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RESEARCH ARTICLE

# Biofunctional properties and production of peptides from fermented cheddar cheese whey fractions using *Limosilactobacillus fermentum*

**Bhagyashree Das[1], Kunal Gawai[2], Kaushik Das[3], Hiral Kumari Patel[3] and Subrota Hati[3](✉)**

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**Abstract:** This investigation was conducted to analyse the antioxidant activity, proteolytic activity and ability of short-chain fatty acids (SCFAs) production from fermented cheddar cheese whey fractions inoculated with *Limosilactobacillus fermentum,* MF951096 (M4) @ 2.5% when incubated at 37°C for 48 h. Ultrafiltration membranes with appropriate molecular weight thresholds were used to isolate low-molecular-weight peptides from fermented whey, including the 3/ kDa permeate, 10/ kDa permeate, and retentate fractions. In acidity and pH profile, there was no significant difference observed. Significantly higher peptide content (7.84mg/mL) and antioxidant activity (65.81%) were observed in the 10/ kDa retentate fraction; However, the 10 kDa retentate showed a lactic count among all the fractions. Also we characterized the peptide fractions by analysing their chromatographic peaks using Reverse-Phase High- Performance Liquid Chromatography. The 10/ kDa retentate fraction produced the most SCFA *i.e.* 46.68 ìg/mL acetic acid,21.59 ìg/mL propionic acid, and 5.27 ìg/mL butyric acid, respectively. From this investigation, we concluded that the conversion of dairy waste into bioactive molecules presents a novel approach with conceivable applications in the dairy, food and pharmaceutical sector.

**Keywords**: Fermented cheddar cheese whey fractions, antioxidant, short-chain fatty acids, *Limosilactobacillus fermentum*

[1] Department of Dairy Technology, Parul Institute of Technology, Parul University, Vadodara - 391760, INDIA
[2]Department of Dairy Microbiology, College of Dairy Science, Kamdhenu University, Amreli, Gujarat, India.
[3]Department of Dairy Microbiology, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India.

(✉)Subrota Hati, Dept. of Dairy Microbiology, SMC College of Dairy Science, Kamdhenu University, Anand-388110, Gujarat, India; E-mail address: subrota.hati@kamdhenuuni.edu.in

## Introduction

The functional food and drink sector has been growing substantially over the last decade.The beneficial health effects of functional foods and nutritional products are primarily attributed to bioactive compounds or specific functional groups generated during their metabolic processing in the body (Agyei and Danquah, 2012). Protein hydrolysates are the principal derivatives of dietary proteins, and those exhibiting beneficial physiological effects are referred to as bioactive peptides (BPs). These peptides typically consist of 2 to 30 amino acid residues and also known as low molecular weight peptides (LMWPs) (Fadimu et al. 2022; Ye et al. 2022; Xiao et al. 2022). Although extensively studied over the years, low molecular weight peptides (LMWPs) have not attracted sufficient attention within the scientific community. Notably, this peptide fraction is pivotal in modulating gene expression and participating in a wide range of disease-associated pathways. In Japan, the inclusion of BPs as functional components in food products has been authorized for specific health claims under the designation of Foods for Specified Health Uses (FOSHU) (Ye et al. 2022).On the other hand, the production of BPs and low-molecular-weight peptides (LMWPs) from agricultural waste has recently gained attention as a sustainable and eco-friendly approach. In the dairy industry, casein coagulation during milk processing results in the formation of whey as a major by-product, which constitutes a significant portion of dairy waste.

Global cheese production is estimated at approximately 22.6 million tonnes per year, resulting in the generation of an estimated 180-200 million tonnes of whey annually (Pires et al. 2021;Irazoqui et al.2024).Nearly fifty percent of global cheese whey output is processed and turned into various food and feed products. Around 50% of this processed fraction is used in the form of liquid, 30% is dried out and transformed into powdered cheese whey, 15% is further processed into lactose and its byproducts, and the rest is used to make cheese whey protein concentrates (WPC) (Barba, 2021).

Whey proteins have a globular structure with a homogeneous distribution of nonpolar, polar, and imposed amino acid residues. Whey proteins include β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins (Ig), serum albumin (SA), lactoferrin (LA), lactoperoxidase (LP), and approximately 60 enzymes. Other protein components include glycomacropeptide (GMP), which is released from κ-casein during the beginning phase of enzymatic coagulation (Pires et al. 2021).

Whey proteins, widely recognized for their popularity as nutritional supplements, exhibit a distinct amino acid composition compared to caseins and other proteins, characterized by a higher content of amino acid profile, particularly essential amino acids (EAAs) (Pires et al. 2021; Tang et al.2025). The essential amino acids (EAAs) present in whey protein fraction includes the essential amino acids (EAAs) histidine, leucine, isoleucine, lysine, phenylalanine, methionine, threonine, tryptophan, and valine. Among these leucine, isoleucine, and valine are the branched-chain amino acids (BCAAs) (Tang et al.2025).

Researchers are highly interested in these peptides, as food-derived BPs have been proven to possess multiple biological functions, including antioxidant, antihypertensive, anti-inflammatory, immunoregulatory, antibacterial, anti-photoaging, opioid-like, and other health-promoting activities (Ye et al. 2022)

Wang et al. (2022) reported that peptides have emerged as a distinct class of therapeutic agents in recent years, owing to their unique biochemical properties and significant therapeutic potential. Zhang et al. (2021) reported bioactive peptides have antioxidant and antimicrobial activity. Several researchers have investigated and testified that the whey proteins hydrolysis releases bioactive peptides with various physiological functions, including antioxidant, antihypertensive, antimicrobial, opioid, and antithrombotic activities (Mann et al. 2015).

Numerous research reports mentioned that contribution of whey proteins enhance the activity of glutathione peroxidase, a critical antioxidant enzyme responsible for reducing oxidative stress at the cellular level (Yu et al. 2020). Furthermore, lactoferrin makes up about 1-2% of the entire protein concentration in whey and has excellent antioxidant effects (Barba et al. 2019). The presence of sulfur-containing amino acids, as well as its ability to bind transition metals, are responsible for its ability to scavenge free radicals. Additionally, lactoferrin possesses significant antifungal activity (Barba, 2021).

Das et al. (2025a) examined the effects of incubation period besides culture concentration on the fermentation of cheese whey using the *Lactiplantibacillus plantarum* (KGL3A) culture, with the evaluation of enhancing antioxidative, antidiabetic, and peptide production. Additionally, the fermented whey was analysed using a peptidomic approach. They observed that as the incubation period progressed, antidiabetic activity, antioxidant capacity, and peptide production increased.

Feng et al. (2022) investigated the hydrolysis of whey protein and reported that outcomes in the generation of both short-chain fatty acids (SCFAs) and BPs in the gut, which are associated with a range of potential health benefits. Enzymatic degradation of whey protein into smaller peptides increases its fermentability by gut microbes, thereby promoting increased SCFA production. Additionally, they identified whey protein hydrolysate (WPH) as a valuable dietary supplement, not only rich in essential amino acids but also possessing the potential to support the restoration of the infant gut microbiome.

The objective of the study was to analyse the antioxidant activity, proteolytic activity and peptide production from fermented cheddar cheese whey fractions fermented with the *Limosilactobacillus fermentum*M4 culture, as well as to analyse the short-chain fatty acid profile and lactic acid bacterial count of the resulting whey peptide fractions.

## Material and Methods

### Preparation of fermented whey and whey fractions

Vidya Dairy in Anand, Gujarat, India provided the cheese whey, which was then autoclaved sterilized at 121/ °C for 15 mins. and stored at 5/ ±/ 1/ °C for future use. Pure *Lactobacillus* culture (*Limosilactobacillus fermentum,* M4)(MF951096) was added to the sterilized cheese whey @ 2.5% incubated simultaneously followed by incubation for 48hrs at 37°C. Peptide extraction from the fermented whey was carried out using the methodology developed by Panchal et al. (2020) and Dineshbhai et al. (2022). Fermented cheese whey samples were separated to obtain the supernatant through the process of centrifugation at a constant temperature of 4°C at 16873 g force (max.) for 30 min. The supernatants were collected and fractionated by ultrafiltration using a 10kDa and 3kDa cut-off membranes (Amicon® Milipore ultra centrifugal filters, MERK, Ireland) at 2363g force for 30 min and different peptide fractions of >10kDa, <10kDa, <3kDa were collected from the membranes. Unfermented whey was taken as the control sample, and fractionated whey samples were used as the treatment samples in this study.

### Physico chemical analysis of fermented cheese whey

### Determination of pH

The pH of all cheese whey samples *i.e.* fermented as well as unfermented was measured using a calibrated pH meter (OAKTON pH700, India) according to the method given by the Parmar et al, 2016. The pH meter was calibrated at first using standard buffer solutions at pH 4.0, 7.0, and 9.0. A 10/ mL aliquot of the well-mixed mixture was put into a beaker, and the pH was measured by dipping the electrode immediately in the sample.

**Determination of titratable acidity**

Titratable acidity of fermented and unfermented cheese whey samples was determined using the method specified in the (Parmar, 2016). . A 10 mL sample was transferred to a porcelain dish, and 1.0 mL of phenolphthalein indicator. The mixture was then titrated with 0.1 N NaOH until a faint pink color appeared and persisted for 30 seconds.

Titratable acidity was estimated using the following formula:

Acidity (% Lactic acid) =9 × V × NX

Where,V = Volume of 0.1 [N] NaOH used in mL

N = Normality of NaOH solution

X = Volume of whey sample used for titration (in mL)

**Determination of lactic counts of fractionated whey samples**

Lactobacillus counts were done in fractionated cheese whey samples using the method reported by Hati et al. (2023).. For serial dilutions, 1.0 mL of each sample was transferred into 9 mL of sterile phosphate buffer solution (PBS) and the process was repeated as required to obtain subsequent dilutions. From the appropriate dilutions, 1.0 mL was dispensed into sterile, labeled Petri plates in duplicate. After cooling to 45°C, 15-20 mL of melted de Man, Rogosa and Sharpe (MRS) agar (HiMedia, India make) was added to each plate. The plates were gently twisted and tilted to ensure that the contents were evenly mixed before being allowed to solidify. A second layer (5-7 mL) of the same agar medium was applied to the hardened surface. After solidification, the plates were incubated for 24 hrs at 37°C while inverted. Colonies with normal Lactobacillus morphology were counted, and the findings were presented as log CFU/mL. Colonies exhibiting typical Lactobacillus morphology in spindle shape, appearing off-white to cream-colored, were counted, and the results were expressed as log CFU/mL

**Assessment of antioxidative activity using ABTS method**

The antioxidant activity of both cheese whey samples (unfermented and fermented) was examined using the ABTS [2,22 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)] radical decolorization assay, as described by Das et al. (2025). The ABTS working solution was prepared by combining 88 µL of 140 mM potassium persulfate and 5 mL of 7 mM ABTS stock solution. The combination was then left to remain at room temperature in the dark for 12 to 16 hrs to produce ABTS+ radicals. The solution was then diluted with phosphate-buffered saline (PBS) until it reached an absorbance of 0.70/ ±/ 0.02 at 734 nm.

To perform the experiment, combine 200 µL of whey supernatant with 2.3 mL of ABTS working solution and vortex for 30 sec. A spectrophotometer (Systronics, India) was used to quantify the decrease in absorbance at 734/ nm every 30 sec for 10 mins. Distilled water was utilized as the control, and all measurements were taken in triplicate.

The free radical scavenging capacity of the sample was measured using the following equation:

ABTS radical scavenging activity (%) = (Absorbance control-Absorbance Samples )/ Absorbance Control] × 100

Where,

Absorbance Sample is the absorbance in the presence of test substance, and Absorbance Control is the absorbance of control.

**Assessment of proteolytic activity through o-phthalaldehyde (OPA) method**

The extent of proteolysis in fermented cheese whey (2.5% inoculum, 48/ h incubation) was evaluated by quantifying the release of free amino groups using the o-phthalaldehyde (OPA) method, as described by Hati et al. (2015). As mentioned, 3 mL sample of fractionated and unfermented cheese whey was combined with 5 mL of 0.75% trichloroacetic acid (HiMedia, India), vortexed for 1 min, and filtered using Whatman No. 42 filter paper (UK). The absorbance at 340 nm was measured using a spectrophotometer (Systronics, India) after adding 200 µL of the filtrate to 3 mL of OPA reagent (HiMedia, India) and incubating at 20°C for two mins.

**Peptide separation via reversed phase high-performance liquid chromatography (RP-HPLC)**

Peptide synthesis in fractionated cheese whey was evaluated using RP-HPLC (a Japanese-made Shimadzu LC-20 system). Sample injection was done with a 20 µL loop-equipped micro-injector from Hamilton Bonaduz AG, Switzerland. Gradient elution was carried out using two mobile phases: Eluent A and Eluent B. Eluent A included 0.01% (v/v) trifluoroacetic acid (TFA), while Eluent B was an 80:20 mixture of deionized water and acetonitrile, likewise containing 0.01% (v/v) TFA. The chromatographic separation was carried out at ambient room temperature with a constant flow rate of 0.25 mL/min. Detection was accomplished using a Shimadzu SPD-20A UV/Visible spectrophotometric detector with a wavelength of 214.0 nm, as described by Pipaliya et al. (2024).

**Evaluation of short chain fatty acid of faecal contents**

Short chain fatty acids of the fractionated cheese whey samples were measured by following the protocol suggested by Makwana et al. (2023). Fractionated cheese whey sample was homogenized in 1.8 mL of 0.05 N sulfuric acid followed by centrifugation at 8437 g force for 10 minswhile temperature of centrifugation was

kept at 4°C. Supernatant was collected and filtered passed on through a 0.45 µm membrane before injecting for HPLC analysis. For separation purposed analysis was conducted using a Shimadzu LC-20 (Japan) device with an analytical column [C18] for anion exchange chromatography. Elution was performed with 0.012N $H_2SO_4$ at 45°C. The flow rate of the sample through HPLC column was maintained at 0.4 mL/min All the samples were analysed trice.

**Statistical analysis**

All experimental data were statistically analyzed, with each experiment performed in triplicate. Results are presented as mean/ ±/ standard deviation (SD). Analysis of variance (ANOVA) was conducted to assess significant differences among the treatments at 5% level of significance ($p$/ </ 0.05), following the method described by Steel and Torrie (1980). Appropriate statistical software was used for data analysis.

**Results and Discussions**

**pH and acidity profile of fermented and unfermented cheddar cheese whey**

Titratable acidity of fermented and unfermented cheese whey are shown in Table 1. After 48 hours of fermentation at 37 °C, the 10 kDa retentate showed the greatest pH reduction (pH 4.58), followed by the 10 kDa permeate (pH 4.66) and the 3 kDa permeate (pH 4.70) respectively.

After 48 hrs of incubation at 37/ °C, *Limosilactobacillus fermentum* (M4) produced acidity in sterilized cheese whey.Among the fermented fractions, the 10/ kDa retentate exhibited the highest titratable acidity (0.69% lactic acid), followed by the 10/ kDa permeate (0.65% LA), the 3/ kDa permeate (0.63% LA), and unfermented cheese whey (0.21% LA), respectively.

Melia et al. 2021 prepared fermented cheese whey with probiotic bacteria, *Pediococcusacidilactici with* different fermentation time2 hours intervals. After 20 hours fermentation titrable acidity was 0.61 ±0.05 with pH 5.10. Shukla et al. (2013) observed that fermentation of whey with *Lactobacillus acidophilus* NCDC 015 over a 5-24hrs period resulted in a pH decrease from 4.82 to 3.30, accompanied by an increase in titratable acidity from 0.394% to 1.353%. Previously, Hernandez-Mendoza et al. (2007) reported that whey fermented with *Lactobacillus reuteri* and *Bifidobacterium bifidum* resulted in a pH reduction from 4.85 to 4.50 and an increase in titratable acidity from 0.315% to 0.378% over a 30-day storage period.

**Proteolytic activity of fermented whey peptide fractions**

It was detected that proteolytic activity varied significantly ($p$<0.05) among different membrane treatments of permeate and retentate samples as well as in case of unfermented cheese whey. The proteolytic activity of fermented whey peptide fractions, including the 3 kDa permeate, 10 kDa retentate, and 10 kDa permeate, is illustrated in Figure 1. The highest proteolytic activity was noticed in the 10/ kDa retentate sample (7.84/ mg/mL), followed by the 10/ kDa permeate (7.27/ mg/mL) and the 3/ kDa permeate (7.11/ mg/mL), all of which showed higher activity compared to unfermented cheese whey (1.23/ mg/mL).

For their growth, lactic acid bacteria (LAB) require a continuous supply of amino acids. To meet this demand, Lactobacillus species have evolved an efficient proteolytic system that hydrolyze proteins to release amino acids.

Cell-envelope proteinases (CEPs) initiate protein hydrolysis by cleaving proteins into peptides, typically comprising four to thirty amino acid residues. These peptides are released into the extracellular environment and subsequently transported into the cell via specialized uptake systems, including oligopeptide permeases, ion-coupled transporters for di- and tripeptides, and ATP-binding cassette (ABC) transporters (Raveschot et al.2018).

Table 1. pH, acidity of cheese whey fermentates (3 and 10 kDa permeate and 10 kDa retentate) produced by M4 culture, Mean ± SD of three replicate experiments (n = 3)

| Sample / Treatment | 3KDa Permeate | 10KDa Permeate | 10KDa Retentate | Unfermented |
|---|---|---|---|---|
| pH | 4.7 | 4.66 | 4.58 | 6.2 |
| Acidity (%) | 0.63± 0.02 | 0.65± 0.01 | 0.69± 0.01 | 0.21 ± 0.01 |

Table 2. Characterization of whey peptides generated from fermented cheese whey by RP-HPLC analysis

| Samples | No.of peaks | Retention time range(min.) |
|---|---|---|
| <3kDa | 42 | 27.33 to 49.51 |
| <10kDa | 21 | 27.25 to 48.12 |
| >10kDa | 44 | 24. 51 to 53.04 |

(Mean of three replicate experiments (n = 3)

Chopada et al. (2022) used *Lactobacillus paracasei* and *Saccharomyces cerevisiae* cultures to optimize maximal peptide synthesis from whey protein concentrate (WPC) during fermentation. Longer incubation times considerably improved the enzymatic efficiency of peptide synthesis in both cultures (p/ </ 0.05). After 48 hrs of incubation, *Lactobacillus paracasei* and *Saccharomyces cerevisiae* cultures produced the most peptide (6.50/ mg/mL and 8.59/ mg/mL, respectively).

Padhi et al. (2021) employed a diafiltration membrane to evaluate the degree of hydrolysis in hydrolysates derived from sheep milk whey concentrate (SMWCDF). After6 hrs of hydrolysis, SMWCDF exhibited the highest degree of hydrolysis (15.56%), while the lowest degree (12.07%) was observed at 1 hour.

**Antioxidant activity of fermented whey peptide fractions**

Fermented cheese whey's 10/ kDa retentate fraction showed the highest antioxidant activity (65.81%), followed by the 10/ kDa permeate (56.32%), the 3/ kDa permeate (50.41%), and unfermented cheese whey (5.84%).

A significant variation in antioxidant activity ($p< 0.05$) was observed among the different membrane-treated fractions (permeate and retentate), as well as in comparison to unfermented cheese whey.. The antioxidant activity of ultrafiltered fractions from fermented cheese whey, including the 3 kDa permeate, 10 kDa permeate, and 10 kDa retentate, is presented in Figure 1.

Mansinhbhai et al. (2022) found that ABTS activity of 3 kDa and 10 kDa permeated and retented in hydrolyzed WPC employing alcalase enzyme at pH 8.5, temperature 65ºC for 8 hrs. They observed that the 10 kDa retentate (87.88%) exhibited the highest antioxidant activity, and in agreement with their findings, our study also showed that the 10 kDa retentate sample showed the highest antioxidant activity (65.81%) compared with the 10 kDa permeate, the 3 kDa permeate, and unfermented cheese whey samples. Chopada et al.(2022) investigated antioxidant activity in WPC 80 using both *L. fermentum* (KGL4) and *S. cerevisiae* (WBS2A) culture. After 48h fermentation at 37ºC fermented whey was passed through ultra filtered membrane. They revealed their results that 10kDa retentate had maximum antioxidant activity.

Price et al. (2022) reported that whey proteins exhibit several antioxidant properties. Additionally, whey proteins contain a relatively high amount of cysteine (2.2 g per 100 g of amino acids), which contributes to their antioxidant capacity. Cystine, the oxidized dimer form of cysteine, also serves as a key substrate for the biosynthesis of glutathione (GSH), a major intracellular antioxidant (Yu and Long, 2016).Natural antioxidants are in high demand due to their therapeutic potential in the prevention of major diseases such as cancer, cardiovascular disorders, Alzheimer's disease, and arthritis, as well as the reduction of aging symptoms. The current study focuses on using microbial fermentation to produce antioxidant peptides from cheese whey.

**Lactobacillus counts of fermented whey fractions**

Non-significant differences were observed in viable counts among the whey peptide fractions in the study. The 10kDa retentate demonstrated the highest microbial growth (10.55 log CFU/mL), followed by the 10 kDa permeate (9.42 log CFU/mL) and the 3 kDa permeate (9.39 log CFU/mL)

Silva et al. (2018) studied the microbiological and physico chemical characteristics of probiotic functional carbonated beverage.To produce carbonated beverages, they used *Bifidobacterium animalis* subsp. *lactis BB12* probiotic bacteria. They showed good vitality throughout the storage period, with counts ranging between 7.45 and 6.87 log CFU/mL in the final phases of storage.

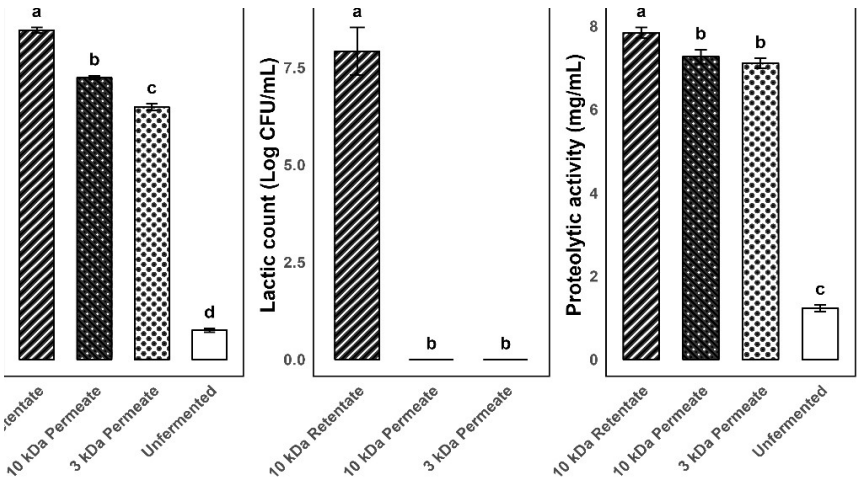


**Fig. 1.** Proteolytic activity (mg/ml), Antioxidant activity (%) and lactic count of cheese whey fragments (3 and 10 kDa permeate and 10 kDa retentate) and unfermented cheese whey sample, Mean ± SD of three replicate experiments (n = 3).

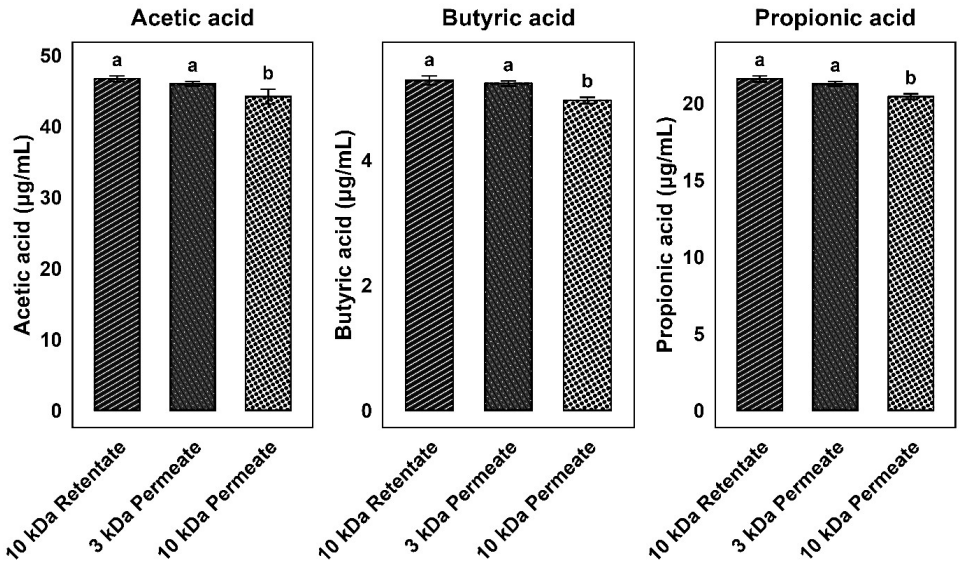


**Fig. 2.** Short chain fatty acid of cheese whey fragments (3 and 10 kDa permeate and 10 kDa retentate) and unfermented cheese whey sample, Mean ± SD of three replicate experiments (n = 3).

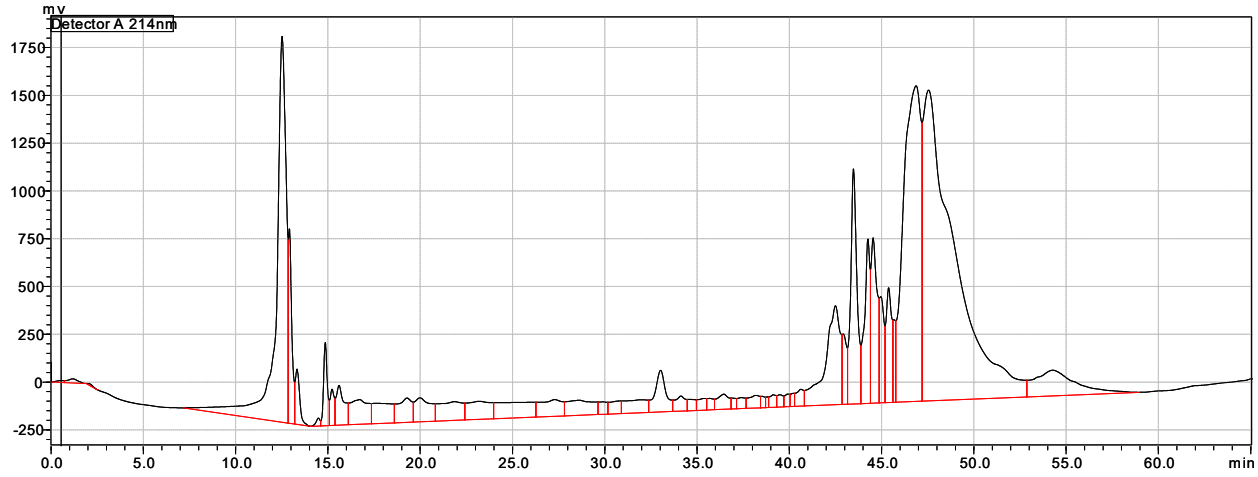


**Fig. 3** RP – HPLC chromatogram of unfermented cheese whey

A total increasing of LAB has been observed and over the period of (20 days) storage it increases from day 0 (8.913 ± 0.009 log CFU/mL in 60 C) today 20 (9.361 ± 0.013 log CFU/mL in 60 C). Saikia and Mishra (2021) developed a fermented whey-based beverage enhanced with lactic acid bacteria and Plumbago zeylanica extract. The formulation included whey, citric acid (0.3%), sugar (7%), and orange flavor (0.02%), and was fermented using a 1% inoculum of *Lactobacillus fermentum* KGL4 and *Lactobacillus plantarum* KGL3A in a 1:1 ratio. Their shelf-life study of beverage revealed a progressive increase in lactic acid bacteria (LAB) counts during storage. Over a 20-day period at 6/ °C, the LAB population increased from 8.913/ ±/ 0.009 log CFU/ mL on day 0 to 9.361/ ±/ 0.013 log CFU/mL on day 20.

**Peptide production through RP-HPLC analysis**

. The RP-HPLC characterization of fermented cheese whey revealed distinct patterns of 3 kDa and 10 kDa peptides, as shown in Figures 3–6. The 10kDa permeate produced the highest-area peaks when compared to the 3kDa permeate and 10kDa retentate. Additionally, the highest number of peaks (42 peaks) was seen in 3kDa permeate chromatograms, which were followed by 10-kDa permeate (21 peaks) and 10kDa retentate (44 peaks) (Table 2). These results are in accordance with results reported by Chopada et al. (2022), who investigated whey protein fermented with *Lactobacillus paracasei* and *Saccharomyces cerevisiae*, followed by fractionation and peptide analysis using RP-HPLC. The researchers reported that the 10 kDa permeate exhibited the uppermost peak intensities compared to the 10 kDa retentate and the 3 kDa permeate. Furthermore, the chromatographic analysis revealed the highest number of peaks in the 3 kDa permeate (67

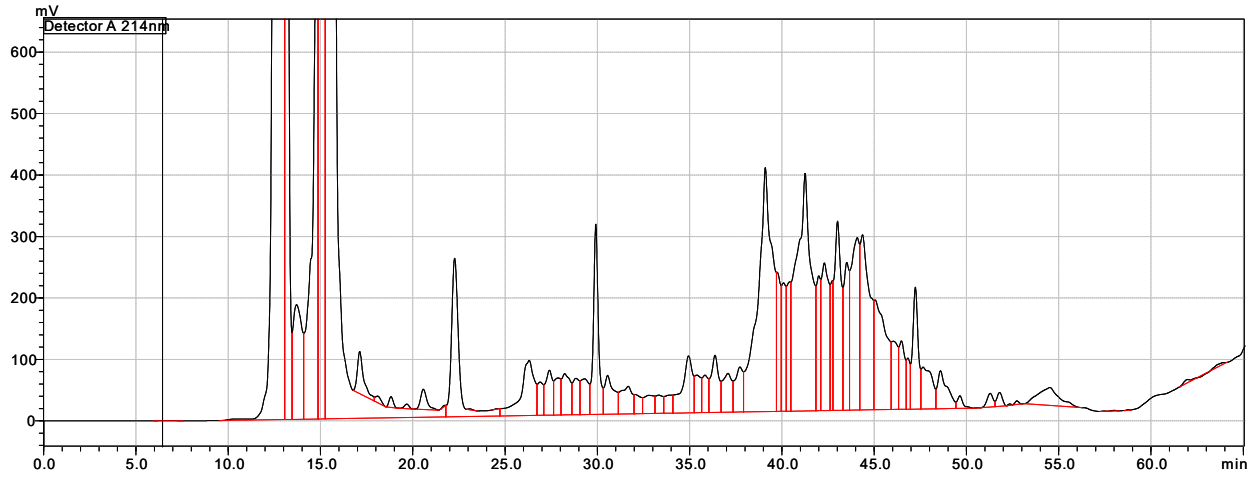


**Fig.4** RP – HPLC chromatogram of 3kDa permeate from whey protein fermented with M4 culture

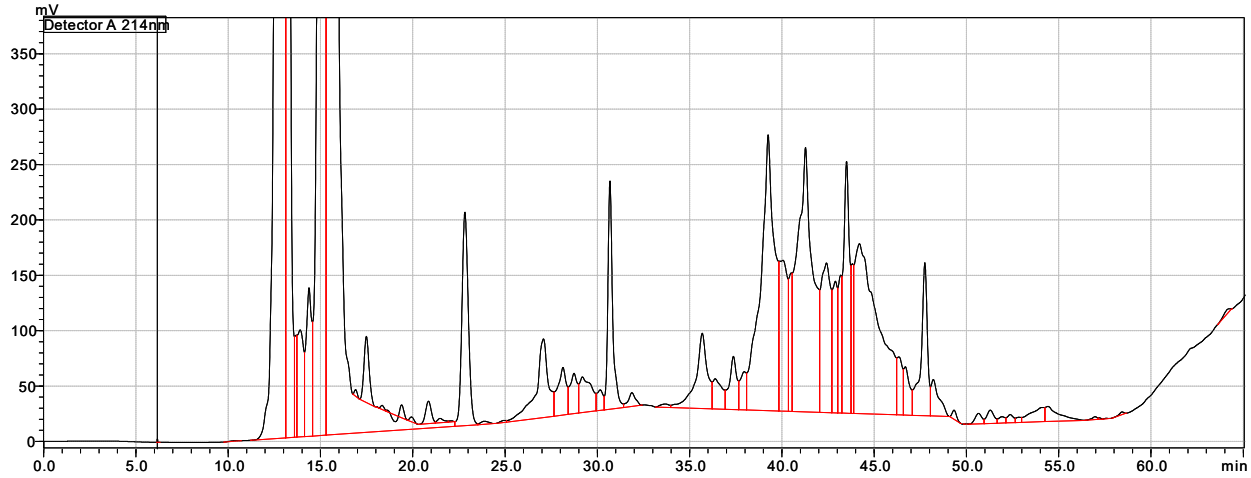


**Fig.5** RP – HPLC chromatogram of 10kDa permeate from whey protein fermented with M4 culture

peaks), followed by the 10 kDa permeate (63 peaks), and the 10 kDa retentate (60 peaks).

The study indicates that smaller molecules in the 3 kDa permeate are more diverse and abundant than larger molecules in the retentate, followed by the 10 kDa permeate and retentate, which is reflected by the greater number of peaks observed in the 3 kDa permeate samples.

**Determination of SCFA of fermented whey peptide fractions**

Short-chain fatty acids (SCFAs) are microbial metabolites produced in the gut as a result of dietary fiber and protein fermentation. Dietary fibers can be classified into soluble types (*e.g.*, pectin and inulin) and insoluble types (*e.g.*, various forms of resistant starches) (Xiong et al. 2022). SCF As are fatty acids with fewer than six carbon atoms, including acetate, propionate, butyrate, pentanoate, malonate, and others. Acetate, propionate, and butyrate are the three most abundant SCFAs, accounting for over 90% of total SCFA production by the gut microbiota (Chen et al. 2020).

In this investigation, SCFA synthesis varied considerably across all peptide fraction samples. The 10/ kDa retentate fraction produced the most SCFAs among all whey peptide fractions, with 46.68 µg/mL acetic acid, 21.59 µg/mL propionic acid, and 5.27 µg/mL butyric acid. SCFA production was not significantly different between the 10/ kDa retentate and 3/ kDa permeate samples. However, the 10/ kDa permeate sample had much lower SCFA levels than the other peptide fractions (Figure 2).

Hati et al. (2019) studied SCFA synthesis in isolated lactic acid bacteria. Lactobacillus strains namely KGL2 (*Lactobacillus fermentum*), RNS4 (*Lactobacillus rhamnosus*), KGL4

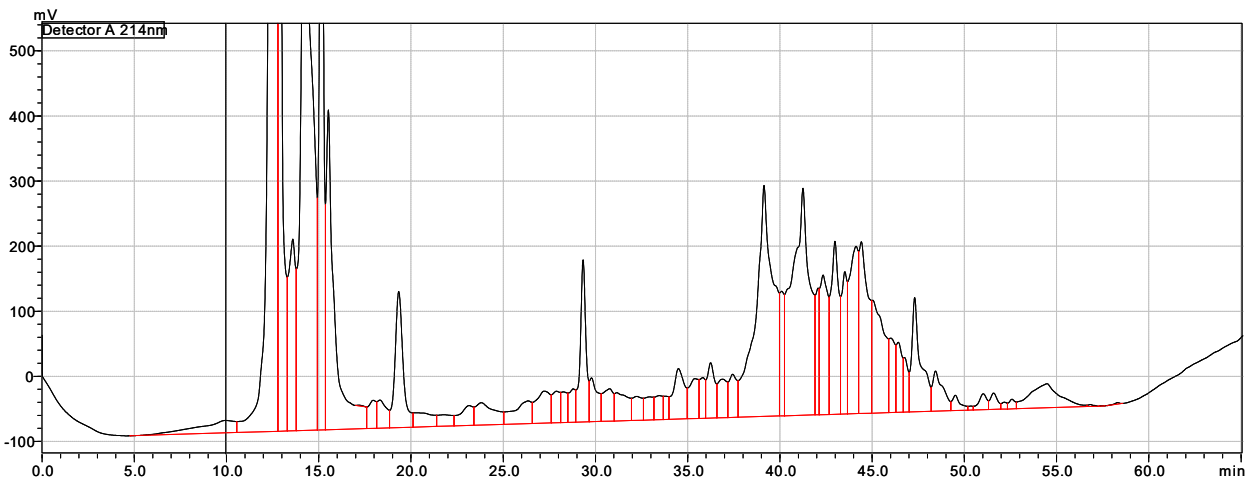


**Fig.6** RP – HPLC chromatogram of 10kDa retentate from whey protein fermented with M4 culture

(*Lactobacillus fermentum*), KGL3A (*Lactobacillus plantarum*), and WTS4 (*Lactobacillus fermentum*) were recovered from traditional rice-based fermented dishes in the Garo Hills region of Meghalaya, India. After 24 hrs of incubation at 37°C, strain RNS4 produced the most SCFA in MRS medium, yielding 5.18 µg/mL lactic acid, 15.41 µg/mL acetic acid, and 0.16 µg/mL butyric acid, followed by KGL2 with 4.39 µg/mL, 0.23 µg/mL, and 12.9 µg/mL lactic acid, butyric acid, and acetic acid, respectively.

## Conclusion

This study addresses the challenge of cheese whey utilization, a major byproduct of the dairy industry, by converting it into bioactive or medicinal compounds and environmentally sustainable alternatives.Cheese whey fermented with the *Limosilactobacillus fermentum* (M4), culture at a concentration of 2.5% and 37°C exhibited significantly highest antioxidant activity and peptide production compared to unfermented cheese whey. The 10/ kDa retentate fraction exhibited the highest peptide content (7.84/ mg/mL) and antioxidant activity (65.81%). Additionally, the fermented cheese whey was found to contain short-chain fatty acids specifically propionic, butyric, and acetic acids along with a high lactic count.The future scope of this research includes large-scale production of the formulated beverage and the isolation of antioxidant peptides for potential applications in the pharmaceutical industry.

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