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RESEARCH ARTICLE

The role of *Lactobacillus casei* bacteria in improving the microbial and sensory properties of fermented millet puree

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Abstract

This study aimed to prepare functional foods from local proso millet puree (from whole grains, crushed grains and millet flour) fermented with *Lactobacillus casei* bacteria and to follow up on its role in improving the properties of the puree. Fermentation was carried out at 37n° C for 24 and 48 hrs using (0, 2, 4, 6 %) pre-activated *Lactobacillus casei* in liquid MRS medium and skim milk, respectively. The results showed an increase in the number of starter bacteria with an increase in the inoculation rate and incubation time. The highest concentration was reached when using millet ground at an inoculation rate of 6 % 24 and 48 hrs after the end of fermentation, as it was 8.86 and 9.75 CFU/g. The pH dropped differently depending on how many bacteria were added and how long the fermentation took. The lowest pH was recorded when 6 % bacteria were added to the flour and after 24 hrs of fermentation, it reached 3.09. There were significant differences at this level (P≤0.05). The total phenol levels went up in both the water-based and alcohol-based extracts of the puree, with the alcohol extract showing the highest levels after the two fermentation periods. The highest levels were found in the pureed millet at a 4 % inoculation rate after 48 hrs, where they were (638.12, 801.58 µg/g), but they also went down. Following 6 % in the whole grain alcoholic extract after 24 and 48 hrs, at 524.28 and 931.72 g/g, respectively, the water-based extract of whole grain puree and crushed extract contained the highest number of tannins. The fermented flour had the lowest amount of tannin. With different inoculation rates and fermentation times, the levels of tannins increased while the levels of phytates varied. After 48 hrs, the puree of flour treatments at all inoculation rates had the highest decrease (275.1, 264.5 and 486.7 g/g, respectively), followed by the whole grain treatments at 4 % inoculation rate (264.5 and 624.2 g/g) respectively. Finally, all the purees in general achieved the highest sensory ratings and general acceptance compared to the control treatments, with significant differences (P< 0.05) among the many sensory attributes used in sensory evaluation.

Keywords: flour; *Lactobacillus casei*; microbial characteristics; millet crushed; millet puree; sensory evaluation; whole grain

Introduction

Cereal crops are the mainstay of human food, as they topped the list of global agricultural production due to their suitability to climate and soil conditions, as well as their ease of handling in terms of service and care, ease of storage and transport and meeting three-quarters of an individual's energy needs and more than half of his protein needs. They are a safety valve that ensures food security and stability in the world (1, 2). Millet belongs to the grass family (Gramineae or Poaceae), which includes nine different genera. It is one of the oldest grains that appeared 10000 years before wheat and rice and it may be the first grain used for domestic purposes. It is a summer grass crop grown for the purpose of producing grains and fodder in most dry areas of the world and is ranked sixth in the world in terms of quantity and production. It enjoys several economic advantages compared to other grains, such as high resistance to pests and diseases, the ability to adapt to climate change, its short growth period (60-90 days), its low water requirements and its protein and fat content that exceeds several other grains. It reached 10.58-11.93 % and 3.77-6.15 %, respectively, in addition to being free of gluten, which made it a favourite crop for many farmers and those suffering from celiac disease, especially in India, Africa and China, as it provided consumers with the energy and protein requirements that they needed. They desperately need it at low prices and various traditional products such as soup and non-traditional products such as baby food are made from it. It has functional properties that allow it to be applied in the production of gluten-free baked goods (3, 4).

Growing awareness of therapeutic nutrition has increased consumer demand for functional and healthy foods that are higher in antioxidants, fibers, minerals, etc. Manufacturers of these products have looked to untapped and unexplored crops, such as millets, which have great potential to grow such functional products. The use of whole grains directly from millet is usually excluded due to their high content of anti-nutritional factors (5, 6). Fermentation is one of the oldest methods used to process grains, such as sorghum and millet. It has been used successfully to make

grains healthier by increasing their protein content and making the protein easier for pepsin to digest. This increases the amount of available lysine and its nutritional value while also lowering the activity of trypsin inhibitors (TIA). Amylase inhibitors and other nutritional inhibitors (7, 8). Many microbial strains have been used to ferment millet, such as Saccharomyces cerevisiae yeast and Lactobacillus lactic acid bacteria, including Lactobacillus casei and Lactobacillus brevis, which have been widely used for this purpose (9-11). We didn't have a lot of scientific or nutritional research on this crop in our country or nearby countries, so we did this fermentation experiment by adding different amounts of Lactobacillus casei bacteria to whole millet grains, crushed millet (100 % extract) and 70 % extracted millet flour. The goal was to lower the nutritional inhibitors and improve the taste of the puree.

Material and Methods

Preparation of proso millet grains and bacteria: Proso millet grains (*Panicum miliaceum*) were purchased from local markets in Baghdad, Iraq. The method described previously (1) was followed in preparing the grains by removing all non-grain contaminants, such as straw and stones, as well as other grains other than proso millet grains, through winnowing and sifting and ending with manual isolation. The grains were washed using running water to remove clay residues, shells and empty (floating) grains and dried in the incubator at 40 °C for 24 hrs. The dry samples were stored in polyethylene bags in the refrigerator at a temperature of 5 ± 2 °C until use in the Grain Technology Laboratory/Department of Food Sciences/College of Agricultural Engineering Sciences/University of Baghdad.

The capsules of the *Lactobacillus caesi* bacteria strain (*Lactobacillius paracasei* CNCM I-1572), which were made by Sofar S.P.A., were used to ferment the millet puree that was being studied. The capsules were activated several times in MRS Broth medium and sterilized skim milk. The activated bacterial inoculum was added to the millet puree (whole grain, crushed grain and flour) at a rate of 0, 2, 4 and 6%.

Proso millet crushed and flour preparation: Crushed whole grain proso millet and 70 % extracted flour were prepared using the (1) method.

Preparation of crushed (ground) millet: The dried proso millet grains prepared above were crushed using a home coffee grinder to produce whole grain ground (powder) with a 100 % extraction rate. The sizes of the particles were standardized by passing all the resulting crushed substances through a No. 70 sieve (with an aperture size of 212 µm) and storing the result in bags at a temperature of -18 °C.

Preparation of millet flour: The moisture of the millet grains was raised to 10 % by adding water to them in two batches for 18 hrs at room temperature, in closed containers, with regular stirring every 2 hrs. Using a laboratory mill from the German company Brabender called the Quaternary Brabender Mill, the technical grinding process on moistened millet grain samples produced flour with a 70 % extraction rate. This process was followed by a sifting process through a No. 70 sieve and the resulting flour was stored in bags at a temperature of -18 °C.

Preparation of proso millet puree: The method of (12) was followed with some modifications. 100 g of intact, crushed and floured Proso millet grains were mixed in sterile, sealed glass jars individually with 100 mL of distilled water [ratio (50:50) (W:V)]. The *L. casei* bacteria inoculum was added at a rate of 0, 2, 4 or 6% by volume of the mixture. The mixture was then left to ferment in glass jars at 37 °C for 24 to 48 hrs.

Experimental design: The parameters were created and distributed according to the table shown below.

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
0	WM10	WM20	GM30	GM40	PM50	PM60
2	WM12 WM	WM22 FM	GM32 GM	GM42 FM	PM52 FM	PM62 FM
4	WM14	WM24	GM34	GM44	PM54	PM64
6	WM16	WM26	GM36	GM46	PM56	PM66

Where it represents: WM10 = whole grain treatment (control) at 0 % inoculation rate after 24 hrs. WM12 = whole grain treatment at a 2 % inoculation rate after 24 hrs. WM14 = whole grain treatment at a 4 % inoculation rate after 24 hrs. WM16 = whole grain treatment at a 6 % inoculation rate after 24 hrs. WM20 = whole grain treatment (control) at 0 % inoculation rate after 48 hrs. WM22 = whole grain treatment at a 2 % inoculation rate after 48 hrs. WM24 = whole grain treatment at a 4 % inoculation rate after 48 hrs. WM26 = whole grain treatment at a 6 % inoculation rate after 48 hrs. GM30 = crushed millet treatment (control) at 0 % inoculation rate after 24 hrs. GM32 = millet-crushed treatment at a 2 % inoculation rate after 24 hrs. GM34 = millet-crushed treatment at 4 % inoculation rate after 24 hrs. GM36 = millet-crushed treatment at 6 % inoculation rate after 24 hrs. GM40 = crushed millet treatment (control) at 0 % inoculation rate after 48 hrs. GM42 = millet-crushed treatment at a 2 % inoculation rate after 48 hrs. GM44 = millet-crushed treatment at 4 % inoculation rate after 48 hours. GM46 = millet-crushed treatment at 6% inoculation rate after 48 hrs. PM50 = millet flour treatment (control) at 0 % inoculation rate after 24 hrs. PM52 = millet flour treatment at a 2 % inoculation rate after 24 hrs. PM54 = millet flour treatment at 4 % inoculation rate after 24 hrs. PM56 = millet flour treatment at 6 % inoculation rate after 24 hrs. PM60 = millet flour treatment (control) at 0 % inoculation rate after 48 hrs. PM62 = millet flour treatment at a 2 % inoculation rate after 48 hrs. PM64 = treatment of millet flour at a 4 % inoculation rate after 48 hrs. PM66 = millet flour treatment at 6 % inoculation rate after 48 hrs (13).

Estimation of the number of *Lb. casei* bacteria: Using the conventional plate technique and solid MRS medium, a previous study has estimated the total number of *Lactobacillus casei* bacteria (14). Plates grown with bacteria were incubated at 35 °C under anaerobic conditions for 24 and 48 hrs of fermentation.

Determination of pH: A portable HANNA digital pH meter was used by placing the bulb of the meter directly in the puree and recording the reading.

Extraction of phenolic compounds: To get phenolic compounds from millet and find out what nutritional inhibitors it has, the methods previously described were utilised (15, 1). Two types of extraction solutions were prepared (aqueous and 70 % alcoholic). 40 mL of each was added separately to 10 g of millet transactions [1:4 (w/v)] in 50 mL tubes, mixed well and incubated in a water bath at a temperature of 60 °C for 20 min. After centrifuging the tubes at 3500 x g for 10 min, the solvent layer with the phenolic extract was taken off and put into new 50 mL tubes that were clean, weighed and labelled. These tubes were then put in the fridge until the tests were done.

Estimation of the total phenolic content: To find out how much total phenolic content was in the extracts being studied, the Folin-Ciocalteu assay at a concentration of 1N and the standard curve of the standard gallic acid at a concentration of 200 µg/ml were both used (1). 1 mL of alcoholic and aqueous extract were added separately in 2 µm into 10 mL test tubes. 7.5 mL of distilled water was added to each tube, then 0.5 mL of Folin-Ciocalteu reagent solution was added, mixing using a vortex after each addition. 1 mL of 7% sodium carbonate solution, Na_2CO_3, was added, then the tubes were closed and incubated in a water bath at a temperature of 25 °C for 5 min. The optical absorption was read at a wavelength of 600 nm after zeroing the device using a control sample with distilled water or alcohol, depending on the extract. The reading for each concentration was subtracted from the blank comparison tube solution, which was made up of 8.5 mL of water, 0.5 mL of reagent and 1 mL of sodium carbonate. The sample's total phenol content was then given as mg gallic acid equivalent/100 g millet weight.

Estimation of tannins: Estimating tannins: The Folin-Ciocalteu assay with a concentration of 1N and the standard curve of the standard tannic acid at a concentration of 200 µg/mL were both used to find out how much tannic acid was in the extracts that were being studied, following the steps outlined (1). Two sets of 0.5 mL alcoholic and aqueous extracts were added to 10 mL test tubes. Next, 2.5 mL of Folin's reagent and 5 mL of sodium carbonate Na_2CO_3 were added and the mixtures were mixed with a vortex after each addition. The tubes were closed and incubated in a water bath at 25°C for 20 min. The optical absorbance was read at a wavelength of 765 nm for each dilution after zeroing the device using distilled water or alcohol, depending on the type of extract. Then, the result for each concentration was subtracted from the blank comparison tube solution, which was made up of 0.5 mL of water, 2.5 mL of reagent and 5 mL of sodium carbonate. This gave the total phenolic content in the sample, which was given as mg tannic acid equivalent/100 g millet weight.

Determination of phytic acid: To find out how much phytic acid was in the samples of proso millet that were being studied, a standard method was used (16). The standard curve for sodium phytate at a concentration of 112 µg/mL was used. 10 mL of an extraction solution containing 2.4 % HCl was added to 0.5 g of dried specimens in 25-mL sealed glass test tubes. This was done twice for each specimen. The specimens were left to extract for 18 hrs on a magnetic stirrer at room temperature, then centrifugation was performed at 11180 xg for 20 min at 25 °C. The liquid was separated and added to other tubes containing 1 g of table salt and mixed well with Vortex, then the tubes were subjected to freezing at a temperature of -20 °C for 60 min. Centrifugation was repeated after thawing the tubes under the same conditions, with 1 mL of the liquid drawn and diluting it 25 times with distilled water. 3 mL of this diluted solution was mixed with 1 mL of pink wade reagent in a test tube and the intensity of the pink color of the diluted tubes was measured in a spectrophotometer at a wavelength of 500 nm. The sample reading was subtracted from the blank comparison tube solution (3 mL of water and 1 mL of reagent).

Sensory evaluation of millet puree: The sensory evaluation questionnaire described by Dey et al. (2017) was followed to evaluate whole-grain, crushed and flour proso millet puree fermented by *Lb. casei* bacteria at a temperature of 37 °C for 24 and 48 hrs according to the characteristics of appearance, texture, hardness and taste. The smell and general acceptance were recorded in the questionnaire at a rate of 10 marks for each characteristic.

Statistical analysis: The Statistical Analysis System (2018) (SAS) was used to look at the data and see how different factors affected the characteristics that were being studied. The study used a completely randomized design (CRD) and the least significant difference test (L.S.D.) was used to compare the differences between the means at the 0.05 level of significance.

Results and Discussion

The impact of the fermentation procedure on the proliferation of the *Lactobacillus casei* bacterium

Table 1 displays the quantities of lactic acid bacteria found in the puree made from whole grains, the crushed puree and the proso millet flour puree. These purees were fermented separately for 24- and 48-hrs using *Lb. casei* bacteria, with inoculation rates of 0, 2, 4 and 6 %. It was seen that the number of bacteria in all treatments (MW10, MW20, MG30, MG40, PM50 and PM60) was much higher after 24 and 48 hrs

Table 1. The initial bacterial count in millet puree fermented with Lb. casei at 24 and 48 hrs of fermentation

Bacterial starter (%)	Starter bacterial count (Log CFU/g)					
	Whole grain millet		Ground millet		Peeled millet	
	24H	48H	24H	48H	24H	48H
0	2.30 [a]	2.44 [b]	2.36 [b]	2.68 [b]	2.38 [b]	2.26 [b]
2	8.32 [a]	8.34 [a]	8.76 [a]	9.22 [a]	8.52 [a]	8.67 [a]
4	8.41 [a]	8.59 [a]	8.80 [a]	9.42 [a]	8.56 [a]	8.70 [a]
6	8.58 [a]	8.49 [a]	8.86 [a]	9.75 [a]	8.75 [a]	8.82 [a]
L.S.D.	2.037 *	2.492 *	2.378 *	2.761 *	2.669 *	2.509 *
	(P≤0.05) *					

of fermentation compared to the control treatments. The values recorded were 2.30, 2.44, 2.36, 2.68, 2.38 and 2.26. The percentage increase after 24 hrs was 6 % and the bacterial count after 48 hrs was 9.75-9.42 cfu/g. There were no notable disparities among the treatments in which the initiator was included. The bacterial counts had the greatest rise in the treatments using crushed millet puree, as opposed to the treatments involving whole grain puree and millet flour puree. The *Lb. casei* bacteria may readily get nutrients due to their greater exposure to a wider surface area compared to the whole grain treatments. Fibers have a more significant function as prebiotics in crushed millet therapies, as opposed to millet flour treatments, where most of the components consist of starch (17, 2).

Arora et al. (2009) (18) found that using ground whole-grain pearl millet increased the amount of *Lb. acidophilus*, a type of probiotic bacteria. These results back up what they found. This increase was attributed to a decrease in pH and an increase in acidity, which occurred because of the breakdown of the cell walls. Enzymes from inside the plant and bacteria broke down chains of starch and proteins. This allowed nutrients that were bound with fiber and phenolic compounds to escape. This, in turn, created an improved environment for growth. Lactic acid bacteria and some yeasts were used in the fermentation of grains to produce specialized (functional) meals that included active components with functional properties. They improve the performance of foods and help probiotics get to where they need to go in a living thing, where they can help it grow and keep a population of viable strains at about 106-107 CFU/g (19-21).

pH: Table 2 shows the pH levels of the three types of proso millet puree that were being studied, along with the rates of addition (0, 2, 4, 6 %) that were seen over 24 and 48 hrs. Overall, it is evident that the pH declines as the inoculation rate and fermentation period increase. The greatest pH values were observed in the whole grain control puree treatment (WM20), while the lowest values were found in the 6% millet flour puree treatment (PM56). The pH reduction seen during fermentation is a result of the use of nutrients, particularly sugars, by the introduced bacteria. This process leads to the formation of organic acids and carbon dioxide. Considering this, the pH reduction in the flour puree was more significant compared to that of the crushed puree and the crushed puree had a bigger pH reduction than the whole grain puree after 48 hrs of fermentation. The most substantial reduction in pH occurred after 24 and 48 hrs of fermenting the flour puree in the treatments (PM56, PM64 and PM62), with pH values of 3.09, 3.62 and 3.69, respectively. These differences between the treatments were statistically significant (P≤0.05).

The pH scale measures the level of acidity in a substance and reflects the presence of several components that might influence it, including sugars, salts and organic acids like lactic and acetic acid. These organic acids are produced via the fermentation of carbohydrates by bacteria that are intentionally introduced. The work of endogenous enzymes, which are naturally present in the grain and exogenous enzymes, which are produced by microorganisms that are introduced or present as contaminants, plays a crucial role in this process. Microorganisms facilitate the decomposition of carbohydrates, proteins and anti-nutrients via the process of fermentation (17). The findings were consistent with the results reported (16). According to their statement, throughout the fermentation process, the pH of the dough decreases as the yeast consumes the sugars, converting them into CO_2 gas and other organic acids. Simultaneously, the dough acquires more reological and nutrients. The results were like those of Thirumangaimannan and (22) study, which showed that the pH value dropped as the fermentation time went up for both pearl and finger millet and maize. This occurred due to the bacteria's secretion of organic acids during the process of breaking down monosaccharides and some polysaccharides.

Quantification of phenolic compounds in both aqueous and alcoholic extracts: It is shown in Tables 3 and 4 how many phenols are in millet puree made with varying amounts of Lb bacteria (0 %, 2 %, 4 % and 6 %) added to water and alcohol-based extracts. Almost throughout the duration of 24 to 48 hrs of fermentation. Table 3 shows that the water extract from the whole grain (WM) and crushed proso millet (GM) treatments, which both had bacteria added to them, had more phenols than the control treatments. The percentage of growth varies based on the amount of the introduced bacteria, as shown in the table. There was a reduction in the overall phenol levels in all flour treatments and in both extracts. The millet puree treatments (GM42, GM44 and GM46) exhibited the most significant rise in total phenol contents after 48 hrs of fermentation. After 24 hrs of fermentation, the treatment (GM36) had the smallest rise, measuring 523.06, 638.12 and 526.02 µg. The increments were 8.12 %, 24.69 %, 14.65 % and 23.00 %, respectively.

Phenol levels exhibited a rise after 24 hrs of fermentation in all addition rates, except for the ground millet mash treatment (GM34). The treatment that showed the greatest growth was MG44 after 48 hrs of fermentation and the percentage increase in the treatments was seen in GM42, GM44 and GM46. Following a fermentation period of 48 hrs, percentages of 17.61, 19.04 and 17.67 % were observed. Similarly, after 24 hrs of fermentation, the whole grain mash in the two treatments (WM12, WM14) had an increase of either

Table 2. pH measurements of millet purée during fermentation with *Lb. casei* bacteria were taken at 24 and 48 hrs

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24h	48h	24h	48h	24h	48h
0	4.87 ^{a}	5.54 ^{a}	4.83 ^{a}	4.53	4.62 ^{ab}	4.24
2	4.04 ^{ab}	4.32 ^{b}	4.32 ^{ab}	4.02	3.86 ^{b}	3.69
4	4.02 ^{ab}	4.00 ^{b}	4.06 ^{b}	4.29	4.72 ^{a}	3.62
6	3.95 ^{b}	3.93 ^{b}	4.23 ^{ab}	3.98	3.09 ^{b}	3.75
L.S.D.	0.873 *	1.026 *	0.702 * (P≤0.05) *	0.698 NS	0.846 *	0.658 NS

Table 3. The phenolic concentrations (in µg/g) in an aqueous extract of millet puree fermented with *Lb. casei* bacteria were measured at 24 and 48 hrs of fermentation

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24h	48h	24h	48h	24h	48h
0	197.63 [b]	291.46 [ab]	405.04 [c]	480.59 [c]	420.84 [a]	475.16 [a]
2%	238.12 [a]	301.33 [ab]	483.06 [b]	523.06 [bc]	329.98 [bc]	315.65 [bc]
4%	245.04 [a]	274.27 [b]	408.49 [c]	638.12 [a]	288.86 [c]	292.44 [c]
6%	239.11 [a]	347.26 [a]	526.02 [a]	563.06 [b]	349.73 [b]	378.86 [b]
LSD	36.74 *	52.68 *	39.82 *	42.861 *	41.57 *	63.72 *
			(P≤0.05) *			

Table 4. The phenolic concentrations (in µg/g) in an alcoholic extract of millet puree fermented with *Lb. casei* bacteria were measured at 24 and 48 hrs of fermentation

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24h	48h	24h	48h	24h	48h
0	605.04 [b]	692.94 [a]	698.37 [b]	648.99 [b]	831.70 [a]	826.77 [a]
2%	732.94 [a]	463.31 [b]	779.36 [a]	787.75 [a]	706.27 [b]	631.70 [c]
4%	699.85 [a]	435.21 [b]	468.00 [c]	801.58 [a]	714.92 [b]	528.49 [d]
6%	451.46 [c]	631.70 [b]	799.11 [a]	788.25 [a]	733.43 [b]	705.28 [b]
L.S.D.	47.38 *	51.02 *	48.09 *	61.28 *	47.95 *	67.44 *
			(P≤0.05) *			

17.45 or 13.55 %. Ultimately, two specific measurements of milled grain mash (GM32 and GM36) observed a rise. Every other therapy showed a reduction in comparison to the control treatment, with percentages ranging from 10.39 to 12.61. This may be attributed to the phenolic compounds being reabsorbed, accumulated, or depleted throughout the fermentation process. By making the millet's surface area bigger so that the bacteria being studied could grow on it during fermentation, phenols were released from the grain's outer layers. The peeling (milling) procedure eliminated these layers and concentrated the endosperm, which is rich in starch. This led to the breakdown of phenols either by fermentation or consumption by bacteria, resulting in their transformation into other compounds.

The results of this investigation were consistent with the findings of Yang et al. (2021) and Gowda et al. (2022). It was shown that removing the outer layer of millet significantly reduced the presence of phenols, flavonoids and phytates, which were originally more concentrated on the outer surface compared to the whole grain. Millet mostly contains phenolic chemicals that are primarily present in bound form. In their study, Dar and Sharma (2011) (23) determined that ethanol is the most effective solvent for extracting phenolic chemicals from grain flour. The optimal temperature for isolating phenolic compounds was found to be 60 ℃, which aligns well with the findings obtained in this work, considering the use of ethanol as the solvent and the temperature conditions used. At 60 ℃, the binding bonds of phenolic compounds break down much more quickly than those of other components. This makes it possible to get more of these extracts overall.

The concentration of tannins in water and alcohol extracts: Tables (5) and (6) show the tannin levels in the water and alcohol extracts of millet puree. These extracts were obtained by adding varying concentrations (0, 2, 4, 6 %) of *Lb. casi* bacteria during fermentation periods of 24 and 48 hrs. The data clearly indicates that the whole grain and crushed grain puree treatments exhibited decreased tannin levels as the percentage of inoculation increased. In contrast to the millet flour puree treatment, larger quantities of tannin were seen as the proportion of inoculation increased in both fermentation periods and extracts.

An analysis in Table 5 shows that the millet puree treatment had the biggest drop in tannins in the water extract, by 6 % (GM36) after 24 hrs of fermentation. After 48 hrs of fermentation, the two whole grain puree treatments, namely the 4% (WM24) and 2% (WM22) concentrations, exhibited the second biggest percentage decrease. The levels of fermentation were measured at 524.28, 997.85 and 1002.42 µg/g, respectively, showing a reduction of 47.45, 28.80 and 24.33 %. The amounts of pureed whole millet and crushed millet (WM16, WM14, WM22, GM34, WM12, WM24, GM32) dropped by a lot with the alcoholic extract (Table 6). The amounts dropped by 44.86 %, 36.00 %, 32.77 %, 23.89 %, 21.47 %, 18.01 % and 17.66 % in that order.

Table 5. The phenolic concentrations (in µg/g) in an aqueous extract of millet puree fermented with *Lb. casei* bacteria were measured at 24 and 48 hrs of fermentation

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24h	48h	24h	48h	24h	48h
0	1215.44 [a]	1451.00 [a]	997.77 [ab]	1201.49 [bc]	438.70 [c]	932.65 [c]
2%	1002.42 [b]	1098.00 [bc]	1131.72 [a]	1260.09 [b]	589.40 [b]	1096.37 [b]
4%	997.85 [b]	1033.12 [c]	970.79 [b]	1362.42 [a]	638.70 [b]	855.91 [c]
6%	1041.49 [b]	1210.79 [b]	524.28 [c]	1156.84 [c]	710.33 [a]	1315.91 [a]
LSD	88.92 *	118.52 *	117.37 *	92.65 *	68.41 *	97.55 *
			(P≤0.05) *			

Table 6. The tannins concentrations (in µg/g) in an alcoholic extract of millet puree fermented with *Lb. casei* bacteria were measured at 24 and 48 hrs of fermentation

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24h	48h	24h	48h	24h	48h
0	1689.86[a]	1575.44[a]	1522.42[a]	1261.02[b]	708.47[b]	1116.84[c]
2%	1327.07[b]	1059.16[d]	1253.58[b]	1549.40[a]	1168.93[a]	1372.65[a]
4%	1081.49[c]	1291.72[c]	1158.70[c]	1156.84[c]	1146.60[a]	1168.90[bc]
6%	931.72[d]	1440.56[b]	1511.26[a]	1173.58[bc]	1177.30[a]	1226.60[b]
LSD	107.573 *	98.15 *	79.51 *	94.78 *	76.02 *	91.48 *
			(P≤0.05) *			

The research's findings supported Kalinova's (2007) claim that peeled grain flour has a 65-80 % decrease in tannin content. This decrease may be attributed to the fact that the bran layers of grains contain 15-40 times more phenols, including tannins, compared to the peeled grains. In their study, Mbithi-Mwikya et al. (2000) observed a significant decrease in tannin concentration in finger millet seeds that were soaked for 8-10 hrs and then sprouted. For them, this wasn't because the tannins were physically lost, but because hydrophobic interactions (complexes) formed between the tannins and seed proteins. There was a drop-in tannin activity and a big rise in *in vitro* protein digestibility (IVPD) because tannins broke down and were released into the germination medium and polyphenol oxidase and other protein-degrading enzyme levels rose. The results of this investigation confirmed the previous findings by Osman (2010) (24) that there were fluctuations (both an increase and a reduction) in the tannin content of pearl millet puree throughout the fermenting process. He ascribed the cause of the rise in tannin concentration to the breakdown of condensed tannins such as proanthocyanidins, while the decline may be traced to their connection with portions of the endosperm. The detection of cotyledon is often not performed regularly since it is insoluble in solvents or may degrade because of the activity of the microbial enzyme phenyl oxidase.

Budhwar et al. (2020) found that Lactobacillus bacteria are more effective than yeast in reducing pH levels. This leads to the breakdown of the grain cell wall, resulting in the release of various biologically active compounds. An increase in hydrolytic microbial enzymes speeds up the fermentation process, which leads to a rapid rise in phenolic compounds like tannins. This process also enhances the level of folic acid by up to seven times and increases phenolic compounds by up to ten times after both germination and fermentation. Additionally, the breakdown of fiber and its reduced content indirectly improve the nutritional value by increasing concentrations of amino acids and vitamins. The continuous fermentation process may explain the higher tannin concentrations in millet flour and other treatments.

Millet puree's phytate content: Table 7 shows the amount of phytate in whole grain, crushed and millet flour samples that were fermented for 24 and 48 hrs with different amounts of inoculation (0, 2, 4, 6 %). The table shows that after 48 hrs of fermentation, the phytate concentration dropped the most in the millet flour puree treatments (PM64), followed by PM62 and PM66, with drops of 59.02 %, 57.38 % and 24.59 %, respectively. Subsequently, the whole grain puree treatments (WM22, WM12 and WM14) were administered after 24 and 48 hrs of fermentation, resulting in reductions of 7.91 %, 6.78 % and 1.70 %, respectively. Phytate content increased in all the other treatments being investigated.

The change in phytate content during the two fermentation processes was different because the inoculum rates were different. This is because of the re-association of inositols and the formation of phytates (inositol hexaphosphate or "IP6"), which were then broken down into smaller inositols (IP1-IP5), which were affected by pH differences. Arora et al. (2009) and (1) say that when the pH goes up, different inositol complexes are formed that break down phytate and change the amount of phytate that is lost. Nasser, Hammood (2019) and Osman (2010) observed that the amounts of phytate varied when the pH decreased during the fermentation of wheat flour and pearl millet dough. The reduction in phytic acid concentration was attributable to the impacts of microbial phytase and millet phytase, Inner Pearl.

According to Bora et al. (2018) (25), phytates are mostly present in the bran layers. The amounts of phytate in whole grain proso millet ranged from 170 to 610 mg per 100 g, which aligns with the findings of this research. The study conducted in (1) revealed that whole grain proso millet had 1767.43 mg/100g of phytates, whereas the extracted flour, comprising 71 % of the total, had 1553.22 mg/100g. This exceeds the findings of the control puree treatments in the trial.

Evaluation of fermented millet puree based on taste: Table 8 displays the aggregate ratings assigned by eight judges to the fermented proso millet puree samples (whole

Table 7. Phytate levels in millet puree that underwent fermentation with *Lb. casei* bacteria at 24 and 48 hrs

Bacterial starter (%)	Whole grain millet		Ground millet		Peeled millet	
	24h	48h	24h	48h	24h	48h
0	624.2[b]	454.9[c]	412.6[c]	518.4[c]	264.5[c]	645.4[a]
2%	581.9[b]	624.2[a]	666.51[a]	592.5[b]	497.2[b]	275.1[c]
4%	613.6[b]	418.9[c]	592.5[b]	708.83[a]	450.1[b]	264.5[c]
6%	719.4[a]	560.7[b]	592.5[b]	613.61[b]	560.7[a]	486.7[b]
LSD	51.48 *	46.55 *	67.09 *	64.21 *	76.95 *	69.71 *
			(P≤0.05) *			

Table 8. Sensory evaluation values in the millet puree after 24 and 48 hrs of fermentation with the bacteria *Lb. casei*

	Whole grains								Ground grains								Peeled grains							
	24h				48h				24h				48h				24h				48h			
properties	%0	%2	4%	%6	%0	%2	%4	%6	%0	%2	%4	%6	%0	%2	%4	%6	%0	%2	%4	%6	%0	%2	%4	%6
Colour and appearancence	5.4[b]	7.2[a]	7.2[a]	7.2[a]	3.6[b]	6.3[a]	7.2[a]	7.2[a]	5.4	5.4	5.4	4.5	4.5[a]	4.5[a]	3.6[b]	4.5[a]	8.1	8.1	7.2	8.1	5.4	6.3	6.3	6.3
LSD	1.287 *				1.653 *				1.071 NS				1.148 *				1.006 NS				0.996 NS			
Hardness and texture	6.3[b]	6.3[b]	5.4[b]	8.1[a]	4.5[b]	8.1[a]	7.2[ab]	6.3[b]	5.4	4.5	5.4	5.4	5.4[a]	3.6[b]	2.7[b]	5.4[a]	6.3	7.2	7.2	7.2	7.2[a]	6.3[a]	5.4[b]	4.5[b]
LSD	1.205 *				1.547 *				1.163 NS				1.568 *				1.067 NS				1.326 *			
Taste and odor	4.5[b]	6.3[a]	6.3[a]	3.6[b]	7.2	8.1	7.2	7.2	4.5	4.5	4.5	4.5	4.5[b]	2.7[c]	3.6[b]	5.4[a]	5.4[b]	8.1[a]	7.2[a]	8.1[a]	6.3[a]	7.2[a]	5.4[b]	5.4[b]
LSD	1.513 *				1,164 NS				1.027 NS				1.348 *				1.504 *				1.761 *			
General acceptance	5.4[b]	6.6[a]	6.3[a]	6.3[a]	5.1[a]	7.5[a]	7.2[a]	6.9[a]	5.1	4.8	5.1	4.8	4.8[a]	3.6[b]	3.3[b]	5.1[a]	6.6[b]	7.8[a]	7.2[a]	7.8[a]	6.3[a]	6.6[a]	5.7[a]	5.4[b]
LSD	1.044*				1.179 *				0.405 NS				*1.194				*0.992				0.835 *			

(P≤0.05) *

grain, crushed and flour) under investigation. The sensory evaluation table indicates that the puree's overall color and appearance are superior to the control treatments for every inoculation rate and fermentation duration. Except for the crushed treatment (GM44), there were no notable variations seen among the treatments, save for a deviation of 3.6 degrees. Regarding the crushed treatments after 24 hrs of fermentation and flour at both periods, there were no notable variations in the fermentation process. The two treatments using whole grains, WM16 and WM22, had the highest hardness measurements, averaging 8.1 degrees. After 48 hrs of fermentation, the crushed and flour treatments exhibited decreased acceptability, except for GM46, which showed similar acceptability to the control treatment. No significant changes were seen in the other treatments.

The taste and flavour profiles exhibited notable differences across the treatments. The sensory acceptability of the two whole grain puree treatments (WM12 and WM14) was the greatest after 24 hrs, with a score of 6.3 for each treatment. However, there were no significant differences in sensory acceptance between the whole grain treatments after 48 hrs. Despite achieving superior ratings in comparison to the same millet puree over a 24-hr period, after a duration of 48 hrs, the treatment using pureed millet (GM46) exhibited the highest values, with a temperature increase of 5.4 degrees. This difference was statistically significant compared to the other treatments. However, no significant changes were seen after a duration of 24 hrs. Within a 24-hr timeframe, all flour treatments showed superior performance compared to the control treatments. However, after 48 hrs, the two treatments, PM62 and PM60, had the highest level of effectiveness. According to Table 8, all fermented treatments had the highest level of sensory approval in comparison to the control treatments, except for the treatments GM42, GM44 and PM66. Also, the fact that there were no big differences in how the crushed puree was treated after 24 hrs of fermentation suggests that the fermentation process improves the millet and makes it more enjoyable to eat.

The findings of this study were like those of Inyang and Zakari (2008), the Fura product made from fermenting and sprouting pearl millet puree got high marks for all the qualities that were tested compared to the control sample.

Conclusion

Millet is a highly nutritious cereal that can be utilised in the production of various fermented bakery products and other food industries as a functional cereal-based product. This can be achieved by incorporating a specific strain of probiotic organisms, to meet the ongoing need for a wide range of healthy, nutritious and functional foods. The use of probiotics in the fermentation process significantly affects the sensory and functional characteristics of grain-based slurries. The levels of different phenolic acids rise, while anti-nutrients decline, leading to enhancements in flavor, color and overall product quality. Thus, from a nutritional perspective, those who eat a quantity of vegetables and fruits that is below the recommended daily consumption may have certain consequences. Fermented foods and beverages include

health-promoting properties and may serve as sources of prebiotics and probiotics. The research demonstrated that *L. casei* bacteria may serve as a starting culture for the fermentation of Proso millet grains. Among the whole grains and flour, the whole grain pulp exhibited the greatest bacterial proliferation, the highest concentration of phenolic compounds and the lowest amounts of tannins and phytates. The flavour of the mixture was enhanced by the addition of the starter, surpassing the flavour of the slurry without it.

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Authors' contributions

AMER study conception and design; ATYAF was microbial activation, identification, cultivation, preservation, millet puree production and data collection. Together were analysis and interpretation of results, draft manuscript preparation and reviewed the results and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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