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RESEARCH PAPER

Preparation and microbiological evaluation of novel fermented whey based corn product

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Abstract All the consumers today have considerable portion of their nutritional needs through fermented foods and beverages. Whey protein offers great functionality as well as many health benefits and could prove to be a key functional ingredient. An economical alternative way to transform whey into valuable products had been through microbial fermentation, for this addition of whey to corn flour in following proportions was done: T1 60:40, T2 65:35, T3 70:30, T4 75:25 and T0 (control) was made with 65 per cent water and 35% corn flour. The prepared product was evaluated for sensory attributes and data were statistically analyzed. The results showed that T1 60:40 was best among all treatments having pH 4.67 and titratable acidity 0.66% LA. The T1 was the most accepted product scored by the judges for the colour and appearance, body and texture, flavour, sweetness and overall acceptability 8.09, 8.27, 7.99, 7.73 and 7.77. From the naturally fermented whey corn batter the LAB (15 *Streptoococci* isolates and 13 *Lactobacilli* isolates) and lactose fermenting yeast (7) were isolated and identified through various biochemical tests. The product was prepared with different combination of starter isolates used as starter culture along with T1 as control. The results showed that PD5 (combination of starter isolates fermented) was best among all treatments and it having pH 5.04 and 0.65% TA. The PD5 scored the sensory value of (7.93, 7.73, 7.76, 7.07 and 7.70). The products with natural and starter isolates fermented were compared. The study concludes that the whey-based fermented corn product was developed in combination of starter culture isolates, demonstrated enhanced sensory attributes and a significantly reduced fermentation time (4 hours) compared to natural fermentation methods.

Keywords: Whey • Corn • Natural fermentation • Starter isolate fermentation

Introduction

Fermented foods are made through the action of beneficial microorganisms or their enzymes, which result in chemical changes and significant modification to the food. All the consumers nowadays are meeting their considerable portion of nutritional needs through fermented food and beverages. Fermented foods form a significant proportion, typically around one- third of human food intake throughout the world (Abou-Zeid, 2016). The fermented foods offer tremendous potential for promoting health, improving nutrition and reducing the risk of various diseases worldwide. The following reasons highlights the role of fermented foods in human health: fermented foods improve digestion, restore the gut balance, rich in digestive enzymes, increase the vitamin content, helps absorbing the nutrients, help to preserve it for longer period of time, inexpensive and enhances the flavour. Infants, children, adults and elderly can consume fermented foods for their good taste and their general nutritional value. Those with special medical needs can turn to fermented foods to provide added nutrition, soothe intestinal disorders, improve immune function and optimize gut ecology. Fermented foods show particular promise in reducing the

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incidence of malnutrition, lactose intolerance, diarrhea and food security.

India is the largest milk producer in the world with 239.3 million tonnes during 2023-24 (NDDB, 2025). The total worldwide whey production has been reported as 153 million tonnes per year, whey production in India is estimated at 100 million kg per annum (Kadyan *et al.,* 2021). Around 80 per cent of whey comes from *paneer* and *channa*, out of which only 2-3 per cent is utilized and rest is drained (Prazeres *et al.,* 2012). It contains nutrients like lactose, whey protein, minerals and vitamins. These nutrients have an indispensable value in human dietary requirements (Singh and Singh, 2012). Whey had constituted about 85-90 per cent of the milk volume used for products manufacture and, it retained about 55 per cent of the milk nutrients. The composition of whey has lactose (5%), proteins (0.85%), minerals (0.53%), fat (0.36%) and water (93%). Whey proteins have high biological value superior to other proteins those of egg, soya and casein of milk, mainly due to high content of branched–chain essential amino acids i.e. isolueucine, leucine and valine (Marwaha and Kennedy, 1988). Fermented whey provides even more bioactive compounds, immunoglobulins, active enzymes and healthy microorganisms that dramatically benefit digestion, immunity and muscle/joint repair. Whey protein has been associated with a range of health benefits, including enhanced post-exercise recovery, improved satiety and weight management, cardiovascular support, anti-carcinogenic properties, wound healing, infection control, infant nutrition, and healthy aging (Smithers, 2008). Whey disposal poses significant environmental concerns due to its high organic load. According to the water pollution research laboratory, whey exhibits a biological oxygen demand (BOD) ranging from 38,000 to 46,000 ppm, and in some cases, up to 76,000 ppm–far exceeding the 200 ppm limit for domestic wastewater. Microbial fermentation offers a cost-effective strategy to convert whey into value-added products, either by producing specific bio-compounds or developing novel functional foods and beverages.

Maize (*Zea mays*) is one the largest produced cereal crops in the world about 1153 MMT in 2022-23 (Grain Market Report, 543/18 May 2023). India produced about 31.65 MMT in 2021-2022 (Director's Review 2022-23: ICAR Indian institute of maize research, New Delhi). Globally, maize is a staple crop, and many people rely on it as a primary source of nutrition. In addition to playing a major role in the human diet, maize is also used as livestock fodder. Maize based fermented foods are being consumed as staple food in African countries and Latin America. Maize is processed to make an assortment of products ranging from high fructose corn syrup to biofuels, all of which play important roles in human society. It occupies an important place as a source of human food (25%), animal feed (11%), and poultry feed (52%), starch (11%), brewery (1%) and seed 1% (Maize-vision-2022-ficci, IIMR-India maize scenario 2023, iimr.icar.gov.in and grain and feed annual 2017, GAIN Report).

The principal constituents of maize are 74.3 per cent carbohydrate, 10.4 per cent protein, 4.2 per cent fat, 6.6 per cent dietary fiber and 2.0 per cent ash. The maize provides 1660 kJ per 100g of energy to the consumers (Mudria *et al.,* 2016). The digestible energy of maize is 87.2 per cent, which is higher than wheat, barley, sorghum, oat and rye. It is a good source of many vitamins and minerals including thiamine, pantothenic acid, folate, dietary fibers, phosphorus and manganese (Kumar *et al.,* 2013; Edward *et al.,* 2016). The colour of the maize is mainly due to the highest amount of lutein, zeaxanthin and anthocyanins (flavonoids) and â-carotene (Winkel, 2021). It also contains cryptoxanthin, a natural carotenoid pigment which has the potential to reduce lung cancer (Khan *et al.,* 2014; Nabi *et al.,* 2020). Maize flour is used to make nutritious bread which is highly palatable and is easily metabolized in the body. When taken at intervals, bread helps to clean the colon (Gwirtz *et al.,* 2014; Saikia *et al.,* 2011). Maize facilitates the removal of toxic food substance and accelerates the passage of faeces through the intestine. The main shortcoming is that most people are not aware of the numerous health benefits of maize, hence fail to include it in their nutrition. Keeping the above facts, the present study was planned with following objectives: Standardization of novel fermented whey based corn product. Isolation and identification of microorganisms from naturally fermented whey based corn batter. Preparation of novel fermented whey based corn product utilizing these isolates.

Materials and methods

Dairy ingredients

Whey: Fresh market milk (Amul) containing 4.5 per cent fat and 8.5 per cent SNF (solids-not-fat) was procured

from the local market in Raipur, Chhattisgarh (India). Whey was obtained as a by-product of *paneer* preparation using the method described by (Aneja *et al.,* 2002).

Skim milk powder: Skim milk powder (Manthan brand) was used for starter culture propagation and purchased from the local market in Raipur.

Non-dairy ingredients

Corn: Fresh yellow corn grains were procured from Raipur local market, sun-dried, and ground into flour.

Culture media: M17 and MRS broth/agar were obtained from HiMedia laboratories Pvt. Ltd. (Mumbai, India), and potato dextrose broth/agar from Central drug house Pvt. Ltd. (New Delhi). Media were prepared with distilled water and sterilized at 121°C (15 lbs pressure) for 15 minutes.

Preparation of novel fermented whey based corn product

Paneer whey was used to prepare a corn batter, following the method of Owusu-Ansah *et al.* (1980) with some modifications; the batter was naturally fermented for 12 hours, and then steamed for 10–15 minutes. After steaming, the product was cooled to room temperature. A schematic flowchart of the process is given below.

The schematic flow chart for product preparation

Whey
↓
Corn flour addition
↓
Mixing
↓
Fermentation (12 to 14 h at room temperature)
↓
Pouring fermented batter in moulds
↓
Steaming for 10-15 min
↓
Novel fermented whey based corn product

Evaluation of fermented whey corn batter

Chemical analysis

pH Measurement: Fermented whey based corn batter pH was measured using a digital pH meter (Ohaus Starter 3100, USA) after standardizing with buffer solutions (pH 4.2, 7.0, 9.2). A sample (corn batter) was placed in a 100 ml beaker and the pH was measured.

Table 1. Treatment details and hypothetic proportions of ingredients to select best treatment of whey and corn combination

Treatments / Ingredients (%)	T0	T1	T2	T3	T4
Water	65	-	-	-	-
Whey	-	60	65	70	75
Corn Flour	35	40	35	30	25

Titratable acidity: Titratable acidity was determined using a modified method of Omemu *et al*. (2007). A 10 g batter was mixed with 100 ml distilled water, allow to settle dense particles for 5 min, and the resulted supernatant decanted. From this, 10 mL titrated against 0.1N NaOH using phenolphthalein as an indicator. Acidity was expressed as % lactic acid using the formula (AOAC, 2003):

% Lactic acid = 9VN/W X Dilution factor

Where, V = Volume of NaOH (mL)
N = Normality of NaOH
W = Weight of sample (g)

Sensory evaluation

The product was evaluated using a 9-point hedonic scale following the method described by (Gupta *et al.,* 1976), where a score of 1 indicated dislike extremely and 9 indicated like extremely. The evaluation criteria included colour and appearance, body and texture, flavour, sweetness, and overall acceptability. A trained panel of eight members, selected from various departments of the college of dairy science and food technology, Raipur, conducted the sensory evaluation.

Isolation of microorganisms from naturally fermented whey based corn batter Isolation and identification of LAB and yeast

Fermented batter was serially diluted and plated on MRS, M17, and PDA agars. Colonies were sub cultured in their respective broths and purified by repeated streaking. Strains were identified to the genus level using a polyphasic approach (morphological identification, catalase test, sugar profiling) and maintained as glycerol stock at -20°C and routinely propagated in respective broth media.

The morphological characteristics i.e. shape and arrangements of cells were determined by both stains i.e. negative straining and gram staining (Lore *et al.,* 2005).

Biochemical characterization

The catalase test was performed by slide method. A drop of 3% H_2O_2 solution (Thermo fisher scientific, Pvt. Ltd*.,* India) was added onto this culture and closely observed for the effervescence, indicating positive test.

Carbohydrate fermentation

Isolates were identified using Hi-carbo kit (HiMedia), containing 12 immobilized carbohydrates in wells viz. Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose, L-arabinose and Mannose. Cultures were incubated to reach OD_{620} >0.5-0.6 (Spectrophotometer; Sytronics, India Ltd.) and were harvested at 10,000 rpm for 10 min at 4°C and washed with saline and re-suspended the pellet in saline (Remi instruments Ltd*.,* India), before testing as per the kit protocol.

Utilization of isolates as starter in different combination

Preparation of starter culture

Best-performing isolates (M17WCB-3, MRS-06, YWCBT-04, YWCB-05, and YWCB-07) were sub cultured in skim milk and incubated at 37°C for M17WCB-3 and MRS-06, 27°C for YWCBT-04, YWCB-05, and YWCB-07.

Preparation of novel fermented whey based corn product by using starter culture

The whey based corn batter was inoculated with isolated cultures in following three combinations

1. M17WCB-3, MRS-06 and YWCBT-04 (PD4)
2. M17WCB-3, MRS-06 and YWCBT-05 (PD5)
3. M17WCB-3, MRS-06 and YWCBT-07 (PD7)

Microbial enumeration

The pour plate method was used to enumerate microorganisms in starter-inoculated and naturally fermented batter after 8 and 12 hours of incubation.

Statistical analysis

Data were analyzed using one-factor and two-factor completely randomized design (CRD) models with SPSS free version software.

Results

The novel fermented product was developed by combining whey and corn flour in varying ratios. Four treatment groups were formulated i.e. T1 (60:40), T2 (65:35), T3 (70:30), and T4 (75:25) of whey to corn flour. A control sample (T0) was prepared using water and corn flour in a 65:35 ratio. All formulations were allowed to ferment at ambient temperature (12–14 hours).

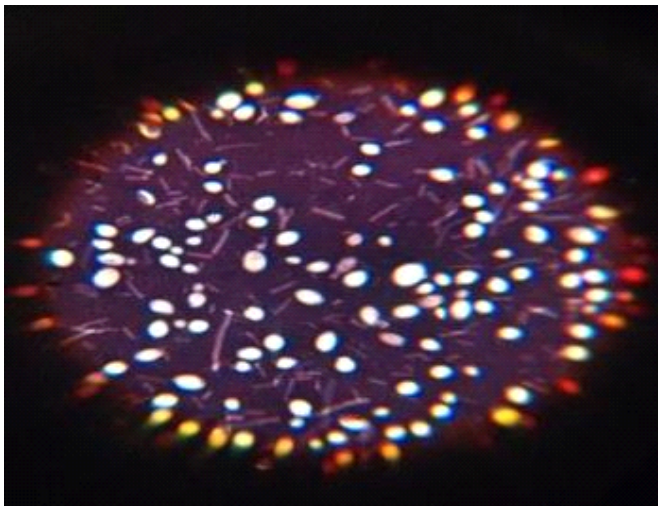

Figure 1. Negative stain of natural fermented whey based corn batter (100X)

pH and titratable acidity of fermented batter

The pH values of the batter across all treatments were compared. The lowest pH was observed in treatment T1 (4.67), while the highest was recorded in treatment T3 (4.81). The control sample exhibited a pH of 5.11. Statistical analysis revealed a significant difference ($p < 0.05$) in pH among all treatments. The lower pH in T1 is attributed due to the high carbohydrate content of maize, primarily in the form of starch. Maize starch consists mainly of two glucose polymers: amylose and amylopectin, which serve as substrates for microbial fermentation. Titratable acidity (TA) was also significantly different ($p < 0.05$) across treatments. The highest TA was observed in treatment T1 (0.66%), followed by T3 (0.54%),

Table 2. Titratable acidity and pH of natural and starter isolate fermented batter

A. Natural fermentation			B. Isolates used as starter culture fermentation				
Treatment	TA (%)	pH	Treatment	8 hours		12 hours	
				TA (%)	pH	TA (%)	pH
T0	0.44±0.02	5.11±0.10	PD 4	0.65±0.05	5.00±0.20	1.01±0.14	4.45±0.40
T1	0.66±0.05	4.67±0.09	PD5	0.65±0.02	5.04±0.22	1.02±0.05	4.4±0.35
T2	0.63±0.06	4.72±0.03	PD7	0.63±0.00	4.86±0.19	1.03±0.04	4.37±0.23
T3	0.54±0.00	4.81±0.03	Control (T1)	0.53±0.12	5.56±0.02	0.65±0.02	4.84±0.18
T4	0.56±0.04	4.80±0.18					

Values are mean ±SD

whereas the control sample showed the lowest value (0.44%). The increased TA in T1 is likely due to the higher availability of fermentable carbohydrates from whey and maize. These carbohydrates are metabolized by naturally occurring fermentative microorganisms, leading to the production of organic acids and thus contributing to the increased acidity of the batter (Table 2).

Standardization of novel fermented whey based corn product

After 12 hours of fermentation, whey-based corn batter samples were steamed for 10–15 minutes and subjected to sensory evaluation by a panel of eight trained judges using a 9-point hedonic scale. The study included four treatments with varying whey-to-corn ratios (T1 to T4) and a control (T0) using water instead of whey. The results, as shown in Table 3, sensory analysis showed significant differences ($p<0.05$) among treatments for all attributes except sweetness. T1 consistently scored highest in colour (8.09), body and texture (8.27), flavour (7.99), sweetness (7.07), and overall acceptability (7.77), while T4 scored lowest across these attributes. The control sample (T0) showed intermediate values. Sweetness differences were non-significant, possibly due to inconsistent scoring. The superior performance of T1 is attributed to its higher carbohydrate content, which enhanced microbial fermentation involving organisms such as *Streptococci*, *Lactobacilli*, and yeast. This microbial activity improved sensory properties by metabolizing complex carbohydrates into simpler compounds, thereby enhancing body, texture, flavour, and sweetness. Additionally, the high colour score of T1 is likely due to elevated levels of natural pigments such as lutein, zeaxanthin and β-carotene in the maize. The study concludes that T1 is the most preferred formulation for developing a novel fermented whey-based corn product with desirable sensory attributes.

Isolation and identification of microorganisms (Lactic acid bacteria and yeast)

Lactic acid bacteria (LAB) and yeast were successfully isolated from naturally fermented whey-based corn batter using pour plate techniques on selective media: MRS agar, M-17 agar, and PDA agar. Colonies obtained were initially screened based on their morphological characteristics using negative staining, followed by confirmation through Gram staining. Colonies exhibiting Gram-positive reactions were presumptively identified as LAB and yeast subsequently inoculated into corresponding enrichment their respective broths to enhance growth and facilitate further purification. The isolates were then purified using the streak plate method on their respective solid media. A

Table 3. Sensory evaluation of natural fermented whey based corn product

Treatment	Colour and appearance	Body and texture	Flavour	Sweetness	Overall acceptability
T0	7.04±0.46	6.62±0.92	7.04±0.46	6.58±0.33	6.41±0.83
T1	8.09±0.22	8.27±0.39	7.99±0.13	7.06±0.64	7.77±0.31
T2	7.43±0.61	7.38±0.09	7.54±0.68	6.70±0.85	7.08±0.32
T3	6.58±0.20	5.94±0.66	6.77±0.39	5.88±1.10	6.08±0.47
T4	6.39±0.37	5.69±0.81	6.39±0.37	5.87±0.98	5.85±0.48

Values are mean ±SD

total of 15 isolates from M-17 agar, 13 from MRS agar, and 7 from PDA were selected based on distinct colony morphology for further identification through a series of biochemical tests. Catalase testing revealed that all bacterial isolates from M17 and MRS media were catalase-negative, while all seven isolates obtained from potato dextrose medium were catalase-positive. These isolates represent a diverse population of lactic acid bacteria and yeast strains potentially involved in the fermentation process of the whey-based corn batter.

Biochemical characterization of isolates

Preliminary identification of microbial isolates was performed based primarily on their carbohydrate fermentation patterns using the Hi-carbo kit. Acid production from a range of sugars was assessed to classify metabolic capabilities. Isolates from M-17 agar were predominantly homo-fermentative, as evidenced by acid production without gas formation (Table 4). Among these, isolate M17-WCB-3 efficiently metabolized lactose, maltose, fructose, dextrose, galactose, trehalose, sucrose, and mannose, indicating a homo-fermentative profile consistent with thermophilic *Streptococcus* spp. Isolates obtained from MRS medium exhibited both homo- and hetero-fermentative pathways (Table 4). Notably, isolate MRS-06 fermented lactose, fructose, dextrose, trehalose, and sucrose, aligning with the metabolic characteristics typical of *Lactobacillus* spp. yeast isolates, assessed using the same kit, demonstrated vigorous fermentation of most tested carbohydrates, with the production of both acid and gas (Table 4). Specifically, isolates YWCBT-04, YWCBT-05, and YWCBT-07 fermented lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, and mannose. These broad sugar utilization profiles suggest their tentative identification as *Candida* spp. These results underscore the metabolic diversity of the microbial community present in the fermented whey-based corn batter, with potential implications for product flavour, texture, and functional properties.

Preparation and evaluation of fermented whey-based corn batter using different combinations of isolated cultures

A standardized blend of whey and corn (60:40, w/w) was utilized for all experimental treatments. The batter was inoculated with combinations of selected lactic acid bacterial strains with PD4, PD5, and PD7 isolates and compared against a control group, T1 without starter culture. All inoculated batters were incubated at ambient temperature for 8 hours, during which targeted acidification levels indicative of fermentation completion were achieved in all isolate treatments, but not in the control. A visibly enhanced leavening effect was observed in the isolate-inoculated batters compared to the naturally fermented control, highlighting the effectiveness of the selected strains in promoting fermentation and batter development.

Sensory analysis of natural and starter isolates fermented novel whey based corn product

Following the fermentation process, the resulting products were subjected to sensory evaluation to assess the acceptability of the final fermented whey-based corn product. Products fermented with the PD5 isolate combination received the highest scores across all sensory attributes, given in Table 5. Statistical analysis revealed significant differences ($p < 0.05$) among the treatments, indicating that the selection of microbial cultures had a substantial impact on product quality. In contrast, the control sample (T1), which underwent natural fermentation (8 hours), demonstrated inferior sensory performance. The results suggest that controlled fermentation using selected microbial isolates, particularly the PD5 combination, significantly enhances both the physicochemical and sensory qualities of whey-based corn product. These findings offer promising potential for the development of novel functional fermented foods using agro-industrial by-products such as whey.

Enumeration of microorganisms present in combination of starter isolates fermented whey based corn batter

Microbial enumeration was performed on whey-based corn batters fermented with combinations of selected starter isolates after 8 hours of incubation, and compared with naturally fermented control samples (T1) incubated for 8 and 12 hours. Serial dilutions of each sample were plated on PDA for yeast, M17 agar for *Streptococci*, and MRS agar for *Lactobacilli*. Plates were incubated at 27°C for yeast and at 37°C for *Streptococci* and *Lactobacilli*. The microbial counts for *Streptococci*, *Lactobacilli*, and

Table 4. Sugar fermentation profile of M-17/MRS/PDA medium isolates

Isolate/Sugar	Lac	Xyl	Mal	Fru	Dex	Gal	Raf	Tre	Mel	Suc	Ara	Man
M17 WCB-01	+	-	+	+	+	-	-	+	-	+	-	-
M17 WCB-02	+	-	+	+	+	+	+	+	+	+	-	+
M17 WCB-03	+	-	+	+	+	+	-	+	-	+	-	+
M17 WCB-04	+	-	+	+	+	+	-	+	-	+	+	+
M17 WCB-05	+	-	+	+	+	+	-	+	+	+	+	+
M17 WCB-06	+	-	+	+	+	+	-	+	-	+	-	+
M17 WCB-07	+	-	+	+	+	+	-	+	-	+	-	+
M17 WCB-08	-	-	+	+	+	+	-	-	+	+	-	+
M17 WCB-09	+	-	+	+	+	+	+	+	+	+	-	+
M17 WCB-09/10	+	-	+	+	+	+	+	+	+	+	-	+
M17 WCB -11	+	-	+	+	+	+	-	+	-	+	-	+
M17 WCB-12	+	-	+	+	+	+	-	+	+	+	+	+
M17 WCB-12/a	+	-	+	+	+	+	-	+	-	+	-	+
M17 WCB-13	-	-	+	+	+	+	+	-	+	+	-	+
M17 WCB -SD3	+	-	+	+	+	+	-	+	-	+	-	+
Control (*Sreptococcus thermoplilus NCDC 325)*	+	-	-	+	+	+	-	+	-	+	-	+
MRS-01	-	-	-	-	-	-	-	-	-	-	-	-
MRS-02	-	-	-	+	-	-	-	-	-	-	-	-
MRS-03	-	-	-	+	+	-	-	+	-	+	-	-
MRS-04	+	-	-	+	-	-	+	+	+	+	-	-
MRS-05	-	-	-	-	-	-	-	-	-	-	-	-
MRS-06	+	-	-	+	+	-	-	+	-	+	-	-
MRS-07	+	-	-	+	-	+	-	-	-	-	-	-
MRS-08	-	-	-	+	+	-	-	-	-	+	+	-
MRS-09	-	-	-	-	-	-	-	-	-	-	-	-
MRS-10	-	-	-	-	+	-	-	-	-	-	-	-
MRS-11	-	-	-	+	-	-	-	-	-	-	-	-
MRS-12	-	-	-	+	+	-	-	+	-	+	-	+
MRS-13	-	-	-	-	-	-	-	-	-	-	-	-
Control *(Lactobacillus acidophilus* NCDC 13)	+	+	+	+	+	+	+	+	+	+	+	+
YWCBT-01	-	+	+	+	+	+	+	+	+	+	+	+
YWCBT-02	-	+	+	+	+	+	+	+	+	+	+	+
YWCBT-03	-	+	+	+	+	+	+	+	+	+	+	+
YWCBT-04	+	+	+	+	+	+	+	+	+	+	+	+
YWCBT-05	+	+	+	+	+	+	+	+	+	+	+	+
YWCBT-06	-	+	+	+	+	+	+	+	+	+	+	+
YWCBT-07	+	+	+	+	+	+	+	+	+	+	+	+
Control *(Candida krusei)*	+	-	-	-	+	+	+	-	-	+	-	-

Lac-Lactose, Xyl-Xylose, Mal-Maltose, Fru-Fructose, Dex-Dextrose, Gal-Galactose, Raf-Raffinose, Tre-Trehalose, Mel-Melibiose, Suc-Sucrose, Ara-L-Arabinose and Man- Mannose

Table 5. Sensory evaluation of starter isolates fermented whey based corn product

Treatment	Colour and appearance	Body and texture	Flavour	Sweetness	Overall acceptability
PD4	7.91±0.06	7.80±0.10	7.70±0.10	7.70±0.20	7.56±0.20
PD5	7.93±0.05	7.73±0.05	7.76±0.20	7.73±0.15	7.70±0.10
PD7	7.71±0.10	7.55±0.43	7.56±0.15	7.33±0.30	7.50±0.17
Control (T1)	7.23±0.25	6.98±0.27	7.00±0.10	7.03±0.15	6.93±0.11

Values are mean ±SD

Table 6. Enumeration of microbes present in natural and starter isolate fermented batter

Combination of Isolate	Period of Incubation (h)	Average Count (log10 cfu/g)		
		Yeast	*Streptococci*	*Lactobacilli*
PD4	8	9.08	9.20	9.10
PD5	8	9.19	9.78	9.26
PD7	8	9.00	9.25	9.12
Control (Natural)	8	8.13	8.12	7.66
	12	10.04	9.93	9.04

yeast in the batter inoculated with starter isolates after 8 hours were comparable to those observed in the naturally fermented control after 12 hours (Table 6), indicating that the use of starter cultures significantly accelerated microbial growth and fermentation.

Discussion

The present study demonstrated that controlled fermentation of whey-based corn batter using selected starter isolates combinations (PD4, PD5, and PD7) led to significant microbial, physico-chemical and sensory improvements within a shorter fermentation period compared to natural fermentation. These findings align with earlier reports on traditional cereal fermentations, particularly in African fermented products such as *Togwa, Masa, Kenkey* and *Ogi*.

Mugula *et al.* (2003) reported a sharp decline in pH (from 5.24–5.52 to 3.10–3.34) during the fermentation of *Togwa*, accompanied by an increase in titratable acidity (TTA), which is consistent with the present study. Similarly, Enwa *et al.* (2011) observed that maize fermented with starter cultures reached a lower pH range (3.59–3.84) than naturally fermented maize (4.53–4.83) after 24 hours, emphasizing the efficiency of starter cultures in acidification. Our results also agree with those of Sanni and Adesulu (2013), who found a decrease in pH and increase in TTA during traditional and laboratory fermentation of *masa*, with starter cultures promoting faster acid development. The accelerated acidification observed in our starter-fermented batters mirrors the trends reported by Edema and Sanni (2008), who noted that starter cultures significantly increased the TTA in sour maize dough. This rapid acid development can be attributed to the metabolic activity of lactic acid bacteria (LAB), particularly *Lactobacillus* and *Streptococcus* species, which have been shown to produce lactic acid efficiently from lactose and other sugars (Saeed *et al.,* 2013).

Our microbial enumeration data indicated that LAB and yeast populations in the batter inoculated with starter isolates reached levels comparable to those found in naturally fermented samples only after extended incubation. This supports the findings of Sherifah and Aima (2011), who documented similar trends in LAB and yeast load during spontaneous fermentation of maize for *Ogi*, and (Teniola and Odunfa, 2001), who reported high LAB and yeast counts in both natural and starter-culture fermentations of maize slurry. The presence of catalase-negative colonies on MRS and M17 media confirmed the presence of *Lactobacilli* and *Streptococci*, consistent with earlier biochemical classifications (Sharma *et al.,* 2013; Saeed *et al.,* 2013). In contrast, catalase-positive isolates on PDA were identified as yeasts, which align with the descriptions by (Kurtzman and Fell, 1998), who characterized common fermentative yeasts such as *Saccharomyces cerevisiae*, *Candida krusei*, and *Geotrichum* spp.

In terms of sensory evaluation, the PD5-inoculated batter consistently scored higher across all organoleptic parameters. This finding is in agreement with studies by Singh *et al*. (2010); Darade *et al*. (2016), who reported improved flavor, texture, and overall acceptability in whey and cereal, based fermented products using selected microbial inoculants. Although Hounhouigan *et al*. (1999) noted that starter cultures sometimes yielded less flavor compared to traditional methods, our results demonstrate

that well-selected isolates can achieve or even exceed the sensory quality of natural fermentation. The enhanced acidification rates observed in our study parallel the findings of Ganguly and Sabikhi (2012), who reported significant acidification by *Lactobacillus acidophilus* in a composite dairy-cereal substrate. Furthermore, the microbial dynamics observed support the results of Omemu *et al*. (2007) reported that *Saccharomyces cerevisiae*, *Candida krusei, C. tropicalis, Geotrichum candidum, G. fermentans*, and *Rhodotorula graminis* were isolated during the fermentation of maize for *Ogi* production. The titratable acidity was increased with fermentation time about 0.32 ± 0.02 to 0.42 ± 0.02 per cent (0-48 hours) and found that yeast populations contribute not only to acid production but also to flavor development during maize fermentation.

Finally, our sensory results align with research on *Kenkey* product by Halm *et al*. (1996); Teniola and Odunfa (2001), who reported on *Ogi* product comparable or superior acceptability for products fermented with starter cultures, depending on strain selection and substrate composition. Overall, the use of selected starter isolates in whey-based corn batter significantly improved fermentation efficiency, microbial growth, acidification, and sensory quality, thus offering a viable alternative to natural fermentation methods.

Conclusion

In this study, five variants of a novel fermented whey-based corn product were developed with the aim of offering a nutritious, cost-effective food option for populations facing economic and resource constraints. Among the formulations tested, the T1 treatment (60% whey and 40% corn flour) received the highest sensory scores for overall acceptability, as evaluated using the 9-point hedonic scale. Microbial analysis indicated that the use of selected starter culture isolates in the fermentation process was more effective than natural fermentation, resulting in improved product consistency and a reduced fermentation time (8 hours versus 12 hours). This suggests that controlled fermentation using defined lactic acid bacteria (LAB) and yeast strains can significantly enhance both the functional and sensory attributes of the final product. Overall, the findings demonstrate that incorporating starter cultures in the fermentation of whey and corn helps not only to shorten processing time but also yields a product with superior sensory attributes i.e. taste, texture and nutritional value. The consumption of such fermented products could contribute to improved gut health and dietary diversity, affordable option for people, particularly in nutritionally vulnerable communities.

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Conflict of interest

The authors declare no competing or conflict of interest.

Authors' Contributions

In this study Narsimlu Battula done bench work, data analysis and manuscript writing, Dr. Manorama helped with conceptualization, supervising of study, reviewing and editing manuscript. Dr. Sarang Dilip Pophaly helped with conceptualizing, investigation of study, editing and manuscript preparation. Jitesh Tarak helped in bench work of this study.

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