Australian Journal of Crop Science AJCS

Aust J Crop Sci. 20(01):21-27 (2026) | ISSN:1835-2707
https://doi.org/10.21475/ajcs.26.20.01.p67

Assessment of controlled lactofermentation by *Pediococcus acidilactici*, a probiotic strain, on the bioactive compounds of two neglected edible plants used in diabetes treatment

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Submitted:* *29/05/2025

Revised:* *20/09/2025

Accepted:* *14/10/2025

Abstract: This study investigates the effect of controlled lactofermentation using *Pediococcus acidilactici*, a potential probiotic strain, on *Picralima nitida* seeds and *Moringa oleifera* leaves, two edible plants traditionally used to manage diabetes in Côte d'Ivoire. The study aims to demonstrate how controlled lactofermentation using *Pediococcus acidilactici* can enhance the bioactive properties and antidiabetic potential activity of these neglected plants. The results revealed that fermentation improved phytochemical contents of these wild fruits and vegetables. Fermented *Picralima nitida* seeds contained over five times more polyphenols than unfermented seeds (0.82 ± 0.01 mg/ml vs. 0.14 ± 0.01 mg/ml), while fermented *Moringa oleifera* leaves contained 0.92 ± 0.00 mg/ml. DPPH radical inhibition increased by 42.1% in *Picralima nitida* seeds and by 5% in *Moringa oleifera* leaves after fermentation. Anti-inflammatory activity increased by 61.1% in fermented *Picralima nitida* seeds and by 25.5% in *Moringa oleifera* leaves. These results encourage the use of fermented foods in diabetes treatment, which could lead to new products being developed for this condition.

Keywords: Diabetes; Lactofermentation; *Moringa oleifera*; *Pediococcus acidilactici*; *Picralima nitida.*
Abbreviations: DPPH_2,2-diphenyl-1-picrylhydrazyl, HCl_hydrochloric acid, MRS_Man Sharpe Rogosa, H_2SO_4_sulfuric acid, $KMnO_4$_potassium permanganate

Introduction

Diabetes is a metabolic disorder characterized by chronic hyperglycaemia with complications such as myocardial infarction, cardiovascular disease and end-stage renal disease (Gajjala et al., 2015). This disease causes oxidative stress, gut dysbiosis and chronic inflammation (Salguero et al., 2019). Diabetes is widely recognized as a leading cause of death worldwide and is a rapidly expanding disease that has been likened to a pandemic (Iftikhar et al., 2020). For a long time, the management of diabetes mellitus was limited to dietary changes, the administration of insulin or oral hypoglycaemic agents. Although these treatments have been effective, it is important to note that mortality has continued to rise (Holaly et al., 2015). In many cases, mortality is attributed to the limited efficacy of therapeutic molecules currently available (Holaly et al., 2015). In addition, the therapeutic management of this disease by modern medicine using synthetic drugs is expensive in developing countries due to the low income of the population. This resulted in the World Health Organization declaring diabetes as a major public health issue at the 66th Assembly of the United Nations in 2011, and to encourage African countries to develop regional strategies on traditional medicine to conduct research on medicinal plants and promote their optimal use in health systems. Due to this situation, several more sustainable and less costly alternatives have been proposed, including the consumption of fruits and vegetables (Ford & Mokdad, 2001). They are inexpensive and rich in bioactive molecules such as prebiotics, vitamins, minerals, antioxidants and anti-inflammatory agents. Fruits and vegetables help to improve the health of the gut microbiota (Maxner et al., 2020). Scientific studies show that adequate consumption of fruits and vegetables reduces the risk of metabolic diseases (Ford & Mokdad, 2001).

However, processing methods that require heat destroy the bioactive molecules in these foods. Therefore, the search for new alternatives has increased worldwide, in particular the consumption of fermented foods. The fermentation of fruits and vegetables is

booming because of the proven health benefits in terms of chronic diseases (Tan et al., 2023). This mainly lactic fermentation, known as lactofermentation, is a process converting sugars in food into organic acids by lactic acid bacteria, with the possible production of alcohol and carbon dioxide (Fessard, 2017). This process has been used for thousands of years and is thought to increase antioxidant and anti-inflammatory capacity, and improve the digestibility of food. The bioactive compounds of the fermented product are enhanced at the end of fermentation (Fessard, 2017). Lactofermentation is a method of preserving vegetables that extends their shelf life. The process, carried out by strains of lactic acid bacteria, produces lactic acid, which acidifies the environment and inhibits the growth of various pathogenic bacteria. The shelf life of plants is increased by this, even without refrigeration as is the case with fermented cabbage used in the production of sauerkraut, the kimchi (Wacher et al., 2010). In addition to its rich flavour, kimchi is also known for its health benefits, particularly its lactic acid bacteria, which promote good digestion and strengthen the gut microbiome (Wacher et al., 2010). Fermenting plants leads to a significant reduction in antinutritional compounds such as phytates and oxalates, which interfere with the body's absorption of minerals. The strains of lactic acid bacteria identified by Yao et al (2023a) demonstrate the ability to synthesize enzymes that break down antinutritional compounds. The use of these strains in a plant fermentation process could be beneficial to consumer health. Côte d'Ivoire is a country, whose economy is based on agriculture, has a diversity of neglected edible wild plant species that could contribute to the treatment of various metabolic diseases (Kouadio et al., 2022).
Work by our research team has demonstrated the efficacy of *Picralima nitida* seeds, in the treatment of diabetes in rats (Yao et al., 2023b). However, these plants are mainly used for cooking or infusion (Kouakou et al., 2020; Tra Bi et al., 2008) resulting in losses of bioactive compounds. Lactic acid bacterial fermentation, which is rapidly expanding in developed countries, could therefore be considered in Côte d'Ivoire to preserve or improve the functional properties of these foods. The aim of this work is to promote the consumption of lacto-fermented fruits and vegetables in the fight against metabolic diseases, in particular type 2 diabetes and arterial hypertension in Côte d'Ivoire.

Results and discussion

Main characteristics of Pediococcus acidilactici compared to two probiotic strains
Table 1 shows the main characteristics of *Pediococcus acidilactici* compared with two probiotic strains (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) from yoghurt marketed in Côte d'Ivoire.
The *Pediococcus acidilactici* strain showed a higher level of resistance to bile salts than the two probiotic strains. Despite the increased resistance of the yoghurt probiotics to pH 2, titratable acid production remained statistically similar for all three strains. The observation is valid for antioxidant activity as well. In terms of anti-inflammatory activity, the *Pediococcus acidilactici* strain showed higher levels than the two yoghurt strains compared. All three strains have the capacity to produce exopolysaccharides and enzymes such as amylase, cellulase, phytase and tannase. However, the enzyme production capacity of the *Pediococcus acidilactici* strain remains superior, with the exception of amylase. They are all able to inhibit the growth of pathogenic strains, with inhibition diameters ranging from 5.75 ± 0.95 cm to 18.75 ± 1.5 cm. In recent years, research has focused on the use of the *Pediococcus* genus in health and food applications as a probiotic and fermentation agent (Savedboworn et al., 2014). *Pediococcus acidilactici* is one of the species recognized for its efficacy in regulating diabetes (Bai et al., 2021). According to studies conducted by Pradhan and Tamang, 2021, this species stimulates insulin production by secreting secondary metabolites. It also helps to regulate glucose and lipid levels in diabetic patients (Rojo-Bezares et al., 2006). As a fermentative agent, it is widely used in the lactofermentation process of fruits and vegetables. During this process, *Pediococcus acidilactici* breaks down complex compounds such as dietary fibers, starch and protein into simple, easily assimilated compounds (Ajibola et al., 2023; Aka et al., 2020; Bartkiene et al., 2013). *Pediococcus* acidifies the fermentation medium, which creates less favourable conditions for the growth of other microorganisms, especially pathogens, thus contributing to the preservation of these foods (Jawan et al., 2019). Based on this, the *Pediococcus acidilactici* strain isolated from cassava paste fermentation by Yao et al. 2023a was used to ferment *Picralima nitida* fruits and *Moringa oleifera* leaves in this study. The results of the study showed a significant decrease in sugars content (total and reducing) of *Moringa oleifera* leaves and *Picralima nitida* seeds after fermentation (Table 2). This observation could be attributed to the fact that sugars present in these fruits and vegetables, such as starch and cellulose, served as substrates for the growth of the strain. By metabolising these complex sugars, *Pediococcus* acidifies the fermentation environment. Concentrations of bioactive compounds were also improved at the end of fermentation.

Nutritive and anti-nutritive properties of two plants fermented by a Pediococcus acidilactici strain
Table 2 shows nutritional and anti-nutritional characteristics of *Moringa oleifera* leaves and *Picralima nitida* seeds after fermentation by a strain of *Pediococcus acidilactici*. The results show a decrease in the concentration of reducing sugars after fermentation in *Moringa oleifera*, from 0.16±0 to 0.04±0 mg/mL, while that of *Picralima nitida* remained stable at 0.02±0 mg/mL. In *Moringa oleifera*, the total sugars concentration after fermentation dropped from 0.15±0.04 to 0.07±0.01 mg/mL, while in *Picralima nitida*, it remained in a statistically unchanged range of 0.1±0.01 to 0.08±0.01 mg/mL. It was observed that phytates content decreased after fermentation of *Picralima nitida* from 13.95±0.59 to 9.25±0.95 mg/mL. In contrast, the phytates content of *Moringa oleifera* remained almost unchanged, increasing from 19.83±0.2 to 18.47±2.03 mg/mL after fermentation. The amount of oxalates decreased significantly in both fruits and vegetables, for *Moringa oleifera* from 1290.67±50.81 to 410.67±110.73 mg/mL and for *Picralima nitida* from 660±44 to 278.67±25.4 mg/mL. These plants contain antinutrients, particularly phytates and oxalates, which can limit absorption of some minerals or interfere with digestion. Lactofermentation using *Pediococcus acidilactici* reduced the concentration of these anti-nutrients, which could improve the absorption of essential minerals. Fermenting these plants could potentially benefit people with diabetes, particularly by reducing the amount of sugars and anti-nutritional compounds. This could lead to better digestibility and absorption of nutrients, while helping to regulate blood sugar level. In this way, fermented plants can be consumed directly, unlike unfermented plants which must be cooked at high temperatures. Finally, the amount of tannins increased significantly in both fermented plants from 0.36±0.03 to 1.41±0.37 mg/mL for *Moringa oleifera* and from 0.34±0.08 to 0.94±0.03 mg/mL for *Picralima nitida*. After fermentation, polyphenol contents of both fruits and vegetables increased significantly compared to unfermented products: *Moringa oleifera* increased from 0.31±0 to 0.92±0 mg/mL and *Picralima nitida* from 0.14±0.01 to 0.82±0.01 mg/mL. In terms of flavonoids content, there was an increase in all fermented plants compared to those that were not fermented: *Moringa oleifera* from 0.06±0.01 to 0.09±0.00 mg quercitin/mL and *Picralima nitida* from 0,032±0,01 to 0,04±0,00 mg quercitin/mL. Proteins concentration increased after fermentation from 25.71±0.01 to 29.32±0.02 and from 3.58±0.16 to 5.66±0.03 for *Moringa* and *Picralima* respectively (Figure 1).

Table 1. Technological properties of *Pedioccocus acidilactici* compared to yoghurt strains.

Lactic acid bacteria strains		***Pediococcus acidilactici* (PL1)**	**yoghurt strains (*Lactobacillus bulgaricus*)**	**yoghurt strains (*Streptococcus thermophilus*)**
Health properties	Survival rate (%) to pH 2	11.30 ± 1.46^{a}	47.21 ± 0.45^{b}	45.63 ± 1.55^{b}
	Survival rate at 0.3% bile salts	85.25 ± 0.01^{c}	51.55 ± 0.83^{a}	71.35 ± 0.69^{b}
	Cell surface hydrophobicity (%)	74.60 ± 0.01^{b}	66.32 ± 1.41^{a}	76.8 ± 0.50^{c}
	Activity antioxidant (%)	31.80 ± 7.64^{a}	28.1 ± 0.95^{a}	34.75 ± 0.78^{a}
	Anti-activity inflammatory (%)	74.65 ± 8.18^{b}	63.94 ± 0.42^{b}	38.38 ± 0.47^{a}
	Exopolysaccharaides synthesis	+	+	+
Antagonist test (Inhibition diameter in cm)	*Aspergillus*	6.25 ± 1.25^{a}	13.5 ± 1.30^{b}	14.25 ± 2.06^{b}
	Staphylococcus	13.25 ± 2.22^{b}	5.75 ± 0.95^{a}	12 ± 1.83^{b}
	E. coli	7 ± 1.41^{a}	6 ± 0.82^{a}	10.25 ± 0.96^{b}
	Candida	11.75 ± 1.70^{b}	6 ± 0.82^{a}	12.75 ± 1.5^{b}
	Klebsiella	16.5 ± 1.30^{a}	18.75 ± 1.5^{b}	17.25 ± 2.06^{a}
	Salmonella	11 ± 0.81^{a}	11 ± 1.83^{a}	13.25 ± 0.96^{b}
	Pseudomonas	15.75 ± 0.96^{b}	14 ± 1.41^{a}	14.25 ± 2.06^{a}
Enzymatic synthesis and acidification	Amylases (cm)	0.6/M	1.5/M	2.1/M
	Cellulase (cm)	1.8/H	1.5/M	3.1/M
	Phytase (cm)	2.1/H	2.0/M	2.0/M
	Tannase (cm)	1.1/H	0.6/M	0.6/M
	pH at 24h	4.02 ± 0.04^{b}	3.14 ± 0.09^{a}	4.01 ± 0.02^{b}
	Titratable acidity (%)	1.84 ± 0.04^{a}	1.77 ± 0.01^{a}	1.83 ± 0.02^{a}

Producers are divided into three categories: H: high producers (diameter ≥ 1 cm), M: medium producers (diameter ≥ 0.5 cm) and L: low producers (diameter < 0.5 cm). The values correspond to the mean of the standard deviations of the measurements carried out in three replicates. Significantly different at P < 0.05 for values with similar superscript along the line. Groups a, ab, b and c show significant differences (p<0.05).

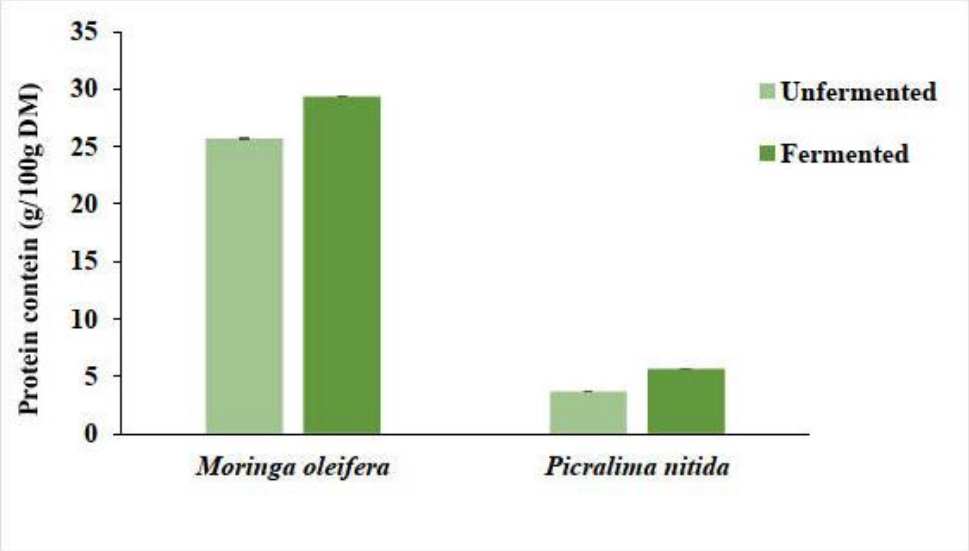

Figure 1. Protein content in fermented and unfermented matrices

Table 2. Nutritive and anti-nutritive characteristics of the two plants after controlled fermentation

Sample		Phytates (mg/mL)	Oxalates (mg/mL)	Tannins (mg/mL)	Total polyphenols (mg/mL)	Flavonoids (mg Quercetin/mL)	Reducing sugars (mg/mL)	Total sugars (mg/mL)
Moringa oleifera	Unfermented	19.83±0.2^{ab}	1290,67±50.81^{ab}	0.36±0.03^{ab}	0.31±0^{ab}	0,06±0,01^{ab}	0.16±0^{ab}	0.15±0.04^{ab}
	Fermented	18.47±2.03^{ab}	410,67±110.73^{a}	1.41±0.37a	0.92±0^{a}	0,09±0,00^{a}	0.04±0^{a}	0.07±0.01^{a}
Picralima nitida	Unfermented	13.95±0.59^{ab}	660±44^{ab}	0.34±0.08^{ab}	0.14±0.01^{ab}	0,03±0,01^{ab}	0.02±0^{ab}	0.1±0.01^{ab}
	Fermented	9.25±0.95^{a}	278,67±25.4^{a}	0.94±0.03^{a}	0.82±0.01^{a}	0,04±0,00^{a}	0.02±0^{ab}	0.08±0.01^{ab}

Data in the table are expressed as mean with standard deviation (±SD). Groups a, ab and b show significant differences (p<0.05).

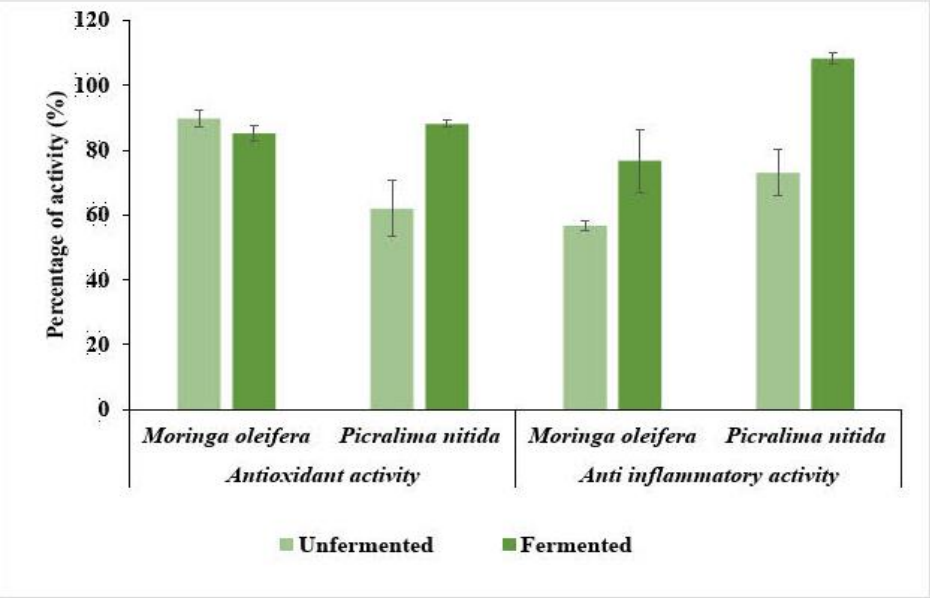

Figure 2. Antioxidant and anti-inflammatory properties of the two plants after controlled fermentation

Table 3. Vitamin and mineral contents of fermented and unfermented matrices.

Sample		Vitamin and mineral contents (mg/100 DM)								
		Vit. C	Vit. D	Vit. E	Vit. A	Cr	Mg	Na	K	Zn
Picralima nitida	Unfermented	1.94± 0.34[a]	0.03± 0.00[a]	0.23 ±0.02[a]	0.28 ±0.03[a]	2.63 ±0.03[a]	7.12 ±0.02[a]	2,36±0, 03[a]	564,3±0 ,2[a]	1.01 ±0.03[a]
	Fermented	3.31± 0.34[b]	0.03± 0.00[a]	0.24 ±0.03[a]	0.22 ±0.02[a]	3.33 ±0.02[a]	9.65 ±0.03[b]	2,57±0, 02[b]	614,57± 0,15[b]	1.23 ±0.03[b]
Moringa oleifera	Unfermented	3.60± 0.17[c]	0.32± 0.02[b]	114.33 ±2.00[b]	17.34 ±2.00[b]	79.80 ±0.20[b]	659.50± 0.30[c]	75,84± 0,02[c]	1002,1± 0,1[c]	2.15 ±0.03[c]
	Fermented	4.58± 0.17[d]	0.34± 0.02[b]	120.35 ±2.00[c]	18.20 ±2.00[b]	89.00 ±0.91[c]	779.50± 0.30[d]	86,5±0, 1[d]	1364,2± 0,2[d]	2.76 ±0.03[d]

The values are means standard deviations of triplicate measurements. Values with similar superscript alphabet letter along the column are significantly different at $P < 0.05$.

Antioxidant and anti-inflammatory properties of the two fermented plants

Figure 2 shows the anti-inflammatory and antioxidant activities of fermented and non-fermented plants. Anti-inflammatory and antioxidant activities are initially present in all matrices. After the fermentation process, it was observed that fermented plants had a higher content of anti-inflammatory compounds than non-fermented plants, with percentages ranging from 55.6% to 69.8% for *Moringa oleifera* and from 67.9% to 109.4% for *Picralima nitida*. These results also confirm that the fermentation approach using the *Pediococcus* strain improves the antioxidant activity of fermented plants compared to non-fermented plants, including *Picralima nitida* (from 61.97±10.52 to 88.06±1.22%) and showed a slight increase in *Moringa oleifera* (from 85.13±3.11 to 89.71±3.04 %). In fact, polyphenol concentrations increased from 0.14±0.01 to 0.82±0.01 mg/mL after fermentation of *P. nitida* fruits, i.e. more than five (5) times in the fermented sample, and from 0.31±0 to 0.92±0 mg/mL in *M. oleifera* leaves, i.e. three (3) times. The fermented products had about three times higher tannin concentrations. These results are justified by the percentage inhibition of the DPPH radical, which showed 42.1% increase of antioxidant activity after fermentation of *P. nitita* seeds and 5% increase of this activity for fermented *M. oleifera* leaves. Anti-inflammatory activity also increased by 61.1% in fermented *P. nitida* seeds and by 25.5% in fermented *M. oleifera* leaves. These results are confirmed by many studies (Sossa-Vihotogbé et al., 2015).

Vitamins and minerals composition

Vitamins and minerals average content of the selected plants is shown in Table 3. After fermentation, the fermented matrices were found to have higher vitamins and minerals content than the unfermented matrices. The vitamin concentrations in *Moringa* increased from 1.94±0.34 to 4.58±0.17 mg/100 dry matter (DM) for vitamin C, from 0.03±0.00 to 0.34±0.02 mg/100 DM for vitamin D, from 114.33±2.00 to 120.35±2.00 mg/100 DM for vitamin E, and from 17.34±2.00 to 18.20±2.00 mg/100 DM for vitamin A. However, statistical analysis showed no significant difference between the mean values for vitamins D and A. For the same matrix, mineral concentrations of determined increased significantly after fermentation, namely chromium (from 79.80 ± 0.20 to 89.00 ± 0.91 mg/100 g DM); magnesium (from 659.50 ± 0.30 to 779. 50 ± 0.30 mg/100 g DM); sodium (from 75.84 ± 0.02 to 86.5 ± 0.1 mg/100 g DM); potassium (from 1002.1 ± 0.1 to 1364.2 ± 0.2 mg/100 g DM) and zinc (from 2.15 ± 0.03 to 2.76 ± 0.03 mg/100 g DM).

For *Picralima* fruit, concentrations of vitamin C and minerals including magnesium, sodium, potassium and zinc increased significantly after fermentation respectively, from 1.94±0.34 to 3.31±0.34 mg/100 g DM; from 7.12±0.02 to 9.65±0.03 mg/100 g DM; from 2.36±0.03 to 2.57±0.02 mg/100 g DM; from 564.3±0.2 to 614.57±0.15 mg/100 g DM; and from 1.01±0.03 to 1.23±0.03 mg/100 g DM. No significant differences were observed between the mean vitamins D, E, A and chromium contents of this fruit after fermentation. The levels of some vitamins (C and E) and minerals (chromium, magnesium, zinc, sodium and potassium) were found to be above the limits set before fermentation. These results show that fermentation with *Pediococcus acidilactici* is beneficial for this fruit and vegetable, as

these compounds are thought to be involved in the treatment of a number of chronic diseases, particularly diabetes. Alfahel et al, (2023) and Deledda et al, (2021) have highlighted the beneficial effects of bioactive compounds on diabetic parameters such as glycaemia and insulin. These compounds stimulate insulin synthesis and help insulin bind to receptors, thereby reducing hyperglycaemia. Previous studies have also shown that *M. oleifera* leaves and *P. nitida* seeds are effective in the treatment of diabetes (Feyisayo & Victor, 2019; Alhassan, 2017; Ishiekwene et al., 2020; Yao et al., 2023b).

Therefore, it is possible that these optimized fermented foods may prove to be highly effective in the treatment of this pathology. Today, fermented foods are becoming more popular than natural foods due to their high nutritional value. In short, lacto-fermentation allows plants to be preserved for longer. This process, carried out by strains of lactic acid bacteria, produces lactic acid, which acidifies the environment and inhibits the growth of many pathogenic bacteria. This extends the shelf life of plants, even without refrigeration, as in the case of cabbage fermented to make sauerkraut. This technique optimizes the bioactive compounds in the food (Varzakas et al., 2017). As a result, the fermented seeds of *P. nitida* and the fermented leaves of *M. oleifera* could be potential substitutes for some compounds used in foods and in the treatment of diabetes. Probiotic fermentation products promote a healthy balance in the intestinal flora. These beneficial bacteria can help to improve digestion, boost the immune system and prevent digestive disorders such as constipation and diarrhoea. During fermentation, *Pediococcus acidilactici* breaks down many complex plant components, making several nutrients more bioavailable.

The fibres in these plants are said to be partially broken down, making them more digestible (Shahidi & Pan, 2022). In addition, the fermentation of these two plants by this probiotic strain increased the levels of many vitamins, such as vitamin C, vitamin E and vitamin A, and minerals, including chromium, magnesium, sodium, potassium and zinc. Lactofermentation could also reduce food waste. By transforming surplus crops or products at risk of spoilage, this process offers a method of recovery that reduces food waste. It also promotes a more natural approach to food preservation without recourse to chemical preservatives (Fessard et al., 2016). In short, lactic fermentation of plants not only improves the preservation and digestibility of food, but also enriches its nutritional profile and creates tastier products that are beneficial for gut health (Fessard et al., 2016). For example, fermented plants could be eaten directly, as opposed to unfermented plants that require high-temperature cooking. This technique would optimize the bioactive compounds in food.

Materials and methods

Plant materials

The biological material consisted of fruits and vegetables (*Moringa oleifera* leaves and *Picralima nitida* seeds) and a starter strain of lactic acid bacteria (PL1). Fruits and vegetables were purchased at the Adjamé market (Abidjan, Côte d'Ivoire) and then transported to the National Floristic Centre of the Félix Houphouët-Boigny University (Abidjan) for identification using analytical flora. The starter strain of lactic acid bacteria strains was isolated from fermented cassava paste and identified as *Pedioccocus acidilactici* by 16S RNA sequencing (Yao et al., 2023a). This starter with probiotic abilities was selected because, in addition to its interesting technological properties compared to the two yoghurt probiotics (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) already published (Yao et al., 2023a), it has the ability to grow on plant matrices.

Lactofermentation process

Fruits and vegetables arriving at the laboratory were inspected for any defects or imperfections, then treated with 70% bleach and carefully rinsed with tap water. The samples were then crushed in a porcelain mortar and ground in a blender with distilled water at a ratio of 1:2 (w/v). The resulting homogenate was inoculated with 10^7 cells of the selected probiotic lactic acid bacterial strains. This probiotic starter (*Pedioccocus acidilactici*) was selected for its interesting technological properties (Yao et al 2023a). The strain was pre-cultured for 24 hours at 30°C in MRS culture medium from pure colonies. A 50 µL volume of the pre-culture was then combined with 50 µL of methylene blue and placed on a Thomas cell counting slide to quantify viable cells. The sample was observed under a light microscope and the cell count was performed directly at ×40 objective, taking into account the quadrants of the Thomas cell. The bacterial cell concentration (cells/ml) was then calculated using the following equation: $NC = n \times 4 \times 10^6 \times fd$ (where NC is the number of cells, n is the average number of cells and fd is the dilution factor).

Subsequently, 30 g of each type of plant material was inoculated with a load of 10^7 cells. Fermentation was carried out for 48 hours at 30°C. After preliminary tests, *Pedioccocus acidilactici* (PL1) was selected for the fermentation of *Moringa oleifera* leaves and *Picralima nitida* seeds. At the end of the fermentation process, the fermented matrices were freeze-dried in 250 mL containers, each containing 30 g in the freeze-dryer (Biobase) set at a temperature of -45°C and a pressure of 1Pa for 48 hours, after freezing at -60°C for 8 hours. The dry product was then ground using a blender and stored for biochemical and nutritional analysis.

Determination of phytochemical compounds, total sugars, antioxidant and anti-inflammatory activities

Phenolic compounds were extracted with methanol and its content was determined by the Folin-Ciocalteu method according to Singleton et al. (1999). Flavonoids were determined by the method described by Meda et al. (2005). Tannins were determined according to Broadhurst and Jones (1978). The anti-free radical activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) as a relatively stable free radical according to the protocol described by Benhammou et al. (2007). In this test, antioxidants reduce the purple DPPH to a yellow compound and the intensity of the colour is inversely proportional to the proton donating capacity of the antioxidants in the medium (Benhammou et al, 2007). The anti-inflammatory activity of the samples was determined using the bovine serum albumin (BSA) proteins denaturation inhibition assay described by Anoop and Bindu (2015). Total sugars content was determined using the phenol-sulphuric acid method described by Dubois et al. (1956). The quantification of reducing sugars was performed according to Benfield (1955), which is based on the reducing properties of sugars.

Anti-nutritional factors determination

The phytates assay was carried out according to the method of Latta and Eskin (1980) using Wade's reagent. One gramme of dried and ground sample was homogenised in 20 mL of HCl (0.65N) with stirring for 12 hours at room temperature. After centrifugation at 12,000 rpm for 40 minutes, 5 mL of the supernatant were removed and added to 3 mL of Wade's reagent. The mixture was then allowed to stand for 15 min and the OD was read on a spectrometer at 490 nm against the control. Finally, a calibration series was performed with sodium phytate at 10 µg/mL.

The method used for the determination of oxalates is described by Day and Underwood (1986). One (1) gramme of sample powder was homogenized in 15 mL of H_2SO_4 (3M) under magnetic stirring for one hour. The resulting mixture was filtered through Whatman paper. The filtrate was hot titrated with a solution of $KMnO_4$ (0.05M) until the colour changed to a persistent pink.

Determination of mineral and protein content

The minerals content was determined using a VARIAN AAS20 air-acetylene flame atomic adsorption spectrophotometer according to the method described by AOAC (1990).

Ground sample quantity of 0.3 g was weighed into a porcelain crucible and then placed in a muffle furnace (Prolabo) at 650°C for 5 h. After cooling, 5 mL of nitric acid (1 mol L^{-1}) was added to the ash obtained and then completely evaporated in a sand bath. To the residue was added 5 mL of hydrochloric acid (0.1 mol L^{-1}) and the whole was returned to the oven at 400°C for 30 min. The final residue was recovered with 10 mL hydrochloric acid (1 mol L^{-1}) and poured into a 50 mL flask. The crucible was rinsed twice with 10 mL hydrochloric acid. The flask was filled to 50 mL with hydrochloric acid. A blank test was performed under the same conditions (IITA, 1981; AOAC, 1990).

The minerals assayed were sodium, potassium, chromium, magnesium and zinc. After mineralization, concentration ranges of each mineral were prepared according to the reference methods for the quantitative determination of chemical elements in plants (IITA, 1981).

Statistical analysis

The characteristics of neglected wild edible fruits and vegetables were measured before and after lacto-fermentation. All experiments were repeated three times and the findings were reported as the mean value ± standard deviation. In order to identify significant variations between the means, a one-factor analysis of variance (ANOVA) was conducted using XLSTAT version 19.0 software. Duncan's test was used at a probability threshold of $\alpha = 0.05$.

Conclusion

This study of Pediococcus acidilactici controlled lacto-fermentation of two neglected edible plants in Côte d'Ivoire highlighted the potential impact of this method on bioactive compounds useful in the treatment of diabetes. After fermentation, the concentration of these bioactive compounds was increased, optimizing the potential anti-diabetic properties of *P. nitida* seeds and *M. oleifera* leaves. The use of a potential probiotic, such as *Pediococcus acidilactici*, not only helped preserve the nutritional qualities, but also enhanced the health benefits, especially in the management of diabetes. These results suggest that these fermented foods could open up new perspectives in the natural management of this disease, while promoting the sustainable use of Ivorian agricultural resources. Future studies should focus on *in vivo* validation of these effects, as well as developing standardized fermented products for dietary interventions.

Acknowledgements

The authors are grateful to the Fund for Science, Technology and Innovation (FONSTI) for the funding of this research project.

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