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Development of fermented wheat flour using indigenous yeast lactic starter consortium

M.K. Anjali[1], Harikesh Manu[2], Archana Chandran[3*] and C.K. Linsha[4]
Department of Dairy Microbiology
College of Dairy Science and Technology, Pookode, Wayanad- 673576
Kerala Veterinary and Animal Sciences University
Kerala, India

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Abstract

This study aimed to develop fermented wheat flour (FWF) using native yeast lactic starter consortium isolated from dahi, a popular Indian fermented dairy product. Yeast and Lactic Acid Bacteria (LAB) were isolated from samples of local household dahi to assess their milk fermentation potential. Molecular methods were used to identify the LAB isolates, while yeast isolates were identified using a carbohydrate fermentation profile. Dahi samples were prepared with LAB isolate Lactiplantibacillus pentosus, and yeast isolate Candida spherica, and their combination showed superior sensory scores. The FWF was prepared using LAB, yeast and their combination, and sensory evaluation of FWF-based soup was performed. The FWF prepared had lower moisture (6%), carbohydrate (71.14%) and calorific value (345.4 kcal) content compared to commercially available wheat flour/atta. Microbiological analysis showed the absence of coliforms, E. coli and Staphylococcus aureus, indicating hygienic preparation and inhibition of spoilage and pathogenic bacteria. The low moisture content and acidic pH (4.4) of the FWF contributed to its storage stability. In conclusion, fermented wheat flour produced using native LAB from dahi is a cost-effective, storage-stable functional food with live beneficial microorganisms, suitable for promoting gut health.

Keywords: *Fermented wheat flour, lactic acid bacteria, fermentation, cereal-based dairy products*

Cereal-based fermented products have a noteworthy place among the dietary nutrients all over the world and they contribute to about one-third of the diet worldwide. Fermentation of cereal is considered the most simple and inexpensive way of increasing their functional, nutritional, therapeutic and sensory properties (Abou-Zeid, 2016).

1. *Teaching Assistant, Department of Dairy Microbiology, CDST, Pookode*
2. *M.Sc. Scholar, Department of Food Science and Technology, School of Health Sciences, University of Calicut*
3. *Assistant Professor and Head, Department of Dairy Microbiology, CDST, Pookode*
4. *Research Assistant, Department of Dairy Microbiology, CDST, Pookode*

** Corresponding author: archanac@kvasu.ac.in, Ph.9744975460*

The growing acceptance and popularity of fermented wheat flour in the global market can be attributed to its ability to provide a gluten-reduced alternative to traditional wheat flour. This development has opened up new possibilities for the production of gluten-free or low-gluten food products, catering to the needs of the gluten-sensitive population. One of the notable advantages of fermented wheat flour is its natural preservation properties. Due to its acidic nature, fermented wheat flour does not require the addition of food preservatives. This natural acidity acts as a preservative, extending the shelf life of food products made with fermented wheat flour. This benefit is significant as it eliminates the need for artificial preservatives, enhancing the overall quality and safety of the flour (Selladurai *et al.,* 2022).

The fermentation process of wheat is considered one of the methods to degrade gluten proteins and to reduce the amount of fermentable short-chain carbohydrate compounds that are responsible for triggering gastrointestinal disorders. Wheat flour is converted into tastier, more attractive, and digestible end products by the sourdough fermentation process using lactic acid bacteria and yeasts naturally occurring in the flour. Nowadays, sourdough fermentation is widely studied due to its potential health effects. Production of postbiotic-like compounds having various health benefits such as non-viable microorganisms, bacteriocins, short-chain fatty acids, biosurfactants, flavonoids, secreted proteins/peptides, amino acids, exopolysaccharides were reported in sourdough fermentation by the symbiotic association of lactic acid bacteria and yeasts (Graca *et al.,*2021; Pérez-Alvarado *et al.*, 2022)

Dahi is an Indian ethnic fermented dairy product produced by bacterial fermentation of milk sugar lactose into lactic acid which gives dahi its characteristic tang and gel-like texture. The microbial consortium in dahi varies widely between regions and remains less explored from a nutritional and sensory point of view (Archana *et al.,* 2022). The aim of this study was to develop fermented wheat flour using dahi prepared from native yeast lactic acid bacteria symbionts.

Materials and methods

Isolation of yeast and lactic acid bacteria (LAB)

Twenty samples of freshly prepared dahi samples were collected and transported to the laboratory at 4ºC in an ice box. Isolation of yeast and lactic acid bacteria from dahi was carried out by pour plating the appropriate dilution of the sample in Potato Dextrose Agar and de Man Rogosa Sharpe Agar, respectively.

Preliminary identification of isolates

Gram staining, simple staining and catalase test were performed for preliminary identification of isolates. The presumptive cultures were streaked to purity and stored at -20ºC in 70 per cent glycerol.

Assessment of milk fermentation potential

The pure yeast and lactic acid bacteria cultures were inoculated into 10 per cent skimmed milk at a 1 per cent level and incubated at 30°C for 16 - 18 h. The isolates giving firm coagulum were selected for further study.

Identification of lactic acid bacteria by molecular method

Molecular identification of ten selected isolates of LAB was done. Total genomic DNA was extracted using the GenElute DNA Extraction Kit (Sigma Aldrich). The 16S rRNA gene was amplified using universal primers 27 F (5' AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' TACGGYTACCTTGTTACGACTT 3') (Pang *et al.*, 2012). The PCR conditions included an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 53.8°C, and extension at 72°C for 1 min; and a final extension at 72°C for 7min. The sequences were purified and sequenced at Gene Spec Pvt Ltd. Kochi. The sequences obtained were searched with the NCBI-BLAST program (https://www.ncbi.nlm.nih.gov/) for their closest relatives/reference strains with a homology of over or equal to 99 per cent.

Identification of yeasts

The carbohydrate fermentation profile of yeast was determined and identified by using API 20C AUX test kit (Biomerieux, France).

Preparation of dahi

Pasteurised toned milk with 3.0 per cent fat and 8.0 per cent SNF was used for the preparation of dahi. Ten strains of lactic acid bacteria and three strains of yeast that fermented milk with good coagulum in 10 per cent skim milk within 18 h were selected as starter cultures. The selected starter cultures in single and combinations (lactic acid bacteria: yeast- 1:1) were inoculated into pasteurized toned milk at 1 per cent level and incubated at 37°C for 16- 18 h. Three strains of LAB (L1, L7, L8) and three yeast (Y6, Y15, Y20) that formed good coagulum within 18 h in toned milk were selected as combinations (lactic acid bacteria: yeast 1:1) for sensory evaluation.

Technological evaluation of dahi

All the dahi samples prepared using single and combination starters were evaluated for acidity (expressed as % lactic acid/LA) and pH. For sensory evaluation, dahi was prepared using single starters (10 lactic acid bacteria starters and three yeast cultures) and nine combination starters. The samples were introduced in identical glass containers to 6 semi trained panelists and the scoring system for sensory attributes was as follows: Flavor (1–45 points), texture (1–30 points), colour (1–10 points), closure (1-5) and acidity (1–10 points). The combination of yeast and lactic acid bacteria with maximum sensory score was used for the preparation of fermented wheat flour (FWF).

Preparation of fermented wheat flour (FWF)

Using the selected yeast and lactic acid bacteria, three samples of dahi coded as A (Only LAB), B (Only yeast) and C (Yeast-LAB consortium) were prepared. For the preparation of FWF, commercial wheat flour and dahi were mixed in a ratio of 2:1 with the addition of 1.5 per cent salt followed by incubation at 40±2˚C for 24 hours (h). Wheat dough was then made into small balls, spread into a round shape and dried in a hot air oven at 50˚C for 24 h in a tray. Dried FWF was then ground into powder form in a mixer grinder. Further, the wheat-based soup was prepared by using the FWF as described by Daglioĝlu (2000). Four hundred grams of FWF was reconstituted in 1 L water and cooked for 30 minutes with occasional stirring. The soup was served hot for sensory evaluation.

Sensory evaluation of wheat-based soup

Sensory evaluation of three samples of soup coded as A (Only LAB), B (Only yeast) and C (Yeast-LAB consortium) made using the prepared FWF was performed by a 9-point hedonic ranking test (9=like extremely and 1=dislike extremely) for colour and appearance, flavour, aroma, odour, aftertaste, mouth feel and overall acceptability. The evaluation was carried out by 6 semi-trained panelists. The FWF that gave soup with the highest overall acceptability score was subjected to chemical and microbiological analysis.

Chemical and microbiological analysis of fermented wheat flour

Proximate analysis of the FWF was determined as per AOAC (2005). The sample was stored in sealed packets at room temperature until analysis. For the estimation of moisture, 5g of FWF was taken in a porcelain dish followed by drying in a hot air oven at 105°C ± 2°C for at least 2 h. The moisture content was calculated from the difference in weight of the sample before and after drying as per AOAC (2005). Quantitative estimation of total carbohydrates present in FWF was done using the phenol-sulphuric acid method. 100 mg of FWF was taken in boiling tubes and 2.5N HCl was added in it to hydrolyse the sample. Boiling tubes were kept in the water bath for 3 h and cooled to room temperature. After cooling it was neutralised by adding solid sodium carbonate until effervescence ceases. The whole volume was made up to 100 ml by adding distilled water and centrifuged, the supernatant was used as the sample (Agrawal *et al.,* 2011). Protein content was estimated by Kjeldahl method with the quantification of total nitrogen

according to AOAC (2005). Fat was estimated by Soxhlet extraction method. Energy value was determined by the calorimetric method in which a portion of the sample is burnt and the heat released is captured to a known amount of water. The energy released was calculated by using the equation, Energy released (J) = (Mass of water (g) x Rise in temperature (°C) x 4.2) / Mass of sample (g). pH of the FWF sample was determined by weighing 5g of the test sample in a conical flask and 100 ml of distilled water was added. After shaking for a minute, it was allowed to settle for 1h. An appropriate amount of the clear aqueous solution from the flask was transferred into a beaker and pH was measured using a calibrated pH meter.

For microbial analysis, 10g of sample was aseptically transferred to a flask containing 90 ml sterile normal saline (0.85% NaCl) to make the 10^{-1} dilution. Serial dilutions were made and total viable count, LAB count, yeast and mould count, coliform count, *E. coli* count and *Staphylococcus aureus* count were determined according to standard methods and the results were expressed as Log Colony Forming Units per gram (log CFU/g) (AOAC, 2000).

Results and discussion

Preliminary identification of yeast and lactic acid bacteria (LAB) isolates

Among the 20 samples analysed an average count of 6.07 Log CFU/mL and 8.95 Log CFU/mL of yeast and lactic acid bacteria were observed respectively. Twenty isolates of LAB and 20 isolates of yeast were phenotypically identified. All of the LAB isolates were Gram-positive and catalase negative. Twenty yeast isolates were identified by morphological features after simple staining.

Assessment of milk fermentation potential

Three isolates of yeast and ten isolates of lactic acid bacteria were found to ferment sterile skim milk within 16-18 h yielding a perfect coagulum.

Identification of lactic acid bacteria by molecular method

Ten isolates were identified up to species level using a 1465 bp PCR amplified product of 16S rRNA gene sequence. Out of the 10 LAB isolates, four showed 99 per cent sequence homology with the existing *Lactiplantibacillus plantarum* (L17, L18, L19, L20), four towards *Lactiplantibacillus pentosus* (L7, L8, L14, L16) species and two *Limosilactobacillus fermentum* (L1, L2) species reported in the NCBI ((Fig.1).

Identification of yeasts

The three yeast isolates were identified as *Candida kefir, Candida rugosa* and *Candida spherica* (Table 1).

Technological evaluation of dahi

The pH of dahi made with lactic acid bacteria, yeast and their combination (1:1) was in the range of 3.7- 5.6, 5.2-5.7, and 3.9-5.8 respectively. Titratable acidity varied from 0.45 - 2.25 per cent LA for LAB, 0.42- 0.6 per cent LA for yeast and 0.30- 2.16 per cent for combination. The results of pH and acidity are shown in Table 2.

Curd prepared with *Lactiplantibacillus pentosus* (L7), *Candida spherica* (Y20) and a combination of *Lactiplantibacillus pentosus* and *Candida spherica* (Y20 L7) was found to have organoleptically superior score in sensory

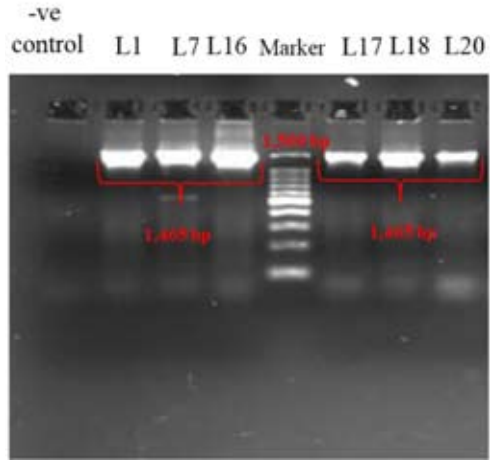

Fig. 1. PCR amplified product of 16S rRNA using universal primer 27 F and 1492R.

Table 1. Result of identification of yeast API 20C AUX kit (Biomeurix, France)

Yeast isolates	Substrate																				Identified as per api web ™ software
	Control	D-Glucose	Glycerol	Calcium 2-Keto-Gluconate	L-Arabinose	D-Xylose	Adonitol	Xylitol	D-Galactose	Inositol	D –Sorbitol	Methyl-α-D-Glucopyranoside	N-Acetyl-Glucosamine	D-Cellobiose	D-Lactose	D-Maltose	D-Saccharose	D- Trehalose	D-Melezitose	D-Raffinose	
Y 6	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	+	*Candida kefir*
Y 15	-	+	+	-	-	+	-	-	+	-	+	-	+	-	-	+	-	-	-	-	*Candida rugosa*
Y 20	-	+	+	-	-	+	-	-	+	-	+	-	-	+	+	+	+	+	-	-	*Candida spherica*

evaluation (Table 3). The pH of the curd prepared with *Lactiplantibacillus pentosus* and the combination of *Lactiplantibacillus pentosus* and *Candida spherica* was found to be in the range reported by Praseeda *et al.* (2006) in market curd samples, although the acidity was slightly higher. Three samples of FWF were prepared using curd made with L7, Y20 and Y20L7. Further, FWF-based soup was prepared using the three samples of FWF.

Sensory evaluation of FWF-based soup

The result of sensory evaluation is shown in Table 4. The mean scores of colour and appearance, aroma, odour, aftertaste, mouth feel and overall acceptability were highest for sample A followed by sample C and then sample B. Since Sample A was found to be best with higher acceptance according to the sensory score (9/10), FWF made with only LAB was subjected to further chemical and microbiological analysis.

Chemical and microbiological analysis of fermented wheat flour

The results of the chemical analysis are given in Table 5. The moisture level in the FWF was found to be 6 per cent, which is below the maximum limit set by FSSAI, 2011 (Food Safety and Standards Authority of India), which defines the maximum moisture content in wheat flour/atta as 14 per cent indicating favourable conditions for preserving the quality and safety of the FWF. Moisture content is a critical factor that influences microbial growth and the shelf life of flour. High moisture levels could promote the growth of microorganisms, including bacteria, yeast, and mould leading to spoilage and potential health risks (Saranraj and Geetha, 2012).

Chemical parameters such as carbohydrate, protein and fat of the product were found to be 71.14 per cent, 9.71 per cent and 3.26 per cent, respectively. The pH and energy values were determined as 4.4 and 345.4 kcal respectively. The study conducted by Sharoba *et al.* (2013) reported higher content of moisture (11.99%), carbohydrate (86.04%), protein content (11.85%) and lower fat content (1.06%) compared to our study. The increase in fat content observed during fermentation can be attributed to the breakdown of large fat molecules into smaller fatty acid units by lipolytic enzymes. This increase can also be caused by fat from dead microflora or the inability of fermenting microflora to use the fat in the food as an energy source (Felix and Francis, 2019). Further, the reduction in carbohydrate content observed with fermentation could be attributed to the breakdown and utilisation of fermentable sugars by lactic acid bacteria for their energy, growth and other metabolic activities (Ogodo *et al*., 2017). Thus, compared to commercially

Table 2. Mean values of pH and acidity of curd made with different isolates

Sl. No	Isolates	pH	Acidity (%LA)
1	Y 6	5.7 ±0.52	0.42±0.01
2	Y 15	5.2±0.70	0.60±0.02
3	Y 20	5.6±0.42	0.43±0.15
4	L1	3.8±0.82	1.46±0.03
5	L2	3.8±0.72	1.985±0.02
6	L7	3.8±0.70	2.25±0.15
7	L8	3.7±0.72	2.07±0.02
8	L14	5.6±0.52	0.48±0.01
9	L16	4.1±0.72	1.29±0.02
10	L17	5.6±0.52	0.50±0.03
11	L18	5.6±0.70	0.58±0.01
12	L19	5.1±0.72	0.46±0.02
13	L20	5.4±0.52	0.58±0.03
14	Y 6 L1	4±0.82	2.11±0.15
15	Y 6 L2	4±0.42	2.22±0.03
16	Y 6 L7	4.2±0.52	2.16±0.15
17	Y 6 L8	4±0.42	1.33±0.03
18	Y 6 L14	5.5±0.52	0.45±0.03
19	Y 6 L16	5±0.70	0.77±0.02
20	Y 6 L17	5.7±0.72	0.51±0.01
21	Y 6 L18	5.7±0.82	0.54±0.03
22	Y 6 L19	5.2±0.42	0.76±0.01
23	Y 6 L20	5.6±0.70	0.51±0.01
24	Y15 L1	3.9±0.72	1.75±0.02
25	Y15 L2	4±0.42	1.71±0.15
26	Y15 L7	4.4±0.72	1.75±0.01
27	Y15 L8	4.1±0.82	1.17±0.03
28	Y15 L14	4.9±0.42	0.71±0.01
29	Y15 L16	4.7±0.70	0.72±0.01
30	Y15 L17	5.6±0.52	0.47±0.02
31	Y15 L18	5.2±0.70	0.70±0.15
32	Y15 L19	5.8±0.52	0.45±0.03
33	Y15 L20	4.6±0.53	0.68±0.01
34	Y20 L1	4±0.43	1.68±0.03
35	Y20 L2	3.9±0.82	1.73±0.02
36	Y20 L7	3.9±0.82	1.73±0.02
37	Y20 L8	3.7±0.42	1.73±0.02
38	Y20 L14	5.4±0.70	0.41±0.15
39	Y20 L16	5.7±0.52	0.31±0.01
40	Y20 L17	4.7±0.42	0.81±0.15
41	Y20 L18	5.6±0.42	0.40±0.03
42	Y20 L19	5.7±0.82	0.42±0.15
43	Y20 L20	5.1±0.52	0.65±0.02

* Values are the means ± standard deviations

available wheat flour/atta, the prepared FWF was found to have a lower energy value and carbohydrate content. This indicated that the prepared FWF can be considered a low-calorie food option and can be advantageous for individuals following a low-carbohydrate or reduced-carbohydrate diet.

The result of the microbial analysis of the product is shown in Table 6. The fresh FWF was free from any coliforms, *E. coli* and *S. aureus*. The total viable count was found to be 1.38 Log CFU/g, which was less than the values observed by Nachi *et al.* (2019).

LAB and Yeast and mould count was found to be 5.9 Log CFU/g and 4.5 Log CFU/g respectively. The absence of coliforms, *E. coli* and *S. aureus* count indicates the hygienic way in which the product was prepared and handled. The low moisture content and pH of the product might have significantly contributed to the inhibition of the growth of spoilage and pathogenic microflora. This is in accordance with the reports of Tamime, (2000) who also reported the absence of coliforms in kishk, a similar dried fermented milk product. Yagoub and Ahmed (2012) reported that the gradual decrease of coliforms and faecal coliforms loads during fermentation may be a result of the activity of the lactic acid bacteria. It's important to note that other factors, such as storage conditions, packaging, and temperature, can also influence microbial growth and shelf life.

Conclusion

From the results of the study, it can be concluded that wheat flour can be effectively used as a substrate for lactic fermentation by native lactic acid bacteria from curd so as to produce functional foods with better nutritional quality. The prepared fermented wheat flour is characterized by low moisture and calorific value. This makes it an economical and storage-stable functional food option. The presence of live beneficial microorganisms further adds to its potential health benefits. Thus, the developed low-acid fermented wheat flour can be suggested as a low-cost, storage stable functional food with live beneficial microorganisms which can contribute to gut health. The study also highlights that the manufacturing process of fermented wheat flour can be easily replicated in households or industrial levels using curd, opening the scope for diversified value addition.

Table 3. Mean sensory score of different curd prepared

Samples		Flavour	Texture	Colour	Closure	Acidity	Overall acceptability
LAB	L1	38	28	8	5	8	87
	L2	36	28	8	5	8	85
	L7	45	30	10	5	10	100
	L8	45	30	8	5	8	96
	L14	34	28	8	5	6	81
	L16	36	26	10	5	8	85
	L17	38	26	10	5	8	87
	L18	45	28	10	5	10	98
	L19	38	28	10	5	8	89
	L20	34	26	8	5	8	81
Yeast	Y 6	38	26	10	5	8	87
	Y 15	38	28	10	5	8	89
	Y 20	45	30	10	5	10	100
Yeast-LAB combination	Y 6 L1	38	28	10	5	8	89
	Y6 L7	45	28	10	5	10	98
	Y6 L8	38	28	10	5	8	89
	Y15 L1	34	26	10	5	8	83
	Y15 L7	45	28	10	5	10	98
	Y15 L8	34	26	10	5	8	83
	Y20L1	39	28	10	5	8	90
	Y20 L7	45	30	10	5	10	100
	Y20 L8	34	26	10	5	8	83

Table 4. Mean sensory score of different fermented wheat flour-based soups prepared

Parameters	Sample A (L7)	Sample B (Y20)	Sample C (Y20L7)
Colour and appearance	9± 0.52	7± 0.67	8± 0.42
Flavour	8± 0.20	7± 0.20	9± 0.67
Aroma	9± 0.20	8± 0.52	9± 0.33
Odour	9± 0.67	7± 0.20	7± 0.52
Aftertaste	8± 0.52	8± 0.42	8± 0.20
Mouth feel	8± 0.42	7± 0.12	8± 0.67
Overall acceptability	9± 0.20	7± 0.22	8± 0.42

* Values are the means ± standard deviations

Table 5. Chemical composition per 100g flour

Parameters	Fermented wheat flour (L7)	Atta (Aashirvaad)
Moisture (%)	6.02 ± 0.04	8.4
Carbohydrates (%)	71.14 ± 0.01	77
Proteins (%)	9.71 ± 1.04	10.9
Fat (%)	3.26 ± 0.20	1.7
pH	4.4± 0.20	7.3
Energy (KCal)	345.4 ± 1.03	367

Note: The results in the above table are means of triplicate value ± standard deviations

Table 6. Microbiological analysis of fermented wheat flour

Parameters	Obtained values
Total viable count	1.38± 0.15 Log CFU/g
Lactic acid bacteria count	5.9 ± 0.03 Log CFU/g
Yeast and mould count	4.5 ± 0.12 Log CFU/g
Coliform count	Nil
E. coli count	Nil
S. aureus count	Nil

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Conflict of interest

The authors report no conflict of interest

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