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Development and characterization of a nutritionally rich traditional fermented product *aktori*

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Abstract

Pseudocereals and millets have emerged as nutrient-dense alternative to conventional cereals, offering significant health benefits, particularly for gut and cardiovascular health. The present study aimed to develop fermented *aktori*, a traditional dish from Himachal Pradesh made with buckwheat flour (*Fagopyrum esculentum*) and finger millet (*Eleusine coracana*) flour, fermented with curd in a 1:1 (w/v) ratio. The fermented batter and product (*aktori*) were analyzed for their physico-chemical characteristics and functional and antioxidant properties along with probiotic potential. The fermented aktori exhibited an enhanced nutritional profile, including high crude fiber (4.22%), protein (11.34%), carbohydrates (44.44%), and phenolic content. The study also explored the probiotic potential of the product, revealing that the isolated bacteria showed strong survivability in both acidic and bile conditions for up to 3 hours. Additionally, these probiotic strains showed strong auto-aggregation, co-aggregation, and hydrophobicity, indicating their potential to contribute to gut health. Therefore, using finger millet (*Eleusine coracana*) flour together with buckwheat flour, followed by fermentation with curd, provides an improved product *aktori* with good nutritional content and probiotic benefits that support gut health.

KEYWORDS

aktori, fermentation, finger millet, gluten-free, gut health, probiotic properties

1 | INTRODUCTION

Traditional fermented foods are indigenous to specific communities, prepared by local people using ancestral knowledge passed down through generations. Across the world, a wide variety of traditional cuisines exist, with fermented foods forming an essential part of many cultures for their role in food preservation, shelf-life extension, and flavor enhancement. According to Marco et al.,[1] fermented foods are those "prepared through controlled microbial growth and enzymatic conversions of major and minor food components". As a result, fermented foods provide various health benefits, including antimicrobial, antifungal, antioxidant, anti-atherosclerotic, anti-diabetic, and anti-inflammatory activities.[2] In recent years, there has been increasing scientific interest in the health-promoting effects of fermentation and the role of associated microorganisms, particularly lactic acid bacteria (LAB). According to the Joint FAO/WHO Expert Committee on Food Additives, the term probiotics refers to live microorganisms which, when administered in adequate amounts,

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confer a health benefit on the host. The core characteristics of probiotics are adequate viable count, live microbial state, and beneficial health effects on the host. They are often used as dietary supplements. *Bifidobacterium* and *Lactobacillus* strains are the most utilized due to their roles in balancing gut flora, enhancing intestinal barrier function, and producing antimicrobial agents, reducing the duration of antibiotic-associated diarrhea, and thus supporting digestive health.[3]

Sustainable food ingredients and healthy living practices have become more popular among consumers than conventional functional meals.[4] This shift reflects a growing awareness of the impact food has on both health and the environment. Two traditional grains that are gaining recognition for their nutritional and functional value are buckwheat and finger millet, both known for their prominent health benefits. Buckwheat (*Fagopyrum esculentum*) is a short-season crop that can grow in a variety of climates, including marginal and rocky soils. It is a gluten-free pseudo cereal from the Polygonaceae family,[5] with a protein content higher than that of common cereals such as rice, wheat, and maize. Rich in lysine and arginine levels, it offers hypolipidemic properties and is valued for its notable nutritional quality and health-promoting benefits. It also contributes to blood pressure regulation and has prebiotic and antioxidant activities. In addition to its strong antioxidant activity, buckwheat has anti-inflammatory, anti-hypertensive, anti-bacterial, anti-diabetic and anti-cancer effects.[6] These are rich in high biological value protein, dietary fiber, minerals, and phenolic compounds, making them suitable for celiac patients.[7]

Similarly, finger millet (*Eleusine coracana*) also known as "ragi," is a prevalent crop in Asia, Africa, and parts of India, recognized for its rich nutrient profile. It contains high levels of protein, micronutrients, fiber, and essential vitamins. It has the highest calcium value among cereals. Its slow digestion rate and high fiber content make it ideal for diabetic patients, as it has a lower glycemic level and provides a feeling of fullness. Consumption of finger millet offers multiple health advantages, including hypocholesterolemic, hypoglycemic, and anti-ulcerative effects.[8] Its growing popularity among consumers is attributed to these health-promoting properties and the presence of functional components such as slowly digestible and resistant starch.[9]

Aktori is a traditional sweet snack from the Lahaul–Spiti region in Himachal Pradesh, resembling Western pancakes. This delicious sweet is typically prepared during festivities and celebrations. It is made by mixing buckwheat flour with wheat flour and pan-frying to achieve a pancake-like texture.[10] *Aktori* has significant market potential due to its alignment with current consumer trends. However, its lack of widespread recognition necessitates a targeted marketing and educational approach. Furthermore, its utilization of locally sourced ingredients not only supports local economies but also reduces the environmental impact of long-distance transportation. This combination of natural ingredients, health benefits, and sustainability forms a strong basis for its introduction as novel product in the functional food market. The present study focuses on developing a fermented *aktori* using a double-layer formulation of buckwheat and finger millet flour to improve its nutritional profile and probiotic potential for supporting gut health. Consequently, a wholesome pancake (*aktori*) enriched with the beneficial components of both buckwheat and finger millet flour was successfully formulated.

2 | MATERIALS AND METHODS

2.1 | Raw materials

Finger millet (*Eleusine coracana*), buckwheat (*Fagopyrum esculentum*), and curd were collected locally from Palampur, Himachal Pradesh. Both grains were manually cleaned, dried, weighed, and ground into flour, after which they were sieved to remove any remaining impurities.

2.2 | Preparation of *aktori*

The raw materials were sieved and batter was prepared by separately mixing buckwheat flour and finger millet flour with an equal amount of curd (1:1), along with water and other ingredients such as sugar and baking powder. Traditionally, curd has been used as a starter culture for various fermented foods. Using equal proportions of flour and curd balances the carbohydrate availability for lactic acid bacteria, ensures the desired batter viscosity, and supports high probiotic survivability. The batter was fermented for 6–7 h, after which it was ready for product preparation. To make the *aktori*, a layer of fermented finger millet batter was first poured onto a pre-heated pan, followed by a layer of fermented buckwheat batter on top, and cooked for 5–6 min. The preparation process is illustrated in Supporting Information S1; Figure S1 (as shown in supplementary file). The microbial cultures isolated from the final product were derived from the curd used as the fermentation starter.

2.3 | Nutritional analysis

The nutritional composition of raw materials, fermented batters, and the final product was analyzed for moisture, ash, fat, protein, and fiber contents using the standard AOAC[11] procedures. Moisture content was determined by oven drying method, in which samples were dried in a hot air oven at 105°C for 1 h, until the constant weight was maintained. Ash content was measured by incinerating a known quantity of the sample in a muffle furnace at 550°C for 5 h. Fat content of the sample was analyzed using a Soxhlet apparatus (SoxTRON-SOX-6) and extraction was done using petroleum ether. Kjeldhal apparatus (KjelTRON apparatus) (AOAC method 981.10) was used for estimating the nitrogen content in a sample. The procedure involved digestion, distillation, and titration, and further protein content was calculated by using a conversion factor of 6.25.

Crude fiber content (AOAC method 962.09) was quantified by the digestion method (acid-alkali) with 1.25% NaOH and 1.25% H_2SO_4 followed by incineration of the residue in a muffle furnace. Carbohydrate content was determined by the difference method as described by Kassegn[12], that is, % carbohydrate = [100 − % (protein + fat + moisture + ash + fiber)].

2.3.1 | Water absorption index (WAI)

For WAI, 1 g sample was mixed with 10 mL distilled water in pre-weighed falcon tubes, mixed properly and then centrifuged at 3500 rpm for 15 min.[13] After collecting the supernatant in pre-weighed petri-plates, the residue left in tubes was reweighed.

2.3.2 | Water solubility index (WSI)

WSI was calculated by drying the supernatant collected from the WAI samples using the method of Kumari et al..[13] The petri-plate supernatant was dried in a hot air oven at 100°C for one-2 hours, cooled, and then re-weighed.

2.3.3 | Oil absorption index (OAI)

In falcon tubes, 1 g of the sample and 10 mL of vegetable oil were mixed together, vortexed for 5 min, and then left to stand at room temperature for 30 min. Then, the sample was centrifuged for 15 min at 3500 rpm, the oil was decanted properly, and samples were reweighed.

2.3.4 | Water activity (a_w)

Water activity was determined using the water activity meter (Aqua lab 4 TE dew point Decagon Devices, Inc.) following the method of Kumari and Gupta.[14]

2.3.5 | pH

The pH was measured using the EUTECH pH700 instrument.

2.3.6 | Total phenolic content (TPC)

The total phenolic content (TPC) was determined using Folin–Ciocalteu method described by Dev and Gupta.[15] Sample extraction was carried out at a 1: 10 ratio of sample to solvent, followed by centrifugation and filtration. For TPC estimation, 1 mL of the extract was mixed with 2 mL of 10% FC (Folin–Ciocalteu reagent) and subsequently with 2 mL of Na_2CO_3 (7.5%). The mixture was incubated for 30 min and the absorbance was measured at 765 nm using spectrophotometer, with gallic acid as the standard.

2.3.7 | Total flavonoid content (TFC)

The TFC was estimated by $AlCl_3$ method with some modifications.[16] 75 μL of a 5% $NaNO_2$ solution was added to the extract mixture. Then, upon adding 150 μL of 10% $AlCl_3$ to the mixture, 750 μL of 1M NaOH was added, the mixture was incubated for 15 min, and the absorbance was recorded at 510 nm, with rutin as a standard.

2.3.8 | HPLC quantification of phenolic compounds

Using a membrane syringe filter (0.22 μm pore size), the extracted samples of buckwheat and finger millet flour, batter, and product (*aktori*) were prepared for HPLC. The Agilent 1260 Infinity II HPLC system (Agilent Technologies, USA) and a diode array detector with separation column (LIChrospher®100 RP-18, 5 μm) at a 30°C (column temperature) were used for the analysis. With some minor adjustments, the mobile phase was made up of water and acetonitrile with 0.1% (v/v) and 0.05% (v/v) formic acid, respectively, in a constant gradient system to measure the flavonoids and phenolic acids under the conditions described by Dadwal et al..[17] A 10 μL injection volume and a 1 mL/min flow rate were utilized to separate the targeted phenolics, and wavelengths of spectral absorption were observed between 210 and 280 nm. Using reference standards of gallic acid, catechin, caffeic acid, vanillic acid, p-coumaric, and t-cinnamic, chromatograms were examined at 280 nm.

2.3.9 | Phytic acid determination

It was determined using Megazyme's K-PHYT kit.[18] This quantitative technique involves the extraction of inositol phosphates by acid, followed by phytase and alkaline phosphatase treatment, which enables the release of all phosphates from the myo-inositol phosphate. Colorimetric method is then used to determine the total amount of phosphate released, which is expressed as *g* phosphorus/100 g of the sample material.

2.4 | Probiotic characteristics

2.4.1 | Isolation and purification of isolates

For isolation, 1 g sample was inoculated in MRS (de Man Rogosa Sharpe) broth and kept in an incubator at 37°C overnight. After that, serial dilution was carried out (10^7) and dilutions of these MRS agar plates were made. The plates were kept for 48 h at 37°C for growth and the colonies were monitored. Repeated streaking on MRS agar plates was carried out to purify the colonies, which was confirmed morphologically and by Gram Staining.

2.4.2 | Acid and bile salt tolerance

The isolated strains were assessed under acidic environments according to Yadav et al.[19] with some modifications. The isolates were cultivated in the MRS broth at 37°C for 24 h and then, to pellet down, cultures were centrifuged at 8000 rpm (4°C) for 10 min. The pellets were washed twice using a phosphate buffer saline (PBS) solution, vortexed, and centrifuged again. The pellets were then resuspended in a MRS solution of different pH, that is, 6.5 (control), 1, 2, and 3 adjusted using 1N HCL. Then, serial dilution and spreading were carried out on the MRS agar plates at 1, 2, and 3 h intervals. The plates were incubated at 37°C and CFU was recorded after 24 h.

The method previously described by Yadav et al.[19] was used for determining bile tolerance, with some modifications. Pellets from the cultures that were grown overnight were harvested and reconstituted in the MRS broth with varying bile salt concentrations (ox-gall), viz 0.5% and 1%. After that, serial dilution and spreading was carried out at 1, 2, and 3 h and the plates were incubated at 37°C. CFU was counted after 24 h.

2.4.3 | Hydrophobicity

Hydrophobicity was measured according to the method described by Yadav et al.,[19] with slight modifications. Overnight grown cultures were taken and then centrifuged to pellet down. The pellets were washed with a PBS buffer (twice) and reconstituted in the same buffer. Then, the OD of the isolated strain was set to 0.5 at 540 nm. The culture suspension was then mixed with chloroform and n-hexane, after which it was vortexed. For phase separation, the culture was kept for 1 h at room temperature. The absorbance of the aqueous phase was then observed and hydrophobicity was measured using Equation (1)

$$\text{Hydrophobicity}\ (\%) = \frac{\text{Initial OD} - \text{Final OD (aqueous phase)}}{\text{Initial OD}} \times 100 \quad (1)$$

2.4.4 | Auto-aggregation and co-aggregation

For auto-aggregation, overnight grown cultures were used and centrifuged to obtain pellets. Pellets were then washed and reconstituted in a phosphate saline (PBS) buffer. The absorbance of culture was maintained at 0.5 at 600 nm (using PBS buffer). The culture suspension was kept at room temperature and OD was observed at hourly intervals upto 5 h. It was measured using Equation (2).

$$\text{Auto-aggregation}\ (\%) = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100 \quad (2)$$

The co-aggregation was estimated according to the method given by Kos et al.[20] with slight modifications. The culture and pathogenic (*Enterococcus faecalis* and *Staphylococcus aureus*) suspension was prepared using a PBS buffer. Similarly, the pathogenic culture suspension was also prepared and the OD of both bacterial and pathogenic cultures was calculated at 600 nm. In a test tube, 2 mL of bacterial culture and 2 mL of pathogenic culture were added and incubated at room temperature for 5 h. After that, spectrometric analysis was performed and calculated using Equation (3).

$$\text{Co-aggregation}\ (\%) = \frac{\left(A_{\text{pathogen}} + A_{\text{probiotic}}\right) / 2 - A\ \text{mix}}{A_{\text{pathogen}} + A_{\text{probiotic}} / 2} \times 100 \quad (3)$$

where, A_{path} = Absorbance of pathogenic bacteria,

A_{pro} = Absorbance of probiotic bacteria, and

A_{mix} = Absorbance of both pathogenic and probiotic bacteria in a single tube.

2.5 | Sensory analysis

Sensory attributes of the product were estimated by using a nine-point hedonic scale (1–9 which represents "dislike extremely" to "like extremely"). A semi-trained panel of 42 participants (15 males and 15 females) aged between 21 and 48 years was recruited from the CSIR-Institute of Himalayan Bioresource Technology, Palampur, and sensory analysis of *aktori* was conducted. The participants graded the *aktori samples* according to their preferences on the basis of a hedonic scale which includes color, flavor, taste, texture, and overall acceptability.[14]

2.6 | Shelf life stability

The *aktori* samples were packed in polyethylene (PE) pouches and zip lock pouches, then stored under both room temperature and refrigerated conditions. Shelf life evaluation was carried out on the 0th, 5th, and 10th days by monitoring water activity and microbial quality. The serial plate dilution method, as described by Kumari and Gupta,[14] which was used for the microbiological study with some modifications, included the enumeration of bacteria, yeast/mold, and *E. coli*. For this method, 1 g of the sample was mixed with sterile 0.85% normal saline solution and 100 µL of suspension was spread onto different media plates, that is, potato dextrose agar (PDA), plate count agar (PCA), and eosin methylene blue agar (EMB). The plates were incubated for 24–48 h at 37°C for *E. coli* and total bacterial count and at 28°C for 72–120 h for total yeast/mold count. The results were expressed as CFU/g.

2.7 | Statistical analysis

The results were statistically determined using one-way ANOVA and the experiments were done in triplicates. The differences between the variables were then examined using the Tukey test. Descriptive analysis was used to examine the mean values in order to perform microbiological evaluation and sensory analysis.

3 | RESULT AND DISCUSSION

Raw materials (finger millet and buckwheat flour), fermented batters, and product (*aktori*) were analyzed for physico-chemical and functional properties and the results are presented in Table 1.

3.1 | Physico-chemical and functional properties of raw materials, fermented batters, and *aktori*

The proximate analysis of flours (Table 1) revealed significant differences ($p < 0.05$) in moisture, fat, ash, fiber, and protein content. Moisture content of buckwheat and finger millet flour was observed to be significant ($p < 0.05$) as 10.96 ± 0.04% and 9.37 ± 0.14%, respectively. The fat and ash content were observed as 3.55 ± 0.47% and 2.46 ± 0.05% for buckwheat flour and 1.35 ± 0.05% and 2.09 ± 0.06% for finger millet flour with buckwheat exhibiting a significantly higher fat content ($p < 0.05$). These findings are consistent with those reported by Sofi et al.[21] and Tripathi et al.[22] for buckwheat and finger millet flour. Buckwheat flour also exhibited a significantly higher ($p < 0.05$) fiber content (4.27 ± 0.75%) than finger millet flour (3.13 ± 0.36%). Similarly, the protein content was also significantly ($p < 0.05$) higher in buckwheat flour (12.49 ± 0.17%) than in finger millet (7.85 ± 0.41%), indicating a superior protein profile of buckwheat. Similar observations for buckwheat flour have been reported by Dziadek et al.[23]; Thakur and Baishya,[5] Grewal[24]; Krishnaswamy and Parameshwari[4] and corresponding results for finger millet were observed by Shingote et al.[25]; Mane and Kadam[26]; Desai et al.[9]; and Kumar et al..[27] The carbohydrate content of buckwheat and finger millet flour was found to be 66.27 ± 0.82% and 76.19 ± 0.54%, respectively.

Similarly, the moisture content of fermented batters from buckwheat (BB) and finger millet (FB) was significantly higher ($p < 0.05$) recorded at 51.64 ± 0.57% and 49.01 ± 0.10% respectively. A decrease in ash and fat content was observed as 1.81 ± 0.31% and 1.72 ± 0.35% and 2.25 ± 0.57% and 1.75 ± 0.01% in fermented buckwheat and finger millet batters, respectively. The slight reduction in fat content is attributed to microbial lipid metabolism during fermentation, where bacteria utilize lipids as an energy source for growth.[28] Significant variations were observed in crude fiber content of BB and FB compared to raw flours, that is, 3.32 ± 0.30% and 2.34 ± 0.17%, respectively. The decrease in fiber content was due to the breakdown (enzymatically) of the fiber during fermentation where lactic acid bacteria utilize fiber as a carbon source for their metabolic activities.[29] Moreover, significant ($p < 0.05$) increase in protein content was observed in the fermented batters compared to raw flours that is, 12.84 ± 0.28% and 8.62 ± 0.05% in buckwheat and finger millet, respectively. This increase in protein content can be attributed to microbial fermentation, which enhances the amino acid profile and overall protein quality in cereal products.[30] The carbohydrate content of BB and FB was observed as 28.87 ± 0.54% and 36.53 ± 0.51%, respectively. The reduction in carbohydrate content compared to raw flours was mainly due to its utilization by lactic acid bacteria during the fermentation process. Additionally, a significant drop in pH was observed after fermentation, decreasing from 6.39 ± 0.01–4.87 ± 0.01 for BB and from 6.81 ± 0.01–4.58 ± 0.01 for FB, which is due to enhanced LAB fermentation activity and increased acid production from microbial activity.[6]

However, in the case of *aktori*, there was a significant decrease ($p < 0.05$) in moisture content with a recorded value of 35.78 ± 0.78%. The ash and fat contents were observed as

TABLE 1 Physico-chemical and functional properties of raw materials, fermented batters, and product (*aktori*).

Parameters		Buckwheat flour	Finger millet flour	Buckwheat (BB)	Finger millet (FB)	Fermented *aktori*
Moisture (%)		10.96 ± 0.04^{d}	9.37 ± 0.14^{e}	51.65 ± 0.57^{a}	49.01 ± 0.10^{b}	35.78 ± 0.78^{c}
Ash (%)		2.46 ± 0.05^{a}	2.09 ± 0.32^{a}	1.81 ± 0.31^{b}	1.72 ± 0.35^{b}	1.59 ± 0.01^{b}
Fat (%)		3.55 ± 0.47^{a}	1.35 ± 0.05^{c}	2.25 ± 0.57^{bc}	1.75 ± 0.01^{bc}	2.62 ± 0.33^{ab}
Fiber (%)		4.27 ± 0.75^{a}	3.13 ± 0.36^{b}	3.32 ± 0.30^{ab}	2.34 ± 0.17^{b}	4.22 ± 0.01^{a}
Protein (%)		12.49 ± 0.17^{a}	7.85 ± 0.41^{d}	12.84 ± 0.28^{a}	8.62 ± 0.05^{c}	11.34 ± 0.37^{b}
Carbohydrate (%)		66.27 ± 0.82^{b}	76.19 ± 0.54^{a}	28.87 ± 0.54^{e}	36.53 ± 0.51^{d}	44.44 ± 0.30^{c}
Water absorption index (g/g)		1.82 ± 0.07^{b}	1.48 ± 0.06^{b}	2.94 ± 0.07^{a}	2.59 ± 0.06^{a}	-
Water solubility index (%)		9.06 ± 0.18^{a}	3.10 ± 0.09^{b}	9.88 ± 0.27^{a}	3.68 ± 0.45^{b}	-
Oil absorption index (g/g)		1.36 ± 0.0^{b}	1.41 ± 0.26^{b}	2.15 ± 0.03^{a}	2.08 ± 0.05^{a}	-
pH	Initial pH	-	-	6.39 ± 0.01^{a}	6.81 ± 0.01^{a}	-
	Final pH	-	-	4.87 ± 0.01^{a}	4.58 ± 0.01^{a}	

Note: *Results are calculated as mean ± SD ($n = 3$). Means in rows are significantly different ($p < 0.05$), indicated by different letters.
Abbreviations: BB, buckwheat flour batter; FB, finger millet flour batter.

1.59 ± 0.01% and 2.62 ± 0.33%, respectively. Crude fiber was higher in *aktori* (4.22 ± 0.01%) when compared to both batters and raw flours, suggesting a higher concentration of fiber components post-processing. The protein content of *aktori* was also relatively higher (11.34 ± 0.33%) due to the inclusion of buckwheat flour, which has a higher protein content compared to finger millet. The carbohydrate content in the final product was reported as 44.44 ± 0.30%.

The WSI and WAI of buckwheat and finger millet flour were found to be 9.06 ± 0.18% and 3.10 ± 0.09% and 1.82 ± 0.07 g/g and 1.48 ± 0.06 g/g, respectively. The OAI (oil absorption index) of buckwheat and finger millet flour was observed as 1.36 ± 0.04 g/g and 1.41 ± 0.26 g/g, respectively. The results are in agreement with the findings of Rani and Kulkarni,[31] who reported similar trends in water and oil absorption capacities. Fermentation of flours led to an increase in WAI, WSI, and OAI, as shown in Table 1. Similar observations were reported by Dev et al.,[32] who found increased WAI and OAI in fermented ragi flour.

Hence, local traditional foods are rich source of bioactive compounds and nutrients, making them ideal for functional food development. They are also well-suited to local environmental conditions. Fermentation as a processing technique further enhances their nutritional content, contributing to the reduction of risks associated with obesity, diabetes, and cardiovascular diseases.[10] Furthermore, promoting their nutritional, environmental, and cultural value can support sustainable food systems, whereas consumer education can boost acceptance and demand. These findings align closely with previous studies on fermented pseudocereals and millets, where fermentation has been shown to substantially enhance nutritional value through increased protein and phenolic content, accompanied by a reduction in anti-nutritional components.[33–35]

3.2 | Total phenolic content (TPC) and total flavonoid content (TFC)

The TPC of flours, fermented batters (buckwheat and finger millet), and *aktori* are depicted in Table 2. After fermentation, the TPC content increased from 5.32 ± 0.11–5.60 ± 0.14 mgGAE/g in buckwheat and from 2.06 ± 0.11–2.94 ± 0.20 mgGAE/g in finger millet. Similarly, the TFC content increased from 2.93 ± 0.09–3.61 ± 0.21 mgRutin/g in buckwheat and from 1.67 ± 0.02–2.58 ± 0.05 mgRutin/g in finger millet after fermentation, as shown in Table 2. Dev et al.[32] also reported an increase in phenolic content after fermenting the finger millet flour. However, in *aktori*, the TPC and TFC content decreased slightly to 3.62 ± 0.08 mgGAE/g and 3.03 ± 0.72 mgRutin/g. This reduction is attributed to the degradation of phenolic compounds during thermal processing, as previously reported by Chlopicka et al..[36]

3.3 | Phytic acid content

Phytic acid content decreased after fermentation in both buckwheat and finger millet batters, that is, from 0.667 ± 0.01–0.321 ± 0.02 g/100 g and 0.515 ± 0.08–0.340 ± 0.03 g/100 g, respectively, as shown in Table 2. According to, Gupta et al.[37] and Dev et al.[32] fermentation reduces the phytic acid content in food grains, primarily due to the action of enzymes produced by microbes during the fermentation process. In *aktori*, the phytic acid content was observed to be 0.314 ± 0.04 g/100 g, which is slightly lower compared to the batters, which may be due to the cooking process, although pan frying has a minimal impact on phytic acid levels.

3.4 | HPLC quantification of phenolic compounds

Phenolic compounds are a class of bioactive chemical entities found in plant-based foods, known for their antioxidant properties and potential health benefits.[38] The phenolic profiles of the samples were evaluated using high-performance liquid chromatography (HPLC), as detailed in Table 3. Vanillic acid was the most abundant phenolic compound in the buckwheat batter (1.14 mg/g), whereas catechin was the predominant phenolic compound in finger millet (1.51 mg/g) followed by *aktori* (0.81 mg/g). In case of *aktori*, only three polyphenolic compounds were detected, with catechin identified as the most abundant.

3.5 | Probiotic characteristics

After pan frying the fermented batters of finger millet and buckwheat into double layered *aktori*, two strains were isolated and identified as gram positive rods based on their morphological characteristics and Gram staining test as presented in Supporting Information S1; Table S1 (data given in supplementary file). The probiotic cell viability was observed to be 10^6 cfu/g. These isolates were subsequently subjected to further evaluation to assess their probiotic characteristics.

TABLE 2 TPC, TFC and anti-nutritional properties of raw materials, fermented batter, and product (*aktori*).

Parameters	Buckwheat flour	Finger millet flour	Buckwheat (BB)	Finger millet (FB)	Fermented *aktori*
TPC (mgGAE/g)	5.32 ± 0.11^{a}	2.06 ± 0.11^{d}	5.60 ± 0.14^{a}	2.94 ± 0.20^{c}	3.62 ± 0.08^{b}
TFC (mgRutin/g)	2.93 ± 0.09^{ab}	1.67 ± 0.02^{c}	3.61 ± 0.21^{a}	2.58 ± 0.05^{bc}	3.03 ± 0.72^{ab}
Phytic acid (g/100 g)	0.667 ± 0.01^{a}	0.515 ± 0.08^{b}	0.321 ± 0.02^{c}	0.340 ± 0.03^{c}	0.314 ± 0.04^{c}

Note: *Results are calculated as mean ± SD ($n = 3$). Means in rows are significantly different ($p < 0.05$), indicated by different letters.
Abbreviations: BB, buckwheat flour batter; FB, binger millet flour batter; TFC, total flavonoid content; TPC, total phenolic content.

TABLE 3 HPLC quantification of the fermented batter and product.

Polyphenols (mg/g)	BB	FB	AK
Gallic acid	0.89 ± 0.05[a]	NQ	NQ
Catechin	0.23 ± 0.01[c]	1.51 ± 0.003[a]	0.81 ± 0.003[b]
Caffeic acid	0.004 ± 0.00[a]	0.002 ± 0.00[a]	0.002 ± 0.00[a]
Vanillic acid	1.14 ± 0.00[a]	0.005 ± 0.00[c]	0.04 ± 0.00[b]
p-coumaric	0.002 ± 0.00[a]	NQ	NQ
t-cinnamic	0.003 ± 0.00[a]	0.004 ± 0.00[b]	NQ

Note: *Results are calculated as mean ± SD ($n = 3$). Means in rows are significantly different ($p < 0.05$), indicated by different letters.

Abbreviations: AK, *aktori*; BB, buckwheat flour batter; FB, finger millet flour batter; NQ, not quantified.

3.5.1 | Acid tolerance and bile tolerance

The probiotic bacteria need to be tolerant to hostile environment found in GI tract in order to colonize the gut and promote health benefits. Probiotic strains must withstand gastric pH (~2.0) for at least 90 min to ensure intestinal delivery. The acid tolerance profile of bacterial isolates at pH levels 1, 2, and 3 is illustrated in Figure 1a,b, with isolate PMZ2 exhibiting a higher survival rate under acidic conditions. Isolate PMZ1 showed slightly lower survivability at pH 1 after 3 h, whereas PMZ2 maintained a higher survivability rate for upto 3 h under highly acidic conditions, with slight variations in viability. This increased acid tolerance in the isolate may be attributed to its greater resistance to acidic pH, potentially due to the constant gradient between intracellular and extracellular pH levels.[39] Similar observations have been reported by[39,40] in traditional fermented dairy and cereal based dairy products. Furthermore, Liong and Shah[41] reported the survivability of probiotic lactobacilli strain at pH 2 for upto 2 h, though with some variations in viability. Bindu and Lakshmidevi[42] also noted slight variation among cultures in their tolerance to acidic pH, with a reduction in viable counts after 60 min of incubation in different food products. Shambhvi and Gupta[43] also reported isolates surviving upto 6 h in acidic conditions within steamed dumplings, with slight variations in their viability.

Another crucial factor in assessing the probiotic potential of isolates is bile tolerance because it influences metabolic activity and microbial colonization in the host's small intestine.[19] In addition to acid resistance, bile salts can damage the bacterial cell membrane, leading to cell death. Therefore, for probiotics to successfully survive passage through the gastrointestinal tract, they must exhibit resistance to bile salts.[42] The survival of the isolates was assessed after exposure to 0.5% and 1% of bile salt for 3 h. Both isolates showed good survival, with minimal impact on CFU counts across different bile concentrations. However, PMZ2 showed higher tolerance to bile salts compared to PMZ1, as shown in Figure 2a ,b. A similar trend was observed by Meena et al.,[44] in a fermented cereal based food product, where probiotic bacteria survived for upto 3 h under different bile concentrations. Shambhvi and Gupta[43] also reported isolates surviving bile concentrations for upto 6 h in steamed dumplings.

3.5.2 | Auto-aggregation and co-aggregation

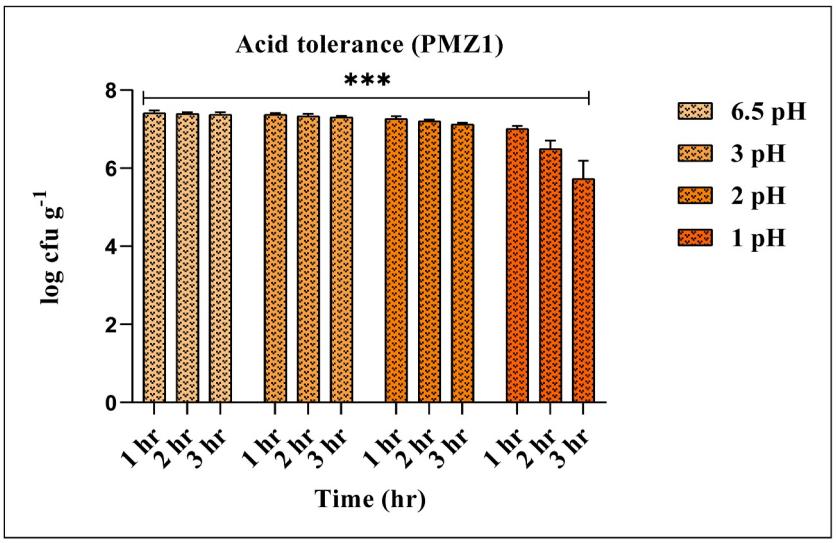
The ability of microbes to interact with one another is termed as auto-aggregation, whereas their interaction with other species or strains is referred to as co-aggregation. Both properties are essential for assessing whether probiotic bacteria can effectively adhere to the oral cavity and gastrointestinal system, where they exert their beneficial effects.[45]

Auto-aggregation helps to increase their adhesion to intestinal epithelial cells and prevents the colonization of harmful microorganisms.[46] The results summarized in Table 4 show that auto-aggregation increased significantly with the increase in incubation time for all the isolates. Specifically, isolates PMZ1 and PMZ2 showed positive results for auto-aggregation with 44.75% and 50.38%, respectively. PMZ2 exhibited higher auto-aggregation as compared to PMZ1. These findings align with those of Vlková et al.[47] who observed adhesion ability in *bifidobacteria* and *clostridia* ranging from 9.1% to 86.0%. Similarly, Guan et al.[48] reported an auto-aggregation rate of 87.74% in bacterial strains derived from fermented milk. Lee et al.[49] have reported that auto-aggregation in various probiotic strains ranged from 41.8% to 92.3%.

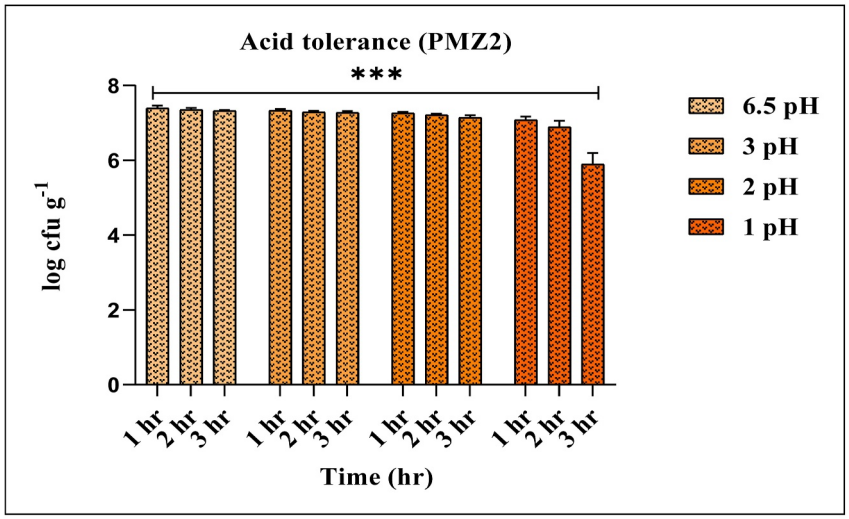
In order to inhibit or kill pathogenic microbes or prevent the colonization of food borne pathogens, probiotic bacteria co-aggregate and secrete antimicrobial compounds.[40] The co-aggregation of isolates with *Enterococcus faecalis* and *Staphylococcus aureus* was observed and PMZ2 showed high degree of co-aggregation with these pathogenic microbes, compared to PMZ1. Similar findings were reported by Vlková et al.[47] who observed adhesion abilities of *bifidobacteria* and *clostridia* ranging from 13.7% to 91.3%. Tuo et al.[50] also observed positive results for co-aggregation ability (21.81%–61%) among various probiotic strains and *E.Coli*. Overall, the results suggest that PMZ1 and PMZ2 possess strong adhesion characteristics, which contribute to their ability to persist in the gastrointestinal tract.

3.5.3 | Cell surface hydrophobicity

The cell surface hydrophobicity of the isolated strains, tested with n-hexane and chloroform is presented in Table 4. According to Del Re et al.,[51] probiotics require a minimum hydrophobicity of 40% to effectively adhere to and interact with host cells. Both isolates showed the highest hydrophobicity with n-hexane, ranging from 98.56% to 97.59%, whereas with chloroform it ranged from 94.04% to 95.26%. Similar results were reported by Vlková et al.,[47] regarding adhesion ability of *bifidobacteria* and *clostridia*, with values ranging from 7.3% to 98.4%. Higher hydrophobicity enhances the bacteria's ability to attach to epithelial cells, influencing their adhesion



(a)



(b)

FIGURE 1 Acid tolerance of bacterial isolates (6.5, 1, 2, 3 pH) (a) survival of isolate PMZ1 in acidic conditions (b) survival of isolate PMZ2 in acidic conditions.

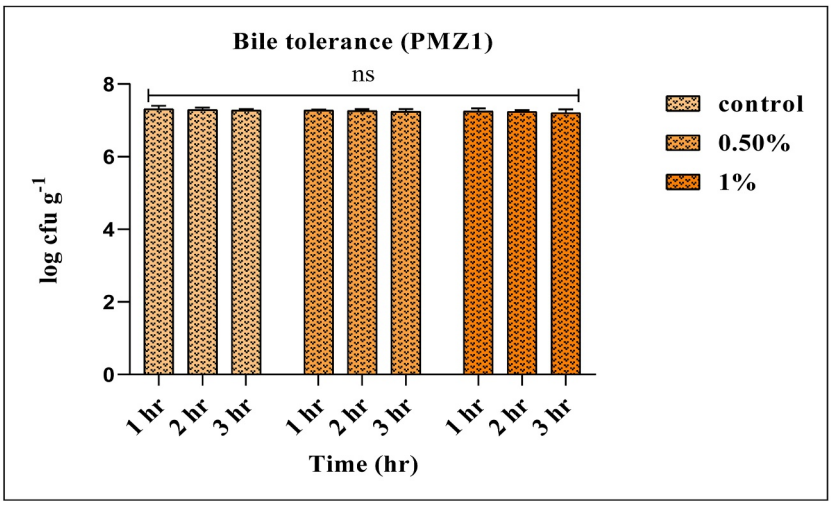
capacity[52] and facilitating the colonization of probiotic bacteria on intestinal epithelial cells as well as strong binding to mucosal cells.[39] Lee et al.[49] also observed comparable cell surface hydrophobicity in various probiotic strains. Additionally, Gandomi et al.[53] reported Hydrophobicity values ranging from 6.58% to 73.3% for *Lactobacillus plantarum* strains isolated from Siahmazgi traditional cheese. Bindu and Lakshmidevi[42] found that isolates showed adherence to n-hexane within the range of 41%–53%.

3.6 | Sensory analysis

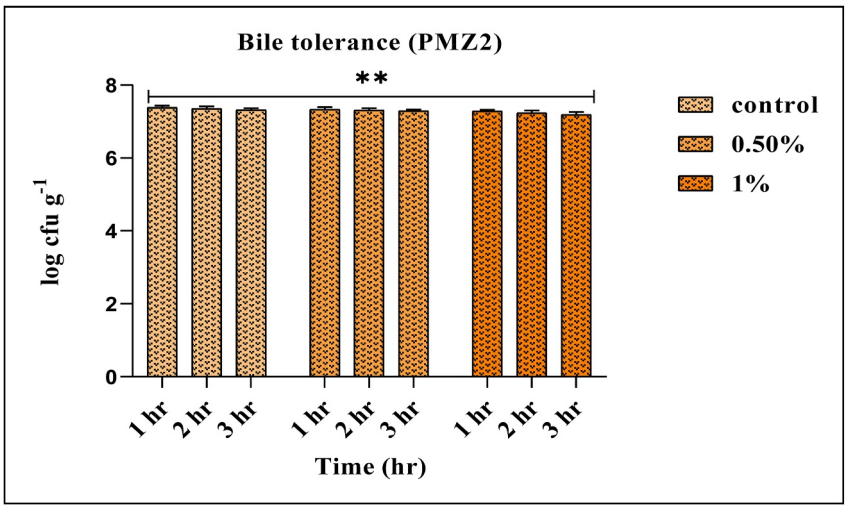
The sensory evaluation of the product *(aktori)* is shown in Figure 3. The color of the product was found to be acceptable with a score of 7. It was also observed that the product exhibited slightly better taste and overall acceptability with the addition of finger millet layer. Although, flavor and taste are important sensory attributes influencing consumers' overall preference for food products, however, color is the primary sensory characteristic that significantly impact their acceptance. Based on these observations, it was concluded that the product was moderately liked by the panel. Furthermore, a mild bitter aftertaste, attributable to buckwheat, was noted but it did not impact overall product acceptability.

3.7 | Shelf life stability

The results shown in Supporting Information S1; Table S2 (data given in supplementary file), indicate that *E.coli* was not detected in any of the product for upto 10 days whether stored at ambient or refrigerated conditions, which indicates good processing and handling techniques during the preparation of the product. However, the total



(a)



(b)

FIGURE 2 Bile tolerance of bacterial isolates (control, 0.5% and 1% bile salt) (a) growth of isolate PMZ1 in different concentrations of bile salts (b) growth of isolate PMZ2 in different concentrations of bile salts.

TABLE 4 Co-aggregation, auto-aggregation, and hydrophobicity of strains isolated from *aktori*.

	Co-aggregation (%)		Auto-aggregation (%)				Hydrophobicity (%)	
Isolates	*E. faecalis*	*Staphylococcus aureus*	1 h	2 h	3 h	4 h	n-hexane	Chloroform
PMZ1	42.22 ± 0.29^{b}	51.23 ± 0.74^{b}	37.37 ± 0.52^{d}	39.50 ± 0.33^{c}	42.49 ± 0.33^{b}	44.75 ± 0.66^{a}	98.56 ± 0.06^{a}	94.04 ± 0.11^{a}
PMZ2	51.18 ± 0.20^{a}	57.11 ± 0.83^{a}	38.41 ± 0.92^{d}	40.51 ± 0.48^{c}	49.25 ± 0.98^{b}	50.38 ± 0.81^{a}	97.59 ± 0.67^{a}	95.26 ± 0.67^{a}

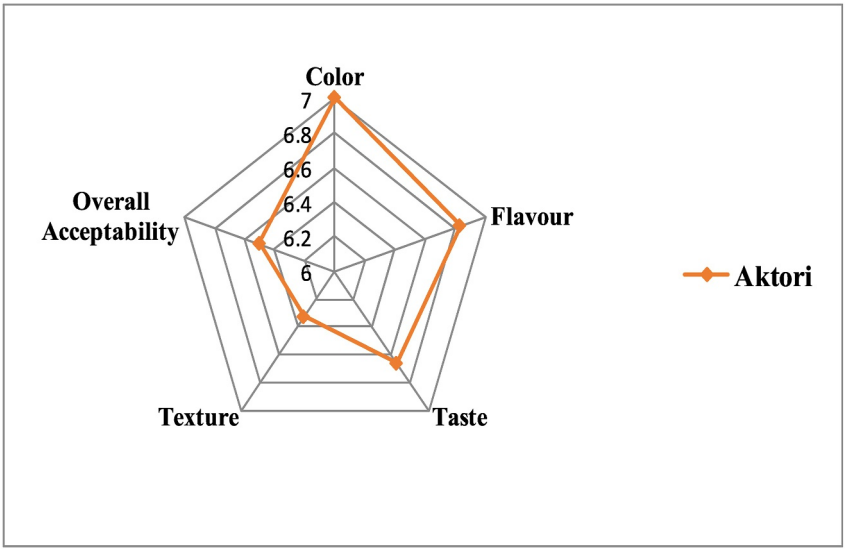
Note: *Results are calculated as mean ± SD (n = 3). Means in columns are significantly different ($p < 0.05$), indicated by different letters for hydrophobicity and co-aggregation, whereas, for auto-aggregation, means in rows are significantly different ($p < 0.05$), indicated by different letters. Abbreviations: *E faecalis*, *Enterococcus faecalis*; PMZ1 & PMZ2, *aktori* strain; *S aureus: Staphylococcus aureus*.

bacterial count remained stable in both packaging materials and conditions. Yeast and mold were not detected on day 0 but emerged by day 5 in both the conditions and packaging materials. The microbial load remained stable during the 10 days of storage in both types of packaging materials, but the sensory acceptability remained for only 2–3 days based on preliminary sensory changes, whereas the microbiological data indicates its stability upto 10 days.

4 | CONCLUSION

In conclusion, *aktori* is a traditional non-fermented dish primarily consumed in the upper region of Himachal Pradesh, typically prepared using buckwheat and wheat flour. Introducing finger millet and applying fermentation process in its preparation enhances the dish's nutritional profile and impart probiotic characteristics

FIGURE 3 Sensory analysis of *aktori*.



compared to traditional *aktori*. The optimized formulation for the development of *aktori* involves adding curd (1:1) to buckwheat and finger millet flour respectively to form batter, followed by fermenting for 6–7 h and subsequent pan frying it in double layers for 5–6 min. In addition to having good probiotic qualities, the fermented *aktori* had significant levels of crude fiber, fat, protein, and carbohydrates. Through fermentation and the use of finger millet together with buckwheat, the nutritional properties were enhanced and probiotic characteristics were developed. The current study suggests that fermented *aktori* is a healthier alternative to traditional *aktori*, offering enhanced nutritional benefits and improved gut health properties. However, the use of cereal grains and natural curd fermentation provides valuable nutritional content, distinguishing it from conventional probiotic foods that often require artificial fortification. Although consumer acceptance of fermented *aktori* may currently be limited but, this combination of traditional knowledge and modern nutritional science positions fermented *aktori* as a novel, culturally significant, and functionally rich alternative in the functional food sector. Therefore, it has the potential to be promoted as a traditional yet innovative health-conscious food product, catering to the growing demand for probiotic-rich, gluten-free, and plant based foods, with future studies focusing on enhancing its sensory attributes and shelf life to enable large-scale commercialization.

AUTHOR CONTRIBUTIONS

Shambhvi: Data curation; formal analysis; writing—original draft. **Mahesh Gupta**: Conceptualization; methodology; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

There was no ethical committee for sensory evaluation in place during this study. Ethical approval for sensory evaluation of food products is also not required. However, verbal consent was appropriately obtained from all taste panellists prior to their participation. All participants were fully informed about the fresh food formulation being assessed, and the evaluation was carried out following standard protocols for the sensory testing of food products.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.