Journal of Food Science and Technology
https://doi.org/10.1007/s13197-024-06023-y

ORIGINAL ARTICLE

Development of nutri-functional paneer whey-based kefir drink

Harisha Devi[1] · Tejinder Pal Singh[1] · Ruby Siwach[2] · Vandana Chaudhary[3]

Revised: 6 June 2024 / Accepted: 21 June 2024

Abstract

Present research focused on biotransformation of paneer whey into a functional fermented product using kefir culture. Out of 9 formulations (S-1 to S-9) tried; S-8, obtained by fermenting FOS (1%) supplemented paneer whey and adding 8% refined sugar, was identified as the most acceptable product. Nutritional analysis revealed the following as per 100 g of product: 44.24 kcal total energy, 8.29 g carbohydrates, 7.19 g sugar, 1.51 g protein, 0.52 g total fat, 0.13 g saturated fat, 0.30 g MUFA, 0.23 g ash, 49.7 mg sodium content, 0.51% (w/w) alcohol and 4.5% (v/v) CO_2. Results revealed a notable decline in pH and a rise in acidity during the early stages of storage followed by stabilization thereafter. Additionally a progressive decrement in lactose content and increase in ethanol was reported owing to the fermentation activity of the diverse microflora in kefir culture. The product exhibited antimicrobial as well as antioxidant activity and also remained stable for 12 days under refrigeration. Microbial stability was further strengthened by the absence of *E.coli* and consistent viable count of lactic acid bacteria and yeast in confirmation with the microbiological standards of fermented milk products. Results indicated that both proteinaceous as well as non-proteinaceous components are responsible for antioxidant activity of the product. Hence, the development of paneer whey-based kefir could relieve hassle of waste management and also provide health benefits.

✉ Tejinder Pal Singh
88tejindersingh@gmail.com

[1] Department of Dairy Microbiology, College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125001, Haryana, India

[2] Department of Dairy Chemistry, College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125001, Haryana, India

[3] Department of Dairy Technology, College of Dairy Science and Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125001, Haryana, India

Highlights

- Paneer whey is generally dumped as waste that increases environmental burden.
- An attempt has been made to bio-transform this waste into a functional product.
- Paneer whey-based kefir drink was standardized and characterized.
- Nutritional facts were validated as per requirements of the regulatory standards.
- Developed product was evaluated for functional properties under in vitro conditions.

Keywords Paneer whey · Kefir · Functional drink · Dairy byproduct · Nutri-functional product · Whey drink

Introduction

Sustainable development goals adopted by United Nation member states has driven everyone's' attention. It has been accepted that it is necessary to coexist in harmony with environment, identify healthier way to reduce carbon footprint, find innovation in waste management and ensuring healthy lifestyle to everyone. As far as Indian dairy industries are concerned, the tremendous growth has been observed in past decades both in terms of production that is from 55.6 million tonnes in 1991-92 to 230.6 million tonnes in 2022-23 and consumption (Milk Production in India | nddb.coop). The dairy products are highly popular among Indian population especially among vegetarians who consider dairy products as an ideal nutritional source.

Paneer is, one of the most popular indigenous dairy products, generally prepared by acid coagulation of milk. Approximately 7% of total milk produced in the country is transformed to paneer in particular (Kapoor et al. 2021). Paneer is also termed as Indian cottage cheese owing to its non melting soft creamy texture and subtle taste. It is popular as a meat alternative among vegetarian population of the country owing to its high protein and calcium content (Smetana et al. 2023). Generally, one liter of milk yields

Published online: 29 June 2024

18-20% paneer and 80-82% paneer whey (Gawande et al. 2023). Hence, paneer whey is a by-product obtained from acid based coagulation of milk which can be designated as acid whey as the pH generally remains less than 5 (Lievore et al. 2015).

Whey generally contains approximately 55% of the milk nutrients which includes 20% of the total milk proteins (Rocha-Mendoza et al. 2021). Researchers have suggested that whey is an affluent source of high quality protein, essential amino acids, minerals and bio-active peptides that can provide various health benefits such as immunomodulation, anti-inflammation, anti-hypertension, and tissue maintenance (Mehra et al. 2021). Emerging evidences of health benefits associated with whey or whey components have drawn interest to develop innovative methods to utilize the functional whey components. Whey is currently being exploited for preparation of whey protein isolates, whey concentrates, de-mineralized whey powder and lactose extraction. However, these productions are restricted to sizable dairy enterprises owing to involvement of costly high-end technologies. The small and medium sized dairy enterprises cannot bear the expenses of installing the necessary equipment to process the limited volumes of whey (Roy et al. 2023). This has created a pressure on dairy industry to find innovative strategies to utilize acid whey particularly in a sustainable manner. Considering that India is a developing country and a significant portion of milk is curdled in unorganized small to medium dairies or in households, this often results in the wastage of nutritionally as well as functionally rich whey.

Kefir is a functional product with distinct characteristics such as acidic-alcoholic taste, creamy texture, and effervescence due to mixed fermentation by lactic acid bacteria and yeast (Vashisht et al. 2023). Kefir is known to possess anti-stress, anti-allergenic, anti-asthmatic, anti-microbial, anti-cancer, immunomodulatory, hypocholesterolemic properties aside from its ability to maintain gut homeostasis (Farag et al. 2020). Such benefits may be attributed to the potential of kefir to modulate gut microbiota and mycobiota, based on the excellent survivability, colonization ability, and microbial interaction of the microorganisms in kefir as well as its rich bioactive components (Kim et al. 2019). Owing to high nutritional and functional value, and increasing consumers' awareness regarding impact of diet on health, whey can be used as a base to prepare a functional fermented drink such as kefir. The kefir drink prepared by fermentation of paneer whey using kefir culture are speculated to be deep-pocketed with various health promoting properties that can help in restoring gut homeostasis. Therefore, considering such limitations and concerns associated with acid whey utilization, the present work proposed the transformation of this by-product into nutri-functional paneer whey-based kefir drink.

Materials and methods

Materials

Standardized milk (4.5% fat and 8.5% SNF; solid not fat) was procured from Milk Plant, Department of Livestock Products Technology, Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. All the chemicals (citric acid, NaOH, Fructo-oligosaccharide (FOS) etc.) used for analysis were purchased from Himedia, Mumbai, India. The kefir culture used for fermentation was obtained from Zoh Probiotics, Mumbai, India. The pathogenic strains of *E. coli* O157:H7 ATCC 43,888 and *Salmonella enteritidis* ATCC 13,076 were procured from College Central Laboratory (CCL), LUVAS. The pathogenic strains were maintained as 25% glycerol stock until use. The pathogenic strains were sub-cultured in Brain Heart Infusion (BHI) broth twice before performing antimicrobial activity.

Extraction of paneer whey

Firstly, the standardized milk was heated to 90°C and then cooled to 70°C followed by addition of preheated (70°C) 2% citric acid solution with continuous but gentle stirring till clear whey which was greenish translucent, separated from the coagulum. Further, the content was kept undisturbed for 10 min to achieve complete separation of coagulum from whey. Lastly, the clear whey was separated from coagulum using double layered muslin cloth. The whey obtained was analyzed for physicochemical parameters such as pH using electrode digital pH meter (EUTECH Instruments, Mumbai), titratable acidity as per BIS (Bureau of Indian Standards) guidelines (1960), total solids as per BIS guidelines (1961), fat as per AOAC (2000) guidelines, total protein content using automatic Kjeldhal digestion and distillation unit (Kel Plus- KES12L, Pelican Industries, Chennai) and lactose by using methodology given by Abu-Lehia (1987).

Development of paneer whey-based kefir

Paneer whey extracted was allowed to cool (25°C) and neutralized to pH 6.5 using food grade NaOH (1 N) to avoid insolubalization of whey proteins which may otherwise create difficulty during processing. The neutralized whey was then filtered through muslin cloth to get clear whey. Further, different concentrations (0.5%, 1% and 1.5%) of prebiotic fructo-oligosaccharides (FOS) were added to the whey followed by heating at 65°C for 30 min in a water bath. Subsequently, the kefir starter culture (Zoh Probiotics, Amazon) was inoculated to FOS supplemented whey samples as per the manufacturers' directions and the fermentation was allowed for 18 h at 25˚C. After fermentation,

the refined sugar (6%, 7%, and 8%) was added to respective fermented whey preparations. The resulting products (S-1 to S-9) were then stored at 4°C overnight before performing sensory evaluation.

Sensory evaluation

The kefir beverages developed were subjected to double blind randomized sensory evaluation using a 9-point hedonic scale. In this, 10 ml of each sample was served to 25 semi-trained judges. The judgement was based on characteristics such as flavour/taste, body and texture, colour and appearance, container and overall acceptability. The study was approved by the competent committee of the University LUVAS, Hisar (LUVAS/PGS/Acad/2022/3229-31) and the consent was taken from each participant before performing the analysis.

Physicochemical analysis of selected product

The product selected on the basis of sensory evaluation results was analysed for physico-chemical parameters. The pH, acidity, fat, lactose content, total solids and total protein content were estimated as per standard protocols mentioned above. However, the viscosity of the samples was assessed using a Brookfield Viscometer Model RVT (Scientific Instrument and Technology, Delhi) at 20 °C. The alcohol content of the product samples was determined using hydrometer based on specific gravity measurement principle (AOAC 2000). The color assessment was done using Chroma Meter CR-400 (Konica Minolta INC., Japan). The product analysis results were also compared with the values obtained for whey and fermented whey (without FOS).

Storage study

In this study, the developed product samples (control fermented whey and selected product; S-8) were kept in refrigeration continuously for 12 days. During this period, the samples were tested for physicochemical and microbiological parameters on every third day (0th day, 3rd day, 6th day, 9th day and 12th day). The physicochemical parameters evaluated are same as mentioned above for developed product. For microbiological parameters, the coliform, yeast and mold and lactic acid bacteria count was estimated using standard pour plating method.

Nutritional facts as per labelling requirement

The samples of developed paneer whey-based kefir were sent to NABL and FSSAI accredited laboratory (Sigma Test and Research Center, Badli, New Delhi) to obtain certification for nutritional facts to be mentioned on product label.

Antimicrobial activity

The antimicrobial activity of the test samples was investigated against *E. coli* O157:H7 ATCC 43,888 and *Salmonella enteritidis* ATCC 13,076 using agar well diffusion method. Firstly, the sterilized BHI agar (2% agar) was poured in petri plates and was allowed to solidify followed by over-layering of the test pathogen (inoculated at the rate of 2%) containing BHI soft agar (0.8% agar). The wells of 6 to 8 mm diameter were punched using sterile cork borer and 50 µL of the test samples were introduced into the respective wells. The plates were observed for zone of inhibition after incubation period of 16–18 h at 37°C.

Antioxidant activity

The antioxidant activity of the samples was evaluated using the DPPH. The DPPH solution was freshly prepared by dissolving 0.39432 g of DPPH in 1 L of methanol and homogenizing in an ultrasonic bath for 30 s. Two ml of test sample was mixed with 20 ml of extracting solvent (methanol: water, 70:30 v/v) and blended thoroughly on a magnetic stirrer followed by storage at 20 ± 1 °C for 4 h in a dark place. Further, it was centrifuged at 10,000 rpm for 10 min and filtered through Whatman™ Grade 2 cellulose filter paper (Diameter: 12.5 cm, Pore Size: 8 µm) (Whatman International Ltd., Maidstone, England). The extract of the samples obtained was used to determine antioxidant activity. The following different cocktail combinations were prepared for estimation of antioxidant activity: 1 ml of extract + 3 ml methanol (control), 1 ml DPPH + 3 ml methanol (blank) and 1 ml of extract + 2 ml methanol + 1 ml DPPH (sample). The cocktails prepared were kept in the dark for 30 min and the absorbance was read at 517 nm using a spectrophotometer (Genesys 10 S UV- Vis, Thermo Scientific). The estimation was done using following calculations:

$$DPPH\ Scavenging\ Activity\,(\%) = \left(1 - \left(\frac{A_1 - A_0}{A_2}\right)\right) \times 100$$

Where, A_0 = Absorbance at 517 nm for control; A_1 = Absorbance at 517 nm for the sample; & A_2 = Absorbance at 517 nm for the Blank Values.

Extraction of bioactive components

Three fractions on molecular weight (MW) basis (> 5 kDa, 5 – 3 kDa, 3 – 1 kDa) were obtained from the developed paneer whey-based kefir and the neutralized whey using

MW cut-off filters. For this, the test samples were loaded to the filter tubes chambers followed by centrifugation for 15 min at 12,000 rpm. The fractions derived were collected in the sample tubes and stored at -20°C till further use. The protein concentrations in obtained fractions were estimated using Lowry method. The fractions obtained were evaluated for antioxidant activity before and after Proteinase K treatment.

Statistical analysis

The experiment data, as when necessary, is presented as the mean ± standard deviation (SD) of different parameters studied in the present investigation. The mean and standard deviation were determined using Microsoft Excel 2007 Software Package, Microsoft Corporation, USA. Statistical analysis was performed on the data obtained from the experiments conducted in triplicate for optimizing the beverage. This included sensory behavior, nutritional and functional analysis. The software used for analysis was IBM SPSS Statistics 25, and the statistical tests used were one-way analysis of variance (ANOVA) with the multiple comparison Tukey Test ($p < 0.05$).

Results and discussion

Parameters studied for paneer whey

The present study focused on biotransformation of dairy by-product paneer whey into nutri-functional product. The paneer whey obtained by acidification of standardized milk was evaluated for various physicochemical parameters such as pH, acidity, lactose, protein, fat and total solids. The pH for obtained whey was found to be 5.39 ± 0.28 which confirms that the whey obtained was acid whey in accordance to Lievore et al. (2015). The acidity, lactose content and protein content in the paneer whey extracted was 0.19 ± 0.02%, 5.43 ± 0.12% and 0.60 ± 0.02%, respectively. Furthermore, the fat and total solids were found to be 0.50 ± 0.01% and 9.45 ± 0.24%, respectively. The variability observed in comparison to available literature for lactose and total solid content of paneer whey could be due to variability in initial milk composition and processing treatments involved. Furthermore, the values for pH, acidity and total solids also vary with the type and amount of acidulant used for coagulation.

Sensory evaluation

The paneer whey obtained was further processed and fermented to obtain nine different products (S-1 to S-9) using varied concentration of prebiotic FOS (0.5%. 1.0% and 1.5%) and refined sugar (6%, 7% and 8%). The prepared product samples were subjected to double blinded randomized sensory evaluation to identify the most acceptable product in terms of flavor, body and texture, color and appearance, and overall acceptability. The results manifested that the mean scores for flavor, body and texture, and overall acceptability were significantly ($p < 0.05$) different among all the kefir samples as depicted in Table 1. However, no significant difference ($p > 0.05$) was observed for color and appearance scores among the prepared kefir samples. Sample S-1was least accepted as this sample showed lowest scoring for flavor and overall acceptability in comparison to other eight samples. The lowest scores of body and texture were observed for S-1 and S-4. In terms of overall acceptability, the lowest scores were observed for S-1, S-2 and S-4. However, no significant difference was observed for S-3, S-5, S-6, S-7 and S-9. Three kefir samples S-5, S-6 and S-9 showed similar scoring for all the five parameters evaluated. Based on the sensory evaluation outcomes, the paneer whey-based kefir drink (S-8), developed by fermenting FOS (1%) supplemented paneer whey, with added (post fermentation) refined sugar to the final concentration of 8% was found to be the most acceptable formulation as it showed significantly ($p < 0.05$) higher scores for taste, body

Table 1 Impact of varying concentrations of FOS and refined sugar on the sensory characteristics of paneer whey-based kefir drink

Composition (FOS & Refined sugar)	Sample	Flavour	Body &Texture	Color & Appearance	Overall Acceptability
0.5% & 6%	S-1	$6.24^{c} \pm 0.52$	$6.76^{b} \pm 0.52$	$7.28^{a} \pm 0.54$	$6.98^{c} \pm 0.46$
1% & 6%	S-2	$7.24^{ab} \pm 0.43$	$7.08^{ab} \pm 0.86$	$7.08^{a} \pm 0.64$	$7.28^{abc} \pm 0.45$
1.5% & 6%	S-3	$6.96^{b} \pm 0.68$	$7.40^{a} \pm 0.76$	$7.56^{a} \pm 0.77$	$7.43^{ab} \pm 0.62$
0.5% & 7%	S-4	$7.00^{b} \pm 0.82$	$6.80^{b} \pm 0.5$	$7.2^{a} \pm 0.76$	$7.20^{bc} \pm 0.45$
1% & 7%	S-5	$7.20^{ab} \pm 1.04$	$7.08^{a\ b} \pm 0.75$	$7.16^{a} \pm 0.85$	$7.31^{ab} \pm 0.53$
1.5% & 7%	S-6	$7.16^{ab} \pm 1.11$	$7.16^{ab} \pm 0.62$	$7.24^{a} \pm 0.88$	$7.34^{ab} \pm 0.47$
0.5% & 8%	S-7	$7.04^{b} \pm 0.73$	$7.12^{ab} \pm 1.01$	$7.32^{a} \pm 0.85$	$7.32^{ab} \pm 0.58$
1% & 8%	S-8	$7.60^{a} \pm 0.76$	$7.48^{a} \pm 0.65$	$7.52^{a} \pm 0.82$	$7.60^{a} \pm 0.55$
1.5% & 8%	S-9	$7.12^{ab} \pm 0.78$	$7.20^{ab} \pm 0.91$	$7.28^{a} \pm 0.84$	$7.35^{ab} \pm 0.59$

Data are Mean ± standard deviation of results from three separate experiments

[abcde] Different symbol means statistically significant difference ($P < 0.05$) within the same column

and texture and overall acceptability. Very few but some researchers have exploited cheese whey (sweet whey) as a substrate for kefir preparation; performed comparative sensory analysis of whey-based kefir and milk kefir (Magalhães et al. 2010; Pereira et al. 2015). However, limited attempts have been made for utilizing acid whey to develop kefir.

Physicochemical parameters studies for final product

The most acceptable paneer whey-based kefir drink (S-8) was investigated for various physiochemical parameters including pH, acidity, lactose, viscosity, total solids, and ethanol content. The pH and acidity of the product was found to be 5.87 ± 0.06 and 0.10 ± 0.01%, respectively. Total solids, lactose and fat content values were 8.81 ± 0.31%, 4.14 ± 0.01% and 0.50 ± 0.01%, respectively. The ethanol content of the product was ascertained to be 0.42 ± 0.02%. The compositional values for paneer whey-based kefir developed in the present study were comparable with the observations reported by Pereira et al. (2015) who developed kefir beverage using whey protein concentrates and concentrated ultrafiltered whey permeates. The viscosity of the developed product was ascertained to be 1.16 ± 0.35cP which was comparatively less than observations reported for whey-based kefir beverage developed by Sabokbar et al. (2015). The use of kefir culture instead of kefir grains in this study was assumed to be the responsible factor for such variability in viscosity.

The compositional parameters of whey, fermented whey (FW) and selected product (S-8) were also compared. It is evident from Table 2 that the pH and acidity values for whey were significantly ($p < 0.05$) different when compared to non-significant values of FW and S-8 which obviously due to fermentation. No significant difference was observed in total solids for FW and S-8, however the values were significantly ($p < 0.05$) lower in comparison to those observed for whey. Such reduction in overall total solids content could be attributed to hydrolytic activities of starter culture. Furthermore, the significant difference for lactose content and protein content was observed among whey, FW and S-8. The ethanol content was not seen in whey but was observed in both fermented products i.e. FW (0.43 ± 0.01%) and S-8 (0.42 ± 0.02%), however, the values were found to be non-significant. This possibly due to the presence of alcohol producing yeast (*Kluyveromyces sp*.) heterofermentative bacteria (e.g. *Lactobacillus kefir*) in the kefir culture (Magalhães et al. 2010).

Table 2 Physico-chemical characteristics of whey, fermented whey and selected kefir product S-8

Parameters	Whey	Fermented whey	S-8
pH	$5.44^{b} \pm 0.03$	$5.90^{a} \pm 0.00$	$5.87^{a} \pm 0.06$
Acidity (%)	$0.19^{a} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.15^{b} \pm 0.01$
Total Solids (%)	$9.45^{a} \pm 0.24$	$8.86^{b} \pm 0.55$	$8.82^{b} \pm 0.31$
Lactose (%)	$5.37^{a} \pm 0.06$	$3.66^{c} \pm 0.02$	$4.14^{b} \pm 0.01$
Protein (%)	$0.62^{a} \pm 0.01$	$0.53^{c} \pm 0.01$	$0.55^{b} \pm 0.01$
Ethanol (w/w %)	Nil	$0.43^{a} \pm 0.01$	$0.42^{a} \pm 0.02$

Data are Mean ± standard deviation of results from three separate experiments

[abcde] Different symbol means statistically significant difference ($P < 0.05$) within the same column

Storage stability

The storage stability of fermented whey (FW) and S-8 were assessed for 12 days at refrigeration conditions during which the samples were analyzed for alterations in various physicochemical (pH, acidity, lactose, ethanol, viscosity, color) and microbial parameters at a regular interval of 3 days (0th, 3rd, 6th, 9th and 12th day). The significant ($p < 0.05$) drop in pH was observed for FW and S-8 during storage period (Table 3). The sudden fall was observed in initial 3 days of storage (0th to 3rd day) for both FW and S-8. After 3rd day, the slight but non-significant variations were seen in pH values. The acidity values also showed sudden significant ($p < 0.05$) increase during initial three days which remain statistically unchanged till 6th day and then again showed significant increase on 12th day for both FW and S-8. The lactose content reduced significantly ($p < 0.05$) for both FW and S-8 during the storage period. The lactose content for FW and S-8 dropped significantly and progressively from 3.66 ± 0.02 (0th day) to 2.76 ± 0.02 (12th day) and form 4.14 ± 0.01 (0th day) to 2.17 ± 0.01 (12th day) during storage, respectively. A significant ($p < 0.05$) difference in lactose content was observed among FW and S-8.

Kefir starter is a consortium of undefined bacterial and yeast species that perform mixed fermentation. The homofermentative lactic acid bacteria are responsible for lactic acid fermentation while others heterofermenters and yeasts are involved in alcoholic fermentation. In fact, facultative and heterofermentative lactobacilli also possess an enzyme, alcohol dehydrogenase, which allows them to produce ethanol via acetaldehyde from lactose metabolism (Magalhães et al. 2010). In this study, the ethanol content of 0.43 ± 0.01% and 0.42 ± 0.02% was observed for FW and S-8, respectively, which significantly ($p < 0.05$) increased during storage. No significant effect of FOS was noticed as the ethanol contents were almost similar in both the samples. Even the ethanol content in FW and S-8 significantly increased up to 6th day which further showed a non-significant increment up to 12th day. Earlier, Wulansari et al. (2021) had also reported successive increments of ethanol content during 14 days storage of the goat milk kefir which supports the observations of the present study.

Table 3 Physico-chemical characteristics of fermented whey (FW) and product S-8 during 12 days storage study

Day	pH		Acidity (%LA)		Lactose (%)		Ethanol (%)		Viscosity (cp.) at 20 °C		Colour Values					
											L		a*		b*	
	FW	S-8	FW	S-8	FW	S-8	FW	S-8	FW	S-8	FW	S-8	FW	S-8	FW	S-8
0th	5.90^{ax} ±0.00	5.87^{ax} ±0.06	0.16^{dx} ±0.01	0.15^{dx} ±0.01	3.66^{ay} ±0.02	4.14^{ax} ±0.01	0.43^{dx} ±0.01	0.42^{dx} ±0.02	1.13^{ex} ±0.00	1.16^{dx} ±0.04	49.56^{bx} ± 5.47	50.79^{ax} ±3.93	-0.88^{ax} ±0.40	-0.70^{ax} ±0.40	2.68^{bx} ±0.28	3.06^{abx} ±0.53
3rd	5.70^{bx} ±0.00	5.67^{bx} ±0.06	0.27^{cx} ±0.00	0.28^{cy} ±0.01	3.55^{bx} ±0.02	3.44^{by} ±0.02	0.49^{cx} ±0.02	0.51^{cx} ±0.03	1.33^{dx} ±0.00	1.33^{cx} ±0.00	56.54^{abx} ± 7.32	54.34^{ax} ±8.94	-1.78^{bx} ±0.01	-1.77^{bx} ±0.12	3.86^{abx} ±0.42	3.72^{abx} ±0.67
6th	5.67^{bx} ±0.06	5.64^{bx} ±0.01	0.28^{bcx} ±0.02	0.30^{bcx} ±0.01	2.99^{cx} ±0.01	2.98^{cx} ±0.02	0.56^{bx} ±0.01	0.57^{bx} ±0.01	1.45^{cx} ±0.02	1.39^{cx} ±0.02	57.25^{abx} ±4.92	61.65^{ax} ±1.85	-1.96^{bx} ±0.16	-1.75^{bx} ±0.04	2.79^{bx} ±1.06	4.34^{abx} ±0.15
9th	5.63^{bx} ±0.01	5.62^{bx} ±0.01	0.30^{abx} ±0.01	0.31^{abx} ±0.01	2.87^{dx} ±0.03	2.39^{dy} ±0.02	0.59^{bx} ±0.02	0.62^{abx} ±0.01	1.66^{bx} ±0.03	1.64^{bx} ±0.02	64.12^{ax} ± 1.36	61.52^{ax} ± 1.75	-2.25^{bx} ±0.05	-2.16^{bx} ±0.01	3.1^{abx} ±0.14	3.28^{bx} ±0.10
12th	5.62^{bx} ±0.06	5.57^{bx} ±0.06	0.31^{ax} ±0.01	0.33^{ax} ±0.02	2.76^{cx} ±0.02	2.17^{cy} ±0.01	0.60^{abx} ±0.01	0.64^{ax} ±0.02	2.12^{ax} ±0.01	2.03^{ax} ±0.06	65.05^{ax} ±3.64	63.12^{ax} ±2.75	-1.84^{bx} ±0.10	-1.47^{bx} ±0.41	4.30^{ax} ±0.13	4.55^{ax} ±0.13

Data are mean ± standard deviation of results from three separate experiments

[abcde] Different symbol means statistically significant difference ($P < 0.05$) within the same column

[xy] Different symbol means statistically significant difference ($P < 0.05$) within the same row between the treatments

No significant difference in viscosity among FW and S-8 samples were observed during storage. However, the successive and significant increment in viscosity values was observed within FW and S-8 samples during 12 days storage as evident from Table 3. The production of exopolysaccharides by kefir starter could be responsible for such changes in viscosity. Previously, Pereira et al. (2015) also noticed an increase in viscosity of kefir developed form whey protein concentrates and concentrated ultrafiltered whey permeates during 7 days storage.

The color of the products was analyzed in terms of L (whiteness), a* (redness) and b* (yellowness) values. The readings ranges from 0 (black) to 100 (white) for L, + 60 (red) to -60 (green) for a* and + 60 (yellow) to -60 (blue) for b* values. As evident from Table 3, the L (whiteness) values kept on increasing significantly in FW from 49.56 ± 5.47 (0th day) to 65.05 ± 3.64 (12th day) during storage. Similarly, the values for L showed significant increment for S-8 during 12 days storage. During the storage, the a* values remained in negative side and the b* values remained on positive side showing the slight green and yellow tinge in FW and S-8 samples. The color parameters showed no significant variation when the readings for FW and S-8 samples were compared on any particular day during storage. Previously, Aidarbekova and Aider (2021) studied the color changes in milk kefir supplemented with different concentrations of whey. They found that the whiteness decreased with increasing concentration of whey and lowering fat content. This probably is due to presence of colloidal proteins (casein micelles) in milk which scatters light. But, in this study the paneer whey was used which lacks colloidal proteins and has more pronounced green color because of riboflavin.

During the storage study, no coliforms were seen which confirms that the product was hygienically prepared, packed and maintained. Absence of coliforms ensures that the product can withstand the microbiological standards of fermented milk products given by FSSAI, India. No significant change in lactic acid bacteria counts were seen as the counts ranged between 8 and 9 log cfu/ml during the 12 days storage. Similarly, yeast and molds counts were found in the range of 5–6 log cfu/ml during storage. These observations explicated that the starter microbes remained viable during 12 days storage. According to the Codex Standards (Commission 2003), kefir must contain minimum 7 log CFU/g of kefir microorganisms and 4 log CFU/g yeasts. Hence, the product developed can satisfy the microbiological standards of both FSSAI and Codex.

Nutritional facts

As per FSSAI Labeling and Display Regulations, 2020 (Food Safety and Standards Authority of India 2020), nutritional information, a description intended to inform the consumer of nutritional properties of the food, must be clearly given on the sample. Considering the importance of labeling, the samples of developed paneer whey-based kefir (S-8) were sent to NABL and FSSAI accredited laboratory (Sigma Test and Research Center, Badli, New Delhi) to obtain certification for nutritional facts to be mentioned on product label. The certified data received from the NABL and FSSAI accredited lab is given in Table 4. The total energy of the product was found to be 44.24 kcal per 100 g product which may be considered as a low-calorie product. However, FSSAI recommended that the total energy should not be more than 40 kcal per 100 g product to declare the product as low-calorie product. The total fat content of the product was 0.52 g per 100 g product (which is less than 1.5 g per 100 g product), therefore, the product can be classified as a low-fat product. The developed product falls under the category of low sodium product as the product showed less than 0.12 g of sodium per 100 g product. The developed product cannot be considered as a protein-rich source for an adult as it does not provide 20% of protein recommended dietary allowance per 100 g of product. The product was found to contain 8.29 g of carbohydrates and 7.19 g of sugar per 100 g of product, respectively. The product did not contain any PUFA (polyunsaturated fatty acids) or dietary fibers. Additionally, the product showed low levels of saturated fats as the values were found to be less than 0.75 g per 100 g of the product. However, MUFA content contributed to 43.33% of the total fat found in the product but still the product falls under low MUFA category. The ash content of the product was found to be 0.23 g per 100 g of product. The alcohol and CO_2 content were estimated as 0.51% by weight and 4.5% by volume, respectively, in the developed product. The data obtained for nutritional facts can be used to design a product label as per FSSAI (Labeling and Display) Regulations, 2020 (Food Safety and Standards Authority of India F 2020).

Table 4 Nutritional facts about the developed paneer whey-based kefir drink (S-8)*

NUTRITIONAL FACTS	
Energy (/100 g)	44.24 kcal
Total Carbohydrates (/100 g)	8.29 g
Total Sugars (/100 g)	7.19
Protein (/100 g)	1.51 g
Total Fat (/100 g)	0.52 g
Saturated Fat (/100 g)	0.13 g
MUFA (/100 g of total fat)	0.30 g
PUFA (/100 g of total fat)	Nil
Ash (/100 g)	0.23 g
Sodium (/100 g)	49.7 mg
Dietary Fibres	NIL
Carbon dioxide (v/v)	4.5
Alcohol % (w/w)	0.51

*As per report of NABL / FSSAI accredited laboratory

Antimicrobial properties

Antimicrobial activity is one amongst many other functional properties that have been associated with kefir. This activity has been attributed to the ability of its starters to produce antimicrobial components during fermentation (Kim et al. 2019). The paneer whey-based kefir developed in this project was also assessed for its ability to inhibit the growth of pathogenic bacteria, specifically *E.coli* ATCC 43,888 (O157:H7) and *Salmonella enteritidis* ATCC 13,076 using agar-well assay. As evident from the results, no antimicrobial activity was observed for whey samples against tested pathogens. However, both FW and S-8 showed antimicrobial activity against *E.coli O157:H7*. S-8 showed significantly ($p < 0.05$) wider zone of clearance (21.9 mm) in comparison to FW (18.5 mm) that too disappeared after neutralization with NaOH. This depicts that the activity was because of metabolites produced during fermentation such as lactic acid and alcohol. However, the product samples (before and after neutralization) showed no antimicrobial activity against *Salmonella enteritidis*. Previously, researchers have also reported the antagonistic activity of milk kefir against various pathogens and spoilage bacteria (Al-Mohammadi et al. 2021; Kim et al. 2019). There are several components like organic acids, hydrogen peroxide, carbon dioxide, diacetyl and ethanol produced by the bacteria in kefir grains that could inhibit the growth of harmful bacteria (Shen et al. 2018). Besides, certain bioactive peptides also created during fermentation that could have antimicrobial activity. But these bioactive components have potential interactions that can either enhance or hinder their antimicrobial effects. Due to these interactions, kefir exhibits varied antimicrobial activities against different bacteria (Kim et al. 2019). This could be the reason for the selective inhibition observed for *E. coli* in this study. Similar to our observations, others have also reported the reduction of antimicrobial activity post neutralization of milk kefir (Al-Mohammadi et al. 2021).

Antioxidant potential

An antioxidant potential of three samples - whey, FW and S-8 was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity method. As evident from the results, the statistically significant ($p < 0.05$) difference was observed in the antioxidant activities of whey,

FW and S-8. The highest antioxidant activity was seen for S-8 (41.31 ± 0.09%) followed by FW (36.97 ± 0.05%) and whey (4.6 ± 0.16%), respectively. Possible reason for some antioxidant activity in whey could be the presence of whey proteins, urate, lactoferrin and residual vitamin C in smaller proportions. Fermentation has the potential to increase the antioxidant activity of substrates by releasing various metabolic end products. The extracellular proteinases of the LAB can hydrolyze milk proteins during fermentation, leading to the formation of peptides that can contribute to the antioxidative properties of fermented products. Also, the use of starter cultures in fermentation enhances the release of reductants like cysteine found in whey protein peptides (Kadyan et al. 2021). Antioxidants derived from food have the potential to shield the host from the intestinal oxidative stress by lowering the levels of free radicals and influencing the presence of beneficial microbial species in the gut (Lobo et al. 2010). This could possibly be the reason for the higher antioxidant activity in FW and S-8 as kefir starter contains significant amount of lactic acid bacteria and yeast. Other researchers have also reported the higher antioxidant activity in kefir starter fermented products of milk, soya milk or blend of pomegranate juice and whey in comparison to their respective unfermented counterparts (Yilmaz-Ersan et al. 2016).

Bioactive components with antioxidant potential

Various fractions (> 5 kDa, 5 − 3 kDa, 3 − 1 kDa) were extracted from whey and S-8 using molecular weight cut-off filters of different sizes. After extraction, the protein content of each fraction was determined by following lowry method. The highest protein content (36 mg/ml) was found in fraction with > 5 kDa molecular weight obtained from S-8In addition, the fractions with 3–5 kDa (k) and < 3 kDa (k) molecular weight had protein content of 21.43 mg/ml and 16.46 mg/ml, respectively. In case of fractions obtained from whey, the protein contents were 36 mg/ml, 15 mg/ml and 12.83 mg/ml, respectively, for > 5 kDa, 3–5 kDa and < 3 kDa fractions. Overall, the fractions obtained from kefir (S-8) had more protein contents than those obtained from whey.

Before analyzing the antioxidant activity of obtained fractions, the protein concentration in each fraction obtained from product S-8 and whey was adjusted to 1 mg/ml. This was done to standardize the comparison of antioxidant activity between the fractions with respect to protein concentration. The purpose was to determine which fraction contributed the most to the antioxidant activity of the product. Table 5 depicts the results for antioxidant activity of each standardized fraction.

A significant difference in the antioxidant activity among the different fractions standardized to a protein concentration of 1 mg/ml was observed. The kefir (S-8) fraction of > 5 kDa (12.73 ± 0.15%) showed the highest antioxidant activity followed by fractions 3–5 kDa (7.67 ± 0.15%) and < 3 kDa (6.17 ± 0.06%), respectively. The fraction > 5 kDa may contain peptides generated from casein and lactoferrin, which contribute to antioxidant activity. The fraction 3–5 kDa might contain smaller peptides and free amino acids like cysteine and methionine, whereas < 3 kDa may include very small peptides together with trace amounts of vitamin C and uric acid adding to antioxidant activity (Mehra et al. 2021). However, there was no significant difference was seen in the antioxidant activity among the whey fractions. The antioxidant activity in whey fractions was significantly lower when compared with kefir fractions of the corresponding molecular weights. Malta et al. (2022) uncovered antioxidant potential within the peptide fraction of < 10 kDa sourced from milk kefir. These findings exhibited reasonable concurrence with the present investigation.

Proteinase K treatment was conducted on all the three fractions (> 5 kDa, 5 − 3 kDa, < 3 kDa) to determine whether the antioxidant activity was solely attributed to proteins or some non-proteinaceous components were also involved. The antioxidant activity of the treated fractions was then compared to the activity of their respective untreated fractions. The antioxidant activity of Proteinase K

Table 5 Antioxidant activity of protein fractions from whey and kefir standardized to 1 mg/ml protein concentration

Samples	MW Fractions	Protein Concentration (mg/ml)	Anti-oxidant activity for Standardized (1 mg/ml) Fractions (%)	Calculated Anti-oxidant activity for fractions (%)
Whey	> 5 kDa	36	$5.57^{cd} \pm 0.49$	200.52
	3–5 kDa	15	$5.67^{c} \pm 0.06$	85.05
	< 3 kDa	12.83	$5.03^{d} \pm 0.06$	64.53
Kefir (Product II)	> 5 kDa	48.2	$12.73^{a} \pm 0.15$	613.58
	3–5 kDa	21.43	$7.67^{b} \pm 0.15$	164.36
	< 3 kDa	16.46	$6.17^{c} \pm 0.06$	101.55

Data are Mean ± standard deviation of results from three separate experiments

[abcde] Different symbol means statistically significant difference ($P < 0.05$) within the same column

treated fractions was significantly ($p < 0.05$) less in comparison to the activity of their respective untreated counterparts. Additionally, there were significant ($p < 0.05$) differences in the antioxidant activity among different fractions within the treated and untreated samples. The untreated > 5 kDa fraction (30.14 ± 0.51%) showed the highest antioxidant activity followed by the 3–5 kDa fraction (27.72 ± 0.20%) and < 3 kDa fraction (14.64 ± 0.64%), respectively. Antioxidant activities of the fractions were reduced significantly after proteinase K treatment but the treated fractions also showed the antioxidant activity. The highest activity was observed in > 5 kDa fraction (26.09 ± 0.53%) followed by the 3–5 kDa fraction (15.06 ± 0.84%) and < 3 kDa fraction (5.08 ± 0.69%), respectively, among proteinase K treated fractions. This depicts that both proteinaceous as well as non-proteinaceous components in the product are responsible for the observed antioxidant activity. Moreover, there is a need to identify and characterize the proteinaceous as well as non-proteinaceous components responsible for the antioxidant activity of the developed product.

Conclusions

In this study, as targeted, the successful transformation of a dairy by-product (paneer whey) into a nutri-functional paneer whey-based kefir was achieved. The developed product was characterized for various physicochemical and nutritional parameters. The nutritional facts were also validated by the NABL and FSSAI accredited laboratory. The results confirmed that the developed product was low calories, low fat, low sodium, acid-alcoholic product which meet the nutritional and safety standards of the regulatory authorities. Furthermore, the developed product was found to possess the antimicrobial and antioxidant activity. In order to further improve the acceptance by consumer future research can be carried out by incorporating natural flavors or extracts such as vanilla, strawberry, or citrus that is recognized for their ability to enhance the taste of dairy products, This could potentially improve the sensory experience of the kefir drink without negatively impacting its health advantages. Hence, the development of paneer whey-based kefir is an easiest, economic and healthier way to reduce carbon footprint, relieve hassle of waste management and also provide alternate means to improve human health.

Author contributions Harisha Devi: Investigation, Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. Tejinder Pal Singh: Investigation, Conceptualization, Methodology, Supervision, Project administration, Writing – final draft layout, Validation. Ruby Siwach: Conceptualization, Writing – review & editing. Vandana Chaudhary: Conceptualization, Writing – review & editing. All authors read and approved the final manuscript.

Funding Not Applicable.

Data availability Data will be made available on request.

Code Availability Not Applicable.

Declarations

(i) the work described has not been published before (except in the form of an abstract, a published lecture or academic thesis),
(ii) it is not under consideration for publication elsewhere,
(iii) its submission to JFST publication has been approved by all authors as well as the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out,
(iv) if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder, and.
(v) JFST will not be held legally responsible should there be any claims for compensation or dispute on authorship.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abu-Lehia IH (1987) A simple and rapid colorimetric method for lactose determination in milk. Food Chem 24(1987):233–240. https://doi.org/10.1016/0308-8146(87)90155-5

Aidarbekova S, Aider M (2021) Use of Electro-activated whey as ingredient in fermented milk production: Proof of the Concept of the Technological feasibility. ACS Food Sci Technol 1(7):1349–1359. https://doi.org/10.1021/acsfoodscitech.1c00230

Al-Mohammadi AR, Ibrahim RA, Moustafa AH, Ismaiel AA, Abou Zeid A, Enan G (2021) Chemical Constitution and Antimicrobial Activity of Kefir Fermented Beverage. Molecules 26(9):2635. https://doi.org/10.3390/molecules26092635

AOAC (2000) Official Methods of Analysis. 17th Edition. In: The Association of Official Analytical Chemists, Gaithersburg, MD, USA

BIS (1960) Indian Standard Methods of Test for Dairy Industry Part I. In Rapid Examination of Milk

BIS (1961) Indian Standard methods of test for dairy industry part II. In Chemical Analysis of Milk

Commission CA (2003) Codex standard for fermented milks. In

Farag MA, Jomaa SA, Abd El-Wahed A, El-Seedi HR (2020) The many faces of Kefir fermented dairy products: quality characteristics, Flavour Chemistry, Nutritional Value, Health benefits, and Safety. Nutrients 12(2):346. https://doi.org/10.3390/nu12020346

Food Safety and standards Authority of India (2020) Food Safety and standards. (Labelling and Display) Regulations. In

Gawande H, Arora S, Mittan R, Lule V, Sharma V, Singh AK (2023) Effect of milk protein standardization using high milk protein ingredients on texture, composition and yield of paneer. Appl Food Res 3(1):100286. https://doi.org/10.1016/j.afres.2023.100286

Kadyan S, Rashmi H, Pradhan D, Kumari A, Chaudhari A, Deshwal G (2021) Effect of lactic acid bacteria and yeast fermentation on

antimicrobial, antioxidative and metabolomic profile of naturally carbonated probiotic whey drink. Lwt 142:111059. https://doi.org/10.1016/j.lwt.2021.111059

Kapoor S, Singh M, Vatankhah H, Deshwal G, Ramaswamy H (2021) Production and quality improvement of Indian cottage cheese (paneer) using high pressure processing. Innov Food Sci Emerg Technol 72:102746. https://doi.org/10.1016/j.ifset.2021.102746

Kim DH, Jeong D, Kim H, Seo KH (2019) Modern perspectives on the health benefits of kefir in next generation sequencing era: improvement of the host gut microbiota. Crit Rev Food Sci Nutr 59(11):1782–1793. https://doi.org/10.1080/10408398.2018.1428168

Lievore P, Simões DR, Silva KM, Drunkler NL, Barana AC, Nogueira A, Demiate IM (2015) Chemical characterisation and application of acid whey in fermented milk. J Food Sci Technol 52(4):2083–2092. https://doi.org/10.1007/s13197-013-1244-z

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4(8):118–126

Magalhães KT, Pereira MA, Nicolau A, Dragone G, Domingues L, Teixeira JA, de Almeida Silva JB, Schwan RF (2010) Production of fermented cheese whey-based beverage using kefir grains as starter culture: evaluation of morphological and microbial variations. Bioresour Technol 101(22):8843–8850. https://doi.org/10.1016/j.biortech.2010.06.083

Malta SM, Batista LL, Silva HCG, Franco RR, Silva MH, Rodrigues TS, Correia LIV, Martins MM, Venturini G, Espindola FS, da Silva MV, Ueira-Vieira C (2022) Identification of bioactive peptides from a Brazilian kefir sample, and their anti-alzheimer potential in Drosophila melanogaster. Sci Rep 12(1):11065. https://doi.org/10.1038/s41598-022-15297-1

Mehra R, Kumar H, Kumar N, Ranvir S, Jana A, Buttar HS, Telessy IG, Awuchi CG, Okpala COR, Korzeniowska M, Guiné RPF (2021) Whey proteins processing and emergent derivatives: an insight perspective from constituents, bioactivities, functionalities to therapeutic applications. J Funct Foods 87:104760. https://doi.org/10.1016/j.jff.2021.104760

Pereira C, Henriques M, Gomes D, Gomez-Zavaglia A, de Antoni G (2015) Novel functional whey-based drinks with great potential in the dairy industry. Food Technol Biotechnol 53(3):307–314

Rocha-Mendoza D, Kosmerl E, Krentz A, Zhang L, Badiger S, Miyagusuku-Cruzado G, Mayta-Apaza A, Giusti M, Jiménez-Flores R, García-Cano I (2021) Invited review: acid whey trends and health benefits. J Dairy Sci 104(2):1262–1275. https://doi.org/10.3168/jds.2020-19038

Roy P, Mohanty AK, Dick P, Misra M (2023) A review on the challenges and choices for Food Waste valorization: environmental and economic impacts. ACS Environ Au 3(2):58–75. https://doi.org/10.1021/acsenvironau.2c00050

Sabokbar N, Moosavi-Nasab M, Khodaiyan F (2015) Preparation and characterization of an apple juice and whey-based novel beverage fermented using kefir grains. Food Sci Biotechnol 24(6):2095–2104. https://doi.org/10.1007/s10068-015-0278-6

Shen Y, Kim DH, Chon JW, Kim H, Song KY, Seo KH (2018) Nutritional effects and Antimicrobial Activity of Kefir (grains). J Milk Sci Biotechnol 36(1):1–13. https://doi.org/10.22424/jmsb.2018.36.1.1

Smetana S, Ristic D, Pleissner D, Tuomisto HL, Parniakov O, Heinz V (2023) Meat substitutes: resource demands and environmental footprints. Resour Conserv Recycl 190:106831. https://doi.org/10.1016/j.resconrec.2022.106831

Vashisht P, Chaudhary V, Sharma S, Devi H, Singh TP (2023) Whey Kefir: a sustainable solution to reduce Waste and Improve Health. ACS Food Sci Technol 3(9):1403–1410. https://doi.org/10.1021/acsfoodscitech.3c00229

Wulansari P, Nurliyani N, Endah S, Nofriyaldi A, Harmayani E (2021) Microbiological, chemical, fatty acid and antioxidant characteristics of goat milk kefir enriched with Moringa oleifera leaf powder during storage. Food Sci Technol 42(7):1621. https://doi.org/10.1590/fst.71621

Yilmaz-Ersan L, Ozcan T, Akpinar Bayizit A, Şahin S (2016) The antioxidative capacity of Kefir produced from Goat milk. Int J Chem Eng Appl 7(1):22–26