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RESEARCH ARTICLE

Optimization and characterization of a value-added fermented ready-to-serve whey-based beverage with high antioxidant potential

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Abstract: This study was conducted to optimize the process parameters for developing a Ready-To-Serve fermented whey beverage using pineapple juice and mint and characterize the drink. The levels of pineapple juice (20-40%), sugar (5-10%), and mint powder (1-3%) on pH, DPPH, TSS, and overall acceptability were investigated using a central composite design. The optimized process conditions achieved were 39.42% (v/v) of pineapple juice, 5.75% (w/v) of sugar, and 1.01% (w/v) of *Mentha*. The beverage had 89.41% mg GAE/ml total phenolic content and 83.88% DPPH radical scavenging activity. FTIR spectrum confirmed the presence of the O-H stretch of phenolic compounds between 3200 cm^{-1}– 3518 cm^{-1} and conformational changes in the secondary structure of the whey protein at 1625-1647 cm^{-1} in the fermented sample. The beverage possessed all essential amino acids, the highest being Leu, Val, and Lys and PUFA (ω6/ω3=1.52) and MUFA levels of 6.7±0.96 and 21.57±0.02 mg/100mL, respectively. In addition, hypercholesterolemic fatty acids (C12:0, C14:0, C16:0) were significantly reduced after fermentation. Therefore, a serving of 100mL would contribute 3.53%, 2.96%, and 2.41% towards the RDA of Fe, K, and Mg, for an adult man, respectively. The whey beverage had acceptable microbiological quality on the 30th day of storage under refrigerated conditions. Fermentation with *Pediococcus pentosaceus* NCDC 273 reduced the coliform count by 5.57% and 4.04% and the Y&M count by 2.2% and 2.9% on the 15th and 30th day of storage, respectively.

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Keywords: Antioxidant; Pineapple whey beverage; Sensory quality; Storage stability; Whey

Introduction

Whey is a by-product of cheese or casein production containing about 90% of the milk volume and 55% of milk nutrients (Ryan & Walsh, 2016). However, this nutritive by-product from the dairy industry is the toughest to dispose of due to its high Biological and Chemical Oxygen Demand of around 39-48g/L and 60-70g/L, respectively (Islam et al. 2021; Panghal et al. 2018). Unfortunately, cheese whey is often disposed of as such in water bodies without pre-treatment of the whey, leading to water pollution. The whey output worldwide is approximately 180 million tonnes, with about 1.5 million tonnes of high-value protein and a lactose output of about 8.6 million tons (Rai et al. 2020). However, yearly, the total whey production is increasing by 1-2%, and less than 50% of the total whey produced is effectively utilized worldwide.

Due to its nutritional value, whey can be used to make refreshing and assimilable beverages. Compared to soft drinks available in the market, fruit and whey-based beverages are more likable and healthier due to their refreshing taste (Shukla, 2012). Furthermore, because processing whey to extract lactose and whey protein concentrates is costly, the formulation of refreshing long-life beverages appears to be a logical solution to draining nutritive whey and increasing water pollution from it. Therefore, researchers have investigated the likelihood of utilizing whey as a base ingredient in developing value-added fruit beverages (Ferreira et al. 2019; M'hir et al. 2019).

Pineapple (*Ananas comosus*) is a tropical fruit relished for its sweet acidic taste, distinct flavor, and aroma (Ali et al. 2020). It is ranked third, after banana and citrus, among the fruit production category in the world. Its world production was registered at about 27.82 million tonnes in 2020 (www.statista.com/statistics/298505/global-pineapple-production). Apart from being a good source of polyphenolic compounds such as gallic acid, chlorogenic acid, and ferulic acid, known to exhibit antioxidative, anticarcinogenic, and antimutagenic properties, it also provides benefits against cataracts and cardio-vascular diseases (Baljeet et al. 2013). The enzyme bromelain in pineapple aids digestion

along with antidiabetic, antihypertensive, and anti-cancerous effects (Ali et al. 2020). Pineapple also adds to the supply of dietary fiber (D.F.) to the food matrices that act as 'prebiotics' and improve the host's health by restoring the proliferation and colonization of probiotic bacteria (Dittakan et al. 2018). It is also a good source of specific vitamins such as A, B, and C and minerals such as magnesium, potassium, calcium, and iron (Islam et al. 2021). Mint (*Mentha* spp.) is a prevalent culinary and medicinal herb possessing curative and antioxidative properties that help prevent spoilage of any food. The most common mint plants are peppermint (*Mentha piperata*) and spearmint (*Mentha spicata*). Essential oils from these find several uses in treating headaches, diarrhea, indigestion, colds, and gallstone infections (Smits, 2013).

The present investigation focuses on developing a functional fermented whey beverage blended with pineapple juice and mint powder, fermented by lactic acid bacteria (*Pediococcus pentosaceus* NCDC 273). This strain is known to produce pediocin AcH/PA-1, which belongs to Class II bacteriocins and acts as a primary metabolite possessing antimicrobial activity against *Listeria monocytogenes* and several other pathogens (Lather et al. 2015; Simha et al. 2012). In addition, pediocin AcH/PA-1 produced by this bacterium has also been used to increase the shelf life of several food products, attributed to the production of pediocin, organic acids, and other metabolites (Garsa et al. 2014; Pandey et al. 2019; Verma et al. 2017)

Materials and methods

The study was carried out in the Department of Dairy Science and Food Technology, Institute of Agricultural Science, Banaras Hindu University, Varanasi, Uttar Pradesh, and ICAR-Central Inland Fisheries Research Institute, Barrackpore, Kolkata, West Bengal.

Preparation of ingredients for whey beverage

Chhana whey was prepared from standardized cow milk containing 4% fat and 8.5% SNF, according to Srivastava et al. (2018). Briefly, the milk was procured from the cattle yard, Banaras Hindu University, and after standardization, it was heated to 90°C, cooled to 70°C, and coagulated with 2% citric acid. Whey was collected by filtering the coagulated milk through a clean muslin cloth. A bioprotective culture of *Pediococcus pentosaceus* NCDC 273 was procured from the National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, Haryana. Aseptically, a lyophilized vial was opened and propagated in M.R.S. broth and preserved in 25% glycerol solution at -20°C for further use. Fresh channa whey was pasteurized (in-bottle pasteurization) at 70°C for 30 min.

Experimental Design

Response Surface Methodology was used to optimize the effect of pineapple juice (20-40 %), sugar (5-10 %), and mint powder (1-3%) levels on DPPH activity, TSS, pH, and overall acceptability of the whey beverage. During the investigation, 20 trials (experiments) were generated through Design Expert (7.0.0) software using Central Composite Rotatable Design (C.C.R.D.). Experimental tests suggested were executed, and the responses were fitted to the design. After each experimental trial, the responses were evaluated to see the effect of independent variables on them. A second-order polynomial equation was used to express the responses in terms of the model constant, liner effect of factor, the cross product of factor, the quadratic effect of factor, and residual error, including the experimental errors and lack of fit chosen for the model.

Beverage preparation

After de-crowning and peeling, the pineapple was cut into small pieces. Using a mixer grinder, the small bits were macerated into a pulp and filtered through a clean muslin cloth to extract the juice. The freshly extracted pineapple juice was pasteurized at 60°C for 1-2 mins. Mint powder used in the study was obtained by grinding the dried mint leaves. Next, pineapple juice was added to the whey, followed by sugar and mint powder to prepare the R.T.S. whey beverage. Fermentation was initiated by transferring the inoculum at 1% rate from an overnight fermented skim milk tube with *Pediococcus pentosaceus* NCDC 273. The beverage is then poured into glass bottles and kept for incubation. Based on the literature on this particular bioprotective strain, fermentation time was selected at 16h at 37°C for maximum release of bioprotective metabolites. After fermentation, the bottles were stored under refrigerated conditions.

Physico-chemical analysis

The fermented whey-based beverage infused with pineapple juice and mint powder was analyzed for its moisture, pH, and titratable acidity. The pH of the whey, pineapple juice, and beverage samples was determined directly using a digital pH meter (EUTECH Instruments). The pH and titratable acidity of whey and pineapple juice was recorded to be 5.34±0.003; 0.40±0.07 % lactic acid, and 3.78±0.004; 0.29±0.01 % lactic acid, respectively. The total soluble solids of the whey and pineapple juice, were determined using a digital refractometer (Milwaukee, Romania) and were recorded to be 6.43±0.30 and 8.43±1.11 ° Brix, respectively. The gravimetric method determined the beverage's moisture (on a dry matter basis) (AOAC, 1990). The organic and inorganic matter of the beverage was evaluated gravimetrically by igniting the sample, which had been oven dried until constant weight, in a muffle furnace at 550–600°C. The titratable acidity of the pineapple juice (as % acetic acid) and whey and beverage sample (as % lactic acid) were determined (AOAC, 1990). Five

mL of the sample was titrated against 0.1N NaOH using a few drops of phenolphthalein indicator to an endpoint of faint pink color. The nitrogen and, thereby, the crude protein (C.P.) content of the beverage was measured by the Kjeldahl method (AOAC, 1990) using KEL PLUS Protein Estimation System, Pelican Equipment Ltd., India.

Total Antioxidants

The antioxidant activity of the beverage samples was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Balakrishnan & Agrawal, (2014) with slight modifications. Two mL of the sample was added to 20 mL of 80% methanol solution, followed by proper extraction in a shaking incubator at 37°C for 2hrs at 100 rpm. The extract was centrifuged at 6000 rpm for 20 min at 4°C to collect the clear supernatant. The supernatant was then filtered with Whatman® paper 1. A stock solution of DPPH (0.035 mg DPPH/mL of methanol) was prepared. A volume of 700µl of each sample was added to 700 µl of DPPH stock solution and mixed properly by a vortex machine. Absorbance at 517nm was measured using a spectrophotometer (PerkinElmer) after incubating the samples and control in a dark place at room temperature for 30 min. The antioxidant activity of the beverage samples was determined by DPPH radical scavenging activity in percentage:

$$DPPH\ radical\ Scavenging\ Activity\ (\%) = \frac{Ac - As}{Ac} \times 100$$

where Ac-Absorbance of the control; As-Absorbance of the sample

Total phenolic Compounds

The Total Polyphenolic Content (TPC) of the beverage samples was determined according to Folin-Ciocalteau (F.C.) method described by M'hir et al. (2019) with slight modifications. First, two mL of the beverage sample was added to 20mL of 80% methanol solution. The mixture was left in the shaking incubator for 2hr at 37°C. Next, the extract was centrifuged at 6000 rpm for 20 min at 4°C to collect the clear supernatant. The supernatant was then filtered with a Whatman® filter paper 1. The so obtained filtrate (0.05 mL) was added to 2.75 mL of distilled water. Next, an aliquot of 0.2 mL of F.C. reagent was added to the above mixture, followed by the addition of 0.6mL of 20% sodium carbonate solution. Finally, the above mixture was shaken well using a vortex machine and kept in the dark for incubation at room temperature (30°C) for 30min, after which the absorbance was measured at 750 nm. TPC was calculated by deriving a standard curve where Gallic acid was used for generating the standard curve having concentrations ranging from 100 to 600 mg/mL.

Sensory evaluation

The beverage samples were evaluated for their sensory attributes as described by Sabokbar & Khodaiyan, (2015). The parameters judged were smell, color, flavor, consistency, and overall acceptability by an untrained panel comprising ten panelists from the Department of Dairy Science and Food Technology, Banaras Hindu University, Varanasi, India. An aliquot of 10mL of the prepared beverage was served in transparent bottles. The panelists were asked to record their remarks on the sensory sheet based on a 9point hedonic scale from 1 (extremely disliked) to 9 (extremely liked).

Fatty acid profiling

Fat extraction from the unfermented and fermented beverages was carried out according to the method described by Folch et al. (1957). For that, 50 ml of the drink was homogenized with 300 ml chloroform-methanol mixture in a ratio of 2:1, and 60 ml of water was added to the funnel. For fatty acid methyl esters (FAME), 10 ml fat extract was taken, and FAME was prepared following a standard protocol described by Metcalfe et al. (1966).

The fatty acid compositions of the samples were analyzed by Gas Chromatography-Mass Spectrometry (GC/MS, Thermo Scientific ITQ 900) with a GC (Trace GC Ultra, Thermo Scientific) having a 30 m×0.25 mm × 0.25 µm TR-FAME capillary column and an MS detector (ITQ 900, Thermo Scientific). For the separation of individual fatty acids, the oven temperature program was kept on hold at 50°C initially for 1 min; then, it was raised from 50°C to 150°C with a heating rate of 20°C/min, followed by a hold at 150°C for 15min. Finally, the temperature was increased from 150 to 240°C at a heating rate of 20°C/min with a final hold at 240°C for 3min. As a carrier gas, the inert gas helium was used with a column flow of 1ml/min. The MS conditions maintained were: 70eV ionization voltage, a mass range of 40-400, and the scan time was equal to the GC run time. The particular fatty acids present in the samples were identified and also quantified by comparing their peak areas and retention times to known standards (Fatty acid methyl esters, saturated (ME-19-KT) and fatty acid methyl esters, unsaturated (ME- 14-KT, SUPELCO Analytical) analyzed in the same GC/MS method and also by NIST Library search (version 2.2, 2014).

Amino acid and mineral profiling

The amino acid content of the beverage was evaluated according to the method described by Qu et al. (2002). In 100 ml of drink, 200 mL HCl (6N) was added and kept in an ultrasonic water bath at 90°C for an hour. After cooling, this mixture was transferred to a flask containing 1300 mL acetonitrile added with 1% formic acid. From this admixture, 100 mL was taken and added to 300 mL of saturated ammonium acetate solution to neutralize it. After adding 600 mL ultrapure water to the mixture, a 10-fold diluted sample was finally injected into the HPLC system and analyzed. Formic

acid (1%) and 10 mM ammonium formate in water and acetonitrile were used as mobile phases A and B, respectively. The mineral profile was assessed through ICP-MS (ICP-MS, NexION 1000 Model, PerkinElmer Make, U.S.A.) according to the methodology described by Poitevin (2016).

FTIR spectroscopy

The sample was concentrated using a lyophilizer, and FTIR spectra (Model-Spectrum Two, PerkinElmer) were obtained according to the methodology described by Scholar (2016). Each spectrum was recorded in the 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. The measurements were made for both fermented and unfermented samples. Spectral measurements were repeated 32 times and averaged.

Microbial quality of beverage

Coliform counts using Violet Red Bile Agar medium were determined by incubating the plates at 37ºC for 24-48 hours. For the enumeration of yeast and mold counts, the Potato Dextrose Agar medium was used, and plates were incubated at 25ºC for 3-5 days. These counts were taken on the 0^{th}, $15^{th,}$ and 30^{th} days of storage to assess the shelf life of the beverage at 7ºC.

Statistical analysis

Statistical significance was calculated by t-test using GraphPad Prism. In addition, multifactor analyses of variance (ANOVA) were performed using Design Xpert version 7.0 to determine the statistical significance of differences among samples.

Results and Discussion

The present investigation was conducted to develop and optimize a value-added fermented whey-based beverage blended with pineapple juice and mint powder with different proportions of sugar, pineapple juice, and mint using R.S.M. . Whey fermentation was done with a bio-protective culture of *Pediococcus*

Table 1 Central Composite Rotatable design for the optimization of the fermented whey drink

SL. No		Factors			Responses			
SO	RO	P.Juice	Sugar	MP	DPPH	TSS	pH	OA
10	1	46.82	7.5	2	96.56±0.26	13.86±0.30	4.93±0.003	7
9	2	13.18	7.5	2	81.16±0.55	13.81±0.15	4.39±0.004	6
20	3	30	7.5	2	94.4±1.51	13.64±0.25	4.41±0.003	7
5	4	20	5	3	82.18±0.52	12.28±0.68	4.43±0.007	5
13	5	30	7.5	0.32	93.56±0.89	13.72±0.35	4.36±0.004	8
17	6	30	7.5	2	94.41±1.51	13.67±0.25	4.43±0.003	7
14	7	30	7.5	3.68	86.65±0.32	15.19±0.40	4.52±0.006	4
8	8	40	10	3	86.44±0.60	17.67±0.36	4.66±0.001	5
12	9	30	11.7	2	85.2±0.30	18.75±0.52	4.32±0.003	6
11	10	30	3.3	2	85.36±0.73	9.16±0.37	4.46±0.002	5
15	11	30	7.5	2	94.42±1.51	13.66±0.25	4.40±0.003	7
6	12	40	5	3	95.86±0	12.38±0.2	4.48±0.002	5
1	13	20	5	1	85.45±0.42	12.18±0.20	4.34±0.002	6
18	14	30	7.5	2	94.42±1.51	13.68±0.25	4.42±0.003	7
16	15	30	7.5	2	94.46±1.51	13.66±0.25	4.45±0.003	7
4	16	40	10	1	94.59±0.72	17.64±0.35	4.73±0.002	8
19	17	30	7.5	2	94.45±1.51	13.69±0.25	4.42±0.003	7
3	18	20	10	1	84.21±0.56	17.86±0.15	4.24±0.005	7
2	19	40	5	1	93.78±0.94	12.84±0.2	4.73±0.002	9
7	20	20	10	3	80.56±0.59	17.95±0.15	4.43±0.004	5

Code: SO-Standard Order, RO-Run Order, OA-Overall Acceptability, P.Juice-Pineapple juice, MP-Mint powder, DPPH-2, 2-diphenyl-1-picryl-hydrazyl-hydrate Assay analysis, TSS-Total Soluble Solids, OA: Overall Acceptability

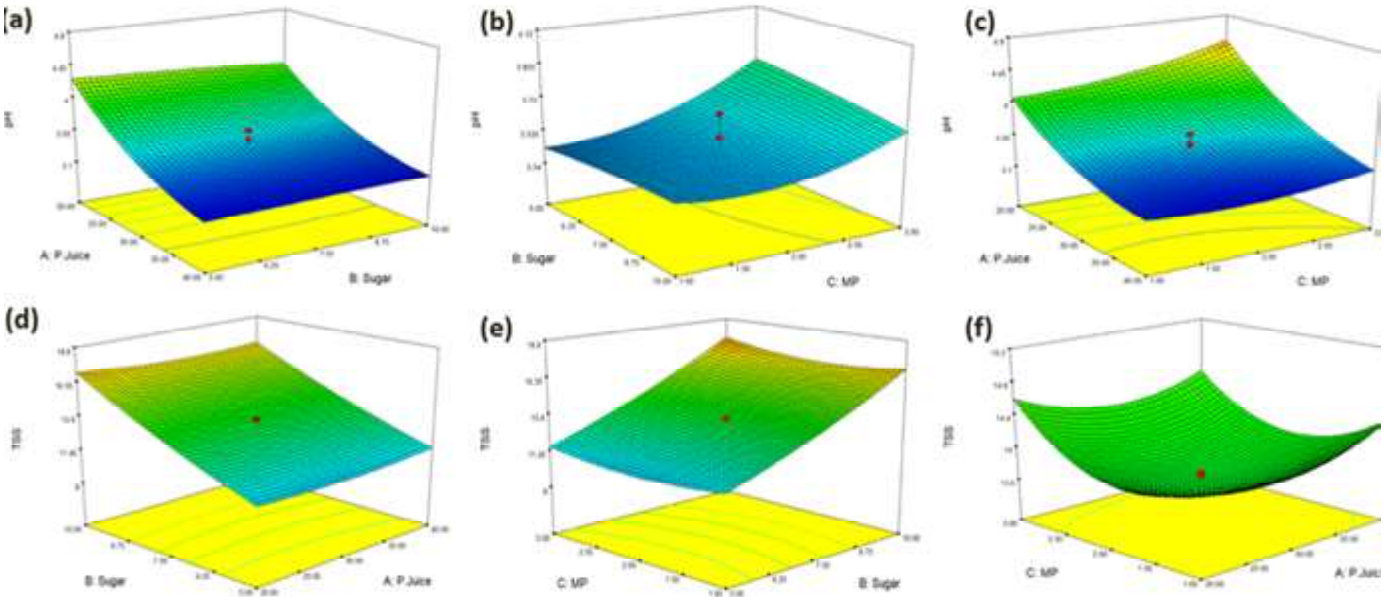

Fig. 1 Response surface plot for (a) pH influenced by the level of sugar and pineapple juice; (b) pH influenced by the level of sugar and mint powder; (c) pH as influenced by the level of mint powder and pineapple juice ; (d) TSS influenced by the level of sugar and pineapple juice; (e) TSS as influenced by the level of mint powder and Sugar; (f) TSS influenced by the level of mint powder and pineapple juice

pentosaceus NCDC 273. This combination of whey pineapple mint fermented with *Pediococcus pentosaceus* NCDC 273 has not yet been used for beverage formulation.

The experiments were designed using Central Composite Rotatable Design. Response Surface Methodology was applied to the experimental data, and parameters taken into account were Pineapple juice (20-40) %, Mint powder (1-3) %, Sugar (5-10) %, and second-order polynomial models were developed (Table 2). In total, 20 formulations were prepared, and the effect of the parameters mentioned above was studied on DPPH radical scavenging activity %, pH, TSS, and overall acceptability (Table 1). The impact of variable quantities of pineapple juice, mint powder, and sugar on responses at linear, quadratic, and interaction levels are given in Table 2. ANOVA was performed for each response to assess the suitability of the selected model (Table 3). All the models displayed statistical significance as indicated by the F value, R^2 values for all models were more than 80%, and lack of fit was found to be insignificant. Therefore, it can be concluded that all models were statistically valid for predicting the response.

Effect of variables on pH

The pH values ranged from 3.18-4.73 for the beverage prepared in this study (Table 1). Acidic pH is an essential parameter for preventing food spoilage by microorganisms. The coefficient of estimation of pH on whey beverages showed that the level of pineapple juice and sugar had a negative effect on pH. On the other hand, the mint powder had a negative impact on the pH but to varying degrees. From Fig. 1(a), it can be observed that with the increase in the level of pineapple juice, a sharp decrease in the pH of the beverage proves the acidic nature of pineapple juice.

In contrast, the sugar level had no such effect on the pH of the beverage. From Fig.1(b), it can be observed that with the increase in the level of mint powder, there is an increase in the pH of the beverage because of the alkaline effect of the mint powder. On the other hand, from Fig. 1(c), it can be observed that with the increase in the level of pineapple juice, there is a sharp decrease in the pH of the beverage.

Effect of variables on TSS

The TSS values ranged from 9.16-17.95 ºBrix (Table 1) after 16hr fermentation at 37°C. TSS mainly measures the sugar content in fruits and beverages. The coefficient of estimation of TSS on the whey beverage showed that the level of pineapple juice, mint powder, and sugar had a positive effect on the TSS but to varying degrees. From Fig. 1(d), it can be observed that the TSS increases sharply with the increase in sugar level. In contrast, with an increasing amount of pineapple juice, there is a slight increase in the TSS of the beverage, considerably lesser than the profound effect of sugar on the TSS of the drink. With increased amounts of mint powder, the TSS of the drink also increased (Fig. 1(e)). However, increasing amounts of pineapple juice initially decreased the TSS, with a slight rise at the end. Similarly, with the increase in mint powder, initially, the TSS decreases somewhat,

followed by a slight increase at the end (Fig. 1(f)). The increase in TSS may be due to the hydrolysis of polysaccharides into monosaccharides and oligosaccharides.

Effect of variables on DPPH

The DPPH values ranged from 80-96% (Table 1) after 16hr of fermentation at 37°C. The coefficient of estimation of DPPH on the whey beverage showed that the level of pineapple juice positively affected DPPH's radical scavenging activity. From Fig. 2(a), it can be observed that with the rise in pineapple juice, the DPPH increases significantly. From Fig. 2(b), it can be observed that DPPH increases considerably with increased levels of both mint powder and pineapple juice. From Fig. 2(c), it is observed that DPPH increases with the concentration of mint powder up to a certain extent, after which it decreases. Antioxidants can delay oxidation in chain reactions, thereby preventing oxidative stress caused due to free radical generation, which can further damage lipids, proteins, and nucleic acids (Jovanovic et al. 2018).

Effect of variables on Overall Acceptability

The coefficient of estimation of the Overall Acceptability of whey beverages showed that the level of sugar and pineapple juice positively influenced the overall acceptability. In contrast, the mint powder had a negative impact on the overall acceptability due to varying concentrations of mint up to a certain level. The terms for the effect of these variables were significant. From Fig. 2(d), it can be observed that with the increase in the level of pineapple juice, there is an increase in the overall acceptability of the beverage. While with the rise in the sugar level, the drink's overall acceptability increases up to a specific limit beyond which it slightly decreases. Still, the increase is considerably lesser than pineapple juice's profound effect on the beverage's overall

Table 2 Analysis of variance and regression analysis

Source	Sum of Squares	DF	Mean Squares	F value	Significant %
pH					
Model	0.027	9	2.992	26.17	**
Residual	1.143	10	1.143		
Lack of fit	1.060	5	2.119	12.65	
Pure error	8.379	5	1.676		
Total	0.028	19			
R^2= 0.9593, Adj R^2=0.9226					
DPPH					
Model	1.62	9	0.18	34.02	**
Residual	0.053	10	5.291		
Lack of fit	0.053	5	0.011	7310.08	**
Pure error	7.236	5	1.447		
Total	1.67	19			
R^2= 0.9684, Adj R^2=0.9399					
TSS					
Model	1.89	9	0.21	27.10	**
Residual	0.078	10	7.755		
Lack of fit	0.078	5	0.016	2763.39	**
Pure error	2.805	5	5.610		
Total	1.97	19			
R^2= 0.9606, Adj R^2=0.9252					
Overall Acceptability					
Model	1.18	9	0.13	29.19	**
Residual	0.045	10	4.480		
Lack of fit	0.045	5	8.961		
Pure error	0.000	5	0.000		
Total	1.22	19			
R^2= 0.9633, Adj R^2=0.9303					

**p<0.0001

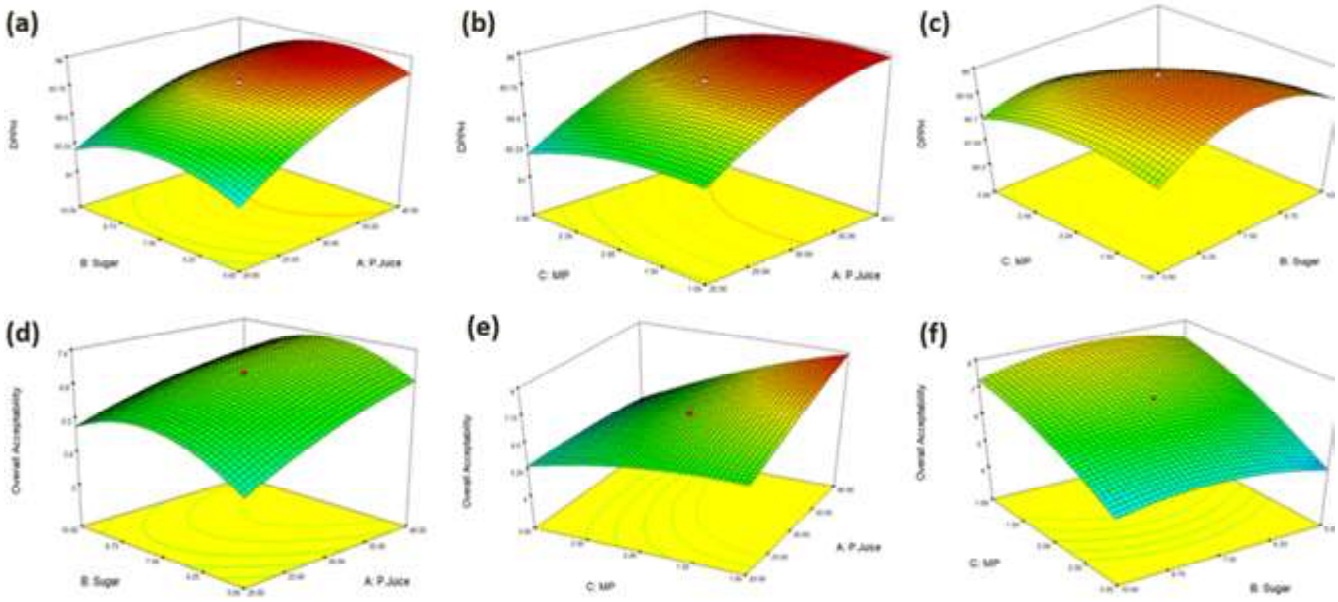

Fig. 2 Response surface plot for (a) DPPH radical scavenging activity influenced by the level of sugar and pineapple juice; (b) DPPH radical scavenging activity influenced by the level of mint powder and pineapple juice; (c) DPPH radical scavenging activity influenced by the level of sugar and mint powder (d) Overall Acceptability influenced by the level of sugar and pineapple juice; (e) overall acceptability influenced by the level of pineapple juice and mint powder; (f) overall acceptability as influenced by the level of mint powder and sugar

Table 3 Analysis of variance as a linear, quadratic, and interaction for each response variable

	Coefficient	Standard Deviation	t.exp	Significant %	Coefficient	Standard Deviation	t.exp	Significant %
Intercept	DPPH				pH			
A-P.Juice	9.72	0.030	9.65	**	2.10	4.361	2.09	**
B-Sugar	0.25	0.020	0.21	**	0.035	2.893	0.029	**
C-MP	-0.046	0.020	-.090	0.0428	-2.799	2.893	-9.246	0.3562
AB	-0.096	0.020	-0.14	0.0006	4.142	2.893	-2.305	0.1828
AC	-0.037	0.026	-.094	0.1826	8.280	3.781	-1.432	0.0533
BC	7.257	0.026	-.050	0.7836	-0.018	3.781	-0.026	0.0009
A^2	-0.070	0.026	-0.13	0.0219	8.280	3.781	-1.432	0.0533
B^2	-0.10	0.019	-0.15	0.0003	0.020	2.817	0.013	**
C^2	-0.17	0.019	-0.21	**	-2.464	2.817	-8.740	0.4022
	-0.078	0.019	-0.12	0.0022	1.736	2.817	-4.540	0.5515
	TSS				Overall Acceptability			
Intercept	3.69	0.036	3.61	**	2.64	0.027	2.58	**
A-P.Juice	4.362	0.024	-.049	0.8584	0.092	0.018	0.052	0.0005
B-Sugar	0.36	0.024	0.31	**	0.014	0.018	-0.027	0.4663
C-MP	0.021	0.024	-.032	0.3954	-0.23	0.018	-0.27	**
AB	-0.021	0.031	-.090	0.5181	-0.021	0.024	-0.074	0.3861
AC	-0.011	0.031	-.080	0.7366	-0.12	0.024	-0.17	0.0006
BC	8.085	0.031	-.061	0.8004	0.021	0.024	-0.031	0.3861
A^2	0.037	0.023	-.015	0.1460	-0.027	0.018	-0.066	0.1579
B^2	0.022	0.023	-.030	0.3653	-0.099	0.018	-0.14	0.0002
C^2	0.065	0.023	.014	0.0183	-0.074	0.018	-0.11	0.0018

**p<0.0001; Coefficients were A, B, C (linear), A^2, B^2, C^2 (quadratic), AB, AC, and BC (interaction) of the model, calculated by software DX7Trial Design expert 7.0.0.

acceptability. On the other hand, with the rise in the level of mint powder, the overall acceptability increases sharply due to the soothing effect of mint on the palate (Fig. 2(e)). From Fig. 2(f), it can be observed that with the rise in the level of sugar, there is a slight increase in the overall acceptability of the beverage up to a specific limit (up to 7.5g), beyond which it decreases slightly, proving that moderate sweetness has a strong influence on the consumer's palate.

Nutritional attributes of the fermented whey beverage

The results of chemical parameters for fermented whey beverage (FPMWB) and Unfermented whey beverage (UFPMWB), such as moisture content, acidity, total soluble solids (TSS), carbohydrate, protein, fat, and ash content of the fermented and unfermented whey beverage have been summarised in Table 4. Several researchers have found similar results for the chemical composition of fruit and whey beverage (AbdulAlim et al. 2018; Cuhna et al. 2022; Islam et al. 2021; M'hir et al. 2019). The beverage's acidity has increased after fermentation due to pineapple juice and acid produced during fermentation. Other studies have observed similar results (Sabokbar & Khodiyan, 2015). The reduced ash content (4.03%) of the fermented whey beverage compared to the unfermented one (4.37%) indicate their possible depletion due to microbial growth. Comparable results have been reported by Islam et al. (2019). The beverage's Total Soluble Solids (TSS) were determined to be approximately 12%, and protein content was around 4% in this study which depends upon the addition of fruit pulp and sugar. Similar results have been obtained by Gimhnai & Liyanage, (2018) and Islam et al. (2021). The phenolic content of the fermented whey beverage and control (unfermented) were 89.41% and 83.34% mg G.A.E./ mL. The results obtained agree with the findings of Balakrishnan & Agrawal, (2014) and Islam et al. (2021). The percentage of DPPH radical scavenging activity of the fermented whey beverage and control (unfermented whey beverage) were found to be 83.88% and 78.17%, respectively, comparable to the results obtained by M'hir et al. (2019). High antioxidant potential, often associated with antiaging and anti-inflammatory effects on human physiology, makes this beverage a healthier option than soft drinks.

FTIR spectra

The FTIR spectra of FPMWB and UFPMWB are presented in Fig. 3(a) and Fig. 3(b), respectively, depicting the predominant

Table 4 Physico-chemical characteristics of the fermented-whey beverage

Parameter	Fermented Beverage (FPMWB)	Control (UFPMWB)
Moisture (% Wet basis)	88.38±1.60^{a}	86.34±0.57^{b}
Dry matter (%)	11.61±1.60^{b}	13.66±0.57^{a}
Ash (%)	4.03±0.85^{b}	4.37±0.69^{a}
Organic matter (%DM basis)	95.96±0.85^{a}	95.63±0.69^{a}
pH	4.13±0.015^{b}	4.34±0.005^{a}
TSS (ºBrix)	12.2±0.1^{a}	12.1±0.1^{a}
Titratable acidity (%)	1.02±0.05^{a}	0.9±0.15^{b}
Crude protein (%)	4.30±0.01^{a}	4.20±0.02^{b}
TPC (mg GAE/mL)	89.41±1.90^{a}	83.34±1.29^{b}
DPPH (%)	83.88±1.77^{a}	78.17±0.99^{b}
Sodium (mg/L)	132.56±0.015^{a}	132.72±0.086^{a}
Potassium (mg/L)	1165.47±0.006^{a}	1036.93±0.044^{b}
Iron (mg/L)	14.66±0.01^{a}	6.72±0.0^{b}
Calcium (mg/L)	27.34±0.02^{a}	25.27±0.02^{b}
Medium chain fatty acid (mg/100mL)	5.8± 0.05^{b}	6.32±0.01^{a}
Long-chain fatty acids (mg/100mL)	49.48±0.27^{a}	41.61±1.08^{b}
Monounsaturated fatty acid (mg/100mL)	24.54±0.2^{a}	21.57±0.02^{b}
Omega 3 fatty acid (mg/100mL)	3.64 ±0.045^{a}	2.6±0.035^{b}
Omega 6 fatty acid (mg/100mL)	7.01± 0.03^{a}	4.01±0.035^{b}
Coliform count (log CFU/mL; % inhibition at 15th and 30th day	1.79±0.04^{a}, 1.97±0.00^{a}	1.69±0.02^{b}, 1.89±0.01^{b} (5.57% ,4.04 %)
Yeast and mold count (log CFU/mL; % inhibition at 15th and 30th day	1.89±0.02^{a}, 1.98±0.00^{a}	1.86±0.02^{b}, 1.92±0.03^{b} (2.2%, 2.9%)

All the values are Mean ± S.D (n = 3). Mean values in a row with different superscript differ significantly from each other (P > 0.05).

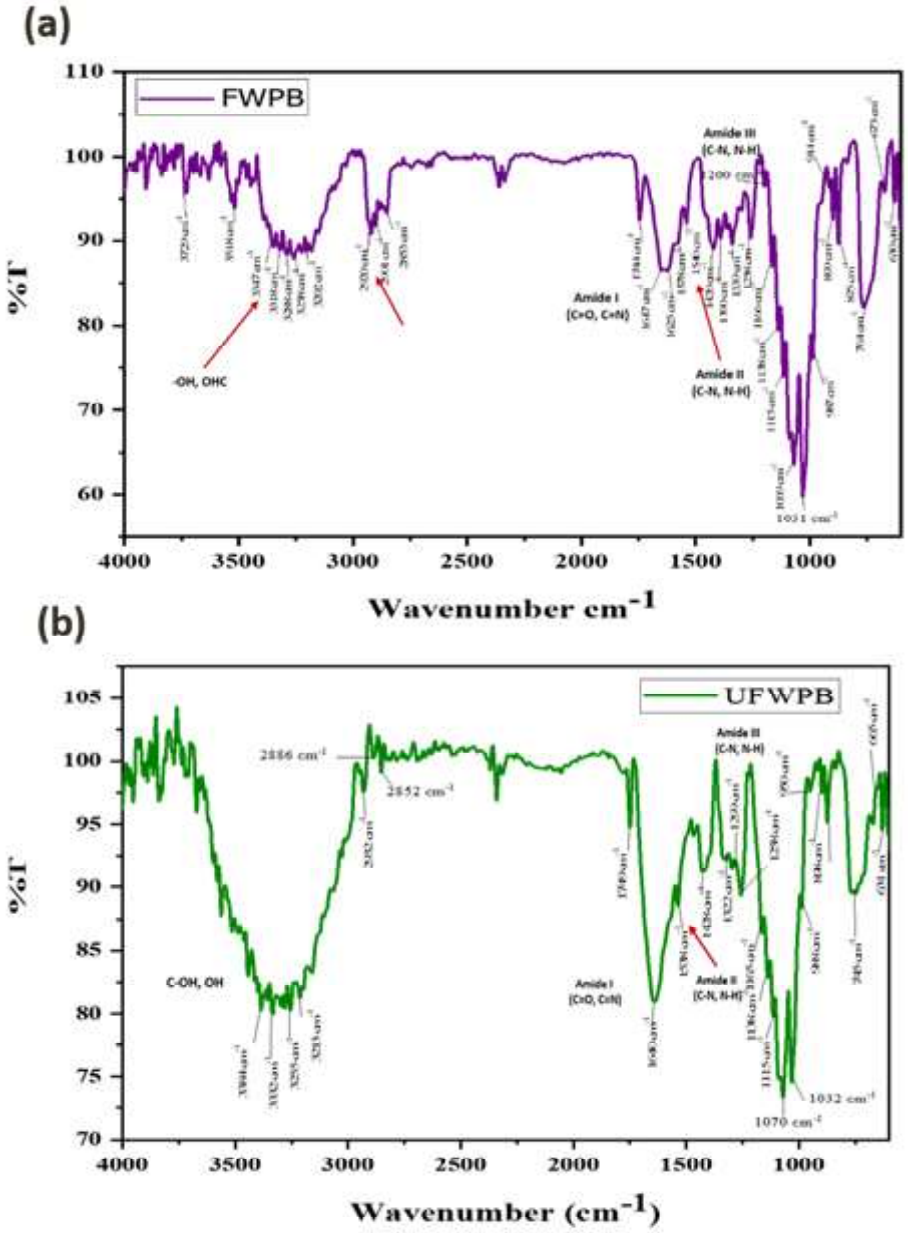

Fig. 3 (a). FT-IR spectrum for Fermented whey beverage; (b). FT-IR Spectrum for unfermented beverage (FWPB-Fermented Whey Pineapple-Mint Beverage, UFWPB- Unfermented Whey Pineapple Mint Beverage)

functional groups present in the beverage and changes in the biomolecules after fermentation. The samples exhibited different absorption bands and heights of the peaks, which may be attributed to the structural changes in proteins' chemical interaction or production of specific metabolites after fermentation.

FPMWB showed increased absorption bands and heights at (1750–1250 cm^{-1} and 2500–3000 cm^{-1}, 3400–3000 cm^{-1}, and 3000–3500 cm^{-1}). Significant differences in the functional groups (Amide-I band) present in the beverage before and after fermentation and comparable results have been reported by Souza et al. (2019) and Wen-Qiong et al. (2021). The Amide-I (C=O coupled with C=N) band at 1640 cm^{-1} in the control sample has been changed to shorter bands appearing at 1625-1647 cm^{-1} in the fermented sample, clearly showing the conformational changes in the secondary structure of the whey protein due to fermentation and interaction with pineapple juice which contains plant polyphenolic compounds and organic acids. The Amide-II band at 1538 cm^{-1}in control and 1540 cm^{-1}in the fermented sample and the Amide-III band at 1258 cm^{-1} in both samples does not show much difference. The bands in the region 3202-3258 $cm^{-1}$of the fermented sample appeared because of stretching vibrations of -O.H. linked to $–NH_2$ (Amide-A), while bands in the area 2901-2932 cm^{-1} are due to asymmetric stretching of $–CH_2$ group (Amide B). The spectral bands occurring between 3200 cm^{-1}– 3518 cm^{-1}clearly demonstrate the presence of the –O.H. group (-O-H stretch) of phenolic compounds. These band ranges are assigned to the stretching vibration of O.H. groups that interact by hydrogen bonding (Heredia-Guerrero et al. 2014). The non-

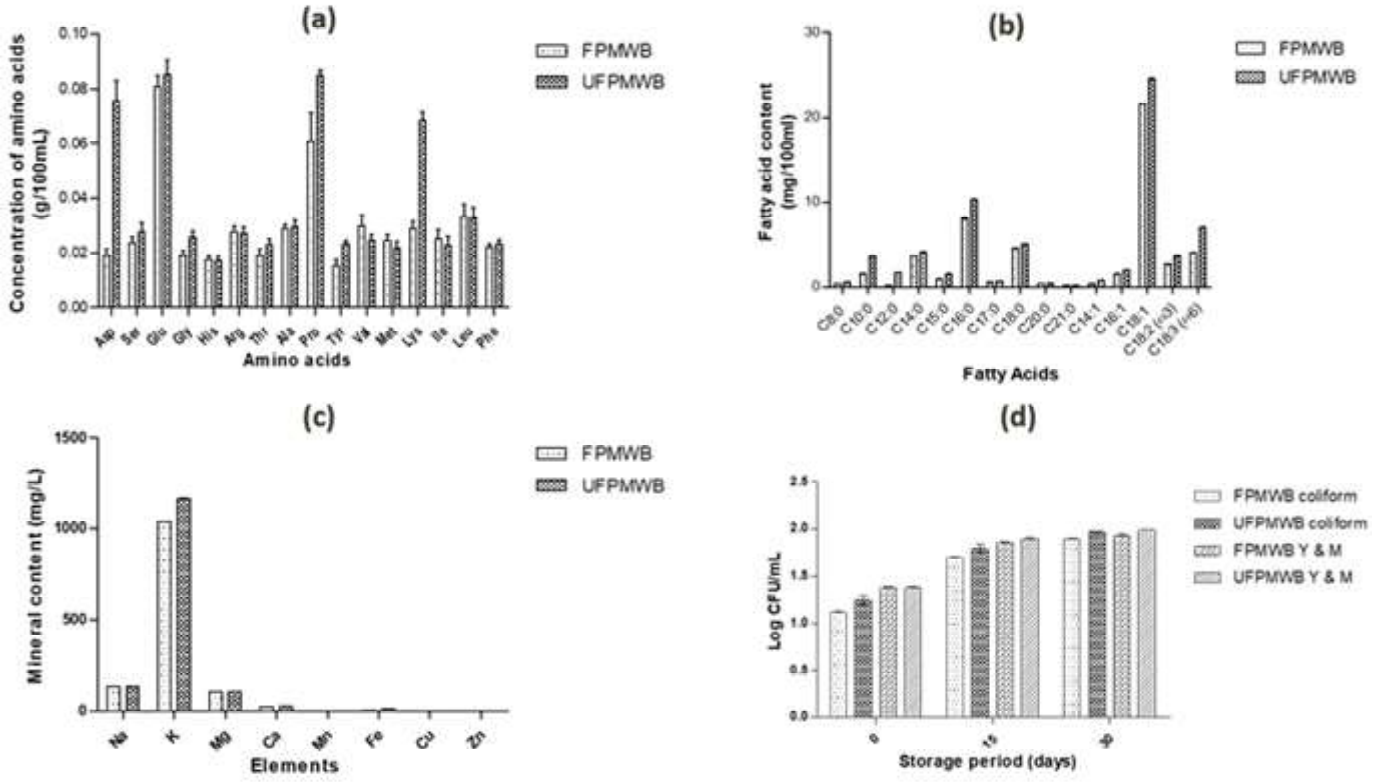

Fig 4 a) Amino acid profile of fermented & unfermented beverage b) Fatty acid profile of fermented & unfermented beverage c) Mineral profile of fermented & unfermented beverage d) Microbial counts of fermented & unfermented beverage (FPMWB- Fermented pineapple-mint whey beverage, UFPMWB- Unfermented pineapple mint whey beverage)

hydrogen bonded or free O.H. groups of alcohols and phenols absorb at 3650-3584 $cm^{-1,}$ which is not visible in these spectra. Apparently, -O.H. groups dominate the fermented sample because fermentation produces more functionalities containing the –O.H. group.

The bands recorded at 1031 $cm^{-1}$with C-O stretch vibration of alcohol functions, 1070 cm^{-1} with C-O, C-C, and C-H stretch vibration, 1165 cm^{-1}, and 1200 $cm^{-1}$with C-O-C ether stretching show the presence of lactose in both the samples. The formation of lactic acid from lactose during fermentation is indicated in the fermented sample by the presence of bands at 1744 cm^{-1} (-C-O stretch), 1420 cm^{-1} (CH_3 bending), and 1390 cm^{-1}(-C.H. bending). The presence of bands at 1744 cm^{-1}in the fermented sample and at 1749 cm^{-1} (-C-O stretch) in the control sample also indicates the presence of other organic acids, including phenolic acids. The bands at 1420 cm^{-1} -1428 cm^{-1} correspond to the stretching vibration of C-C of the aromatic ring, and bands between 900 cm^{-1} -630 $cm^{-1,}$ which result from C-H out of plane deformation, show the presence of aromatic rings such as plant polyphenolic compounds of pineapple juice, and aromatic amino acids of whey protein.

Amino acid, fatty acid, and mineral content

All essential amino acids except tryptophan were found in the fermented and unfermented beverages (Fig.4(a)). Among essential amino acids, the order of concentration was: Leu>Val> Lys>Arg>Met>Ile>Phe>His>Tyr. The concentration of amino acids (especially Asp, Pro, and Lys) is reduced after fermentation as amino acids are consumed by LAB for their growth. The whey beverage had a comparable amino acid content to the results obtained by other researchers (Gulec et al. 2021; Yasmin et al. 2013). Fig. 4(b) shows the fatty acids profile of fermented and unfermented whey beverages. It is clear from the graph that fatty acid content decreased after fermentation. The amount of different types of fatty acid calculated according to Barlowksa et al. (2018) and Florence et al. (2012). Overall, the whey beverage had the highest amount of long-chain fatty acid (41.61± 1.08 & 49.48±0.27 mg/100ml), followed by medium-chain fatty acid (6.32± 0.01 and 5.8± 0.05 mg/100ml) for fermented and unfermented whey beverage. PUFA levels observed in the beverage were 2.6±0.035 & 3.64 ±0.045 mg/100ml (n-3) and 4.01±0.035 and 7.01± 0.03 mg/ 100ml (n-6) for fermented and unfermented whey beverage respectively. The omega-6 to omega-3 fatty acid ratio was 1.52±0.00 and 1.92±0.03 for fermented and unfermented beverages, respectively, showing the positive effects of LAB

fermentation by reducing more omega-6 fatty acid. MUFA levels were 21.57±0.02 and 24.54±0.2 mg/100ml for fermented and unfermented beverages. Similar results have been registered by Silveira et al. (2019). The fatty acids associated with coronary heart disease, termed hypercholesterolemic fatty acid (C12:0, C14:0, C16:0), have been significantly reduced after fermentation, making this beverage healthier.

The whey beverage possessed a fair amount of macro and micro minerals (Fig. 4(c)). Potassium, sodium, magnesium, and calcium concentration ranged from 1.04±0.08, 0.13±0.15, 0.10±0.006 and 0.03±0.02 mg/ml, respectively. The order of concentration of macro-elements was: K> Na> Mg> Ca>Fe. Potassium was highest in macro-minerals and is a healthier choice than sodium due to its hypotensive effects (Machin et al. 2014). The dietary mineral intake for an adult man who consumes 100 ml of the fermented whey beverage would be 3.53%, 2.96%, 2.41%, 0.66%, and 0.25% for Fe, K, Mg, Na, and Ca, respectively, according to the RDA suggested by ICMR, 2020 . Micro-minerals such as Mn, Fe, Cu, and Zn ranged from 1.11±0.006, 6.72±0.01, 0.01±0.001 and 0.60±0.001 mg/L, respectively. Except for Ca, these results corroborated sufficiently with the results of other researchers (Luis et al. 2015; Souza et al. 2019). The mineral content of dairy foods may vary depending on the manufacturing procedure, type, and concentration of ingredients used in the product's manufacturing (Luis et al. 2015).

Shelf-life of beverage

Fermented and unfermented whey beverage had an acceptable amount of coliform and Yeast and molds on the 30th day of storage under refrigerated conditions (Fig 4(d)). The coliform count was recorded as 1.11±0.03, 1.69±0.02, and 1.89±0.01 log CFU/mL, while yeast and mold counts were 1.39±0.02, 1.86±0.02, 1.92±0.03 log CFU/mL at 0th, 15th and 30th day of storage. The percent inhibition in fermented whey beverage due to antimicrobial metabolites of *Pediococcus pentosaceus* NCDC 273 for the coliform count was 5.57% and 4.04%. In comparison, it was recorded as 2.2% and 2.9% for yeast and mold count on the 15th and 30th day of storage, respectively (Table 4). Similar improvements in the microbiological quality of dairy products have been observed previously (Pandey et al. 2019).

Conclusion

The present study illustrates the easy development of value-added healthy fermented RTS beverages from whey, a by-product of the dairy industry. This RTS whey beverage had a refreshing taste and several nutritional attributes, such as high potassium content, total phenolic content and DPPH radical scavenging activity, and a fair amount of PUFA, MUFA, and essential amino acids. Fermentation with a well-known bioprotective culture has improved its nutritional value and shelf life. In India, chhana whey is available in plenty, and this type of RTS beverage presents a logical solution to the problem of environmental pollution due to panner/chhana whey disposal.

Conflict of interest

The authors declare no conflict of interest.

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