol. 2014;190:54-60. doi: 10.1016/j.ijfoodmicro.2014.08.018
46. Mamhoud A, Nionelli L, Bouzaine T, Hamdi M, Gobbetti M, Rizzello CG. Selection of lactic acid bacteria isolated from Tunisian cereals and exploitation of the use as starters for sourdough fermentation. Int J Food Microbiol. 2016;225:9-19. doi: 10.1016/j.ijfoodmicro.2016.03.004
47. Abdelseed BH, Abdalla AH, El-Gasim AYA, Ahmed IAM, and Babiker EE. Some nutritional attributes of selected newly developed lines of sorghum (*Sorghum bicolor*) after fermentation. J. Agric. Sci. Technol. 2011;13: 399-409.
48. Roger T, Leopold TN, Funtong MCM. Nutritional properties and antinutritional factors of corn paste (Kutukutu) fermented by different strains of lactic acid bacteria. Int. J. Food Sci. 2015;1-13.
49. Feil B. Phytic acid. Journal of New Seeds. 2001;3(3):1-35
50. Osman MA, Gassem M. Effects of domestic processing on trypsin inhibitor, phytic acid, tannins and in vitro protein digestibility of three sorghum varieties. Int. J. Agric. Technol. 2013;9(5):1187-1198.
51. Bossi A, Rinalducci S, Zolla L, Antonioli P, Righetti PG, Zapparoli G. Effect of tannic acid on *Lactobacillus hilgardii* analysed by a proteomic approach.J Appl Microbiol. 2007;102(3):787-95. doi : 10.1111/j.1365-2672.2006.03118.x.

APPENDIX