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Development of Functional Nutritional Whey Beverage with Herbal Extract and Its Sensory Quality

Ambitama Guha[1], Aditi Roy Chowdhury[1*]

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ABSTRACT

The dairy industry is one of the biggest Food Industries worldwide. In the dairy industry, lots of whey is during the Paneer manufacturing process. In local sweetmeat production units of India, a huge amount of whey is not handled for preservation and is discarded locally which increases the BOD level of wastewater. The objective of the current work is to make a value-added product utilizing dairy waste with the inclusion of natural preservatives in the form of herbal extracts. Transforming the whey into a value-added product is addressed in the present study. The management of such waste may generate a beneficial product (Herbal whey beverage) with numerous health benefits and it may be recognized as a functional whey beverage worldwide. The methodology of the production of whey was followed using the natural curdling agent in hot conditions with cow milk and cooled down immediately. Herbal extracts were prepared and mixed with whey, maintaining a proper ratio, and different trial runs were done. The final pasteurization step was carried out with a specific time-temperature profile. The final finished product was hot-filled in a 200 ml sterile glass bottle and corked. The production, preservation, and overall shelf-life study were undergone in the current work. The preservation concept was addressed with some natural preservatives using herbs like Cumin, Cinnamon, and Mint for their antimicrobial properties. The observation of the current work satisfactorily claims that the developed beverage is microbially safe for up to 4 weeks when kept in a refrigerated condition. The sensory analysis and chemical analysis also suggest the product's goodness up to 4 weeks with all attributes of sensory parameters like haze, pH, brix, colour, flavour, taste, and mouth-feel. All the operational steps were handled hygienically.

INTRODUCTION

The dairy industry is considered to be one of the largest industries in the food sector all over the world. Lots of valuable products from this sector starting from pasteurized packaged milk, milk powder, ready-to-eat milkshakes, cheese, paneer, butter, ice cream, etc are currently available in the world market. Among these products from milk, cheese has been the largest produced milk-based product that creates a by-product, whey. Whey is the largest produced by-product from milk processing or cheese processing, which is the liquid part that separates after the milk proteins' coagulation. In the Global statistical result, it has been seen that in 2022 cheese production has amounted to about 22.17 million metric tons. Among these, the European Union produces about 10.55 million metric tons of cheese per year claiming itself as the top cheese producer (Statista, 2023, Cheese production worldwide 2015-2023). Out of the total production of liquid whey only 3.2 million metric tons have been utilized by industries to develop some value-added products, whey protein concentrates, isolates, etc. In recent years whey-based beverage production includes whey proteins which have been introduced even in the US market (Chavan *et al.*, 2015), (Miller, 2009).

The expectation for the Global production of cheese has reached 168 million tons of cheese whey and 50% of that whey gets wasted as animal feed, biofertilizer in irrigation systems, and discharged without treatment (Pires *et al.*, 2021). But despite being a by-product whey has been proven to have immense health benefits. Whey can be considered a liquid enriched with nutrients. The composition of whey is equivalent to 94% of water and the total solids remain at 6%. Whey contains lactose (4.5% of total whey), 0.8% proteins, and 0.7% minerals (Pires *et al.*, 2021). Even though whey proteins are present in small amounts they show a high PER value (Protein Efficiency Ratio) and, a high BV value (biological value). The Net Protein utilization score is almost 95 for whey proteins which are the source of α-lactalbumin, β-lactoglobulin, casein-peptides, lactoferrin, immunoglobulin, and lysozyme. These varied compositions in whey proteins give overall immunity, anticancer effects, and show resistance to pathogenic activity. The whey proteins have also recorded antimicrobial properties (Gottschalk, 2005). The other valuable components are mentioned by (Tsermoula *et al.*,2021) as nitrogenous matter like whey protein, non-protein nitrogen, and peptides. The proteins present in whey β-Lactoglobulin and α-lactalbumin are known as immunity proteins. Minor proteins such as lactoferrin which is an iron-binding glycoprotein and found to be present at a 3% level of total whey protein. The functional components present in whey are in the form of oligosaccharides with a minor amount of glucose and galacto-oligosaccharide (Hernández-Ledesma *et al.*,2011).

[1] Department of Food Technology, Techno Main Salt Lake, EM 4/1, Sector-V, Kol-91, India
[*] Corresponding author's e-mail: rcrakhi29@gmail.com

Some functional micronutrients are also present in whey like minerals, mainly calcium, phosphorus, potassium, sodium, chloride, and magnesium. Some vitamins are also found to be present in whey such as Complex B vitamins, especially Riboflavin B2 and Vitamins B12. Polar lipids are also found to be present in whey with an admissible amount (Tsermoula *et al.*, 2021).

When whey has been discarded from industries as run-off effluent (without any treatment), it will impart water pollution like an increase in BOD level, degrading the water quality index. Not only BOD the increased amount of discarded whey may also give rise to the COD level as high as 50gms to 80gms COD L^{-1} or 40gms to 60gms BOD L^{-1}. Impact of a small creamery or sweet meat shop can produce a high amount of BOD value per day. which requires a huge amount of expenditure for treatment, mostly whey is discarded untreated. (de Almeida *et al.*, 2021).

As whey has been proven to be a nutritious liquid, researchers have recognized whey as a co-product rather than a by-product of the cheese, and paneer industries. Whey-based products that are found in the recent market as whey beverages include fermented and non-fermented fruit beverages, alcoholic whey beverages, sports drinks, whey protein powders, and isolates (Papadem & Kotsaki, 2019).

The current study has been focused on developing whey-based beverage with the inclusion of some indigenous herbal extracts. Mint, Cinnamon, and Cumin were selected to prepare the herbal extractive to preserve the whey on the basis of the concept of natural preservation method without the addition of any preservative. These indigenous herbs contain phytochemicals like polyphenols, flavonoids, tannins, alkaloids, terpenoids, lectins, etc. (Viji *et al.*,2015).

Cinnamon has a definite role in lowering blood glucose levels and can modulate insulin levels. It is associated with the reduction of type 2 diabetes. Cinnamaldehyde which is the active component in cinnamon has antimicrobial, antioxidative, and anti-inflammatory effects. Cell structure studies provide proof that it inhibits the growth of bacteria associated with food contaminants (Singletary, 2008).

The antibacterial activity of cumin is well recognized for its active components; alkaloids, flavonoids, glycosides, saponins, and organic acids (citric acid, malic acid, tartaric acid, ascorbic acid, propionic acid) (Clark, 1998). Cumin (cumin aldehyde) is proven to be highly packed with antioxidants and antimicrobial properties that work against both gram-positive and gram-negative bacteria along with some fungi groups (Kedia *et al.*, 2014), (Belal *et al.*, 2017).

Considering the valued nutritious components, functional oligosaccharides, immunity proteins, essential minerals, and vitamins, the whey-based beverage was aimed in this research to serve as a valorized product for the community in the tropical and subtropical zone. In South Asian countries including India, there is a huge market for herbal beverages (Dini, 2019).

The preservation of whey with the natural mode was focused in the present study in a blend of herbal extracts like cinnamon, cumin, and mint. The availability of herbs is common in Indian scenarios and it is also cost-effective. The current study investigates the extended shelf-life study of whey with herbs having their bacteriostatic action with a view to less heat treatment for a quality product.

This research aims towards a green technology and sustainable approach towards the exploitation of whey. The final product is decided as a ready-to-drink beverage with a new preservation mode/concept because of the high demand for beverages in hot and humid climates.

This beverage could be also considered as a valorized product in the post-Corona episode or can drive a parallel beverage sector towards a nutritional aspect compared with carbonated drinks.

MATERIAL AND METHODS

Materials

Standardized and Pasteurized cow milk of a recognized Indian brand with 4.5% fat and 8.5% SNF was purchased for whey extraction, from a local market in Kolkata. Sugar, lemon, and salt were also purchased from a local market in Kolkata, India as well. The choice of Herbs were fresh mint leaves, whole cinnamon, and whole jeera of a local brand in Kolkata, which were also purchased for the preparation of the trials, from a local grocery shop in Kolkata, India.

Preparation of Whey

The whey has been extracted using the traditional acid curdling method, since the ultimate objective is to use all the natural ingredients rather than citric acid, lemon juice has been used as the natural source of citric acid for the coagulation of casein protein. The processing of whey was done by taking 1L of milk, heated up to 80° C followed by the addition of lemon juice for curdling operation. The process was continued for near about 30seconds for the whey to be separated.

After the separation of the whey from the casein protein the separated whey was collected in a sterile beaker using a muslin cloth and then sealed with aluminium foil, and refrigerated. The pH of the raw whey was taken at 25°C with pH paper (Merck Life Science Pvt. Ltd) which came out to be 6 and the brix was measured with a refractometer which came out to be 7°.

Preparation of the Extractives

Three extractives have been prepared that are extractive of mint, cinnamon, and cumin. For the extractive preparation coarsely grounded cumin and cinnamon in a grinder and freshly mashed mint leaves in a mortar pestle, were taken. Each extractive was taken in three separate muslin clothes. In three different beakers, 250ml of water was been heated in a double boiler method up to 60°C. Each of the herbs, packed in muslin cloths, was then

dipped in three separate beakers for 5 minutes maintaining the temperature at 60°C. After the preparation of three extractives, the beakers with the extractives were sealed with aluminium foils and then refrigerated.

Preparation of the Final Beverage

The final beverage was prepared by mixing the whey and the herbal extract in a proper ratio. Salt and sugar have been admixed according to taste. The beverage has been pasteurized for 1 minute at 90°C. After pasteurization, the beverage was filled in a sterile glass bottle of 200ml and then refrigerated at 10±2°C.

Formulation of Trials

Two trials have been done by taking different ratios of whey and herbal extractives. In Trial-1 the proportions of the ingredients were 70ml whey and 30ml of extractives with salt and sugar. In Trial-2 the proportions of the ingredients were 60ml of whey with 40ml of extractives with salt and sugar. Two trials were made based on varying the whey along with the herbal extract proportions on a preliminary basis and the final formulation has been selected based on the sensory scores. The shelf-life study was done on the final product.

Sensory Analysis

The final formulation of the beverage has been finalized using the hedonic scale based test of each trial. The test was carried out among four different age groups: (less than 20), (20- 40), (40-60), and (above 60) making a three-member panel for each age group, the trials were tasted with a triangle test (Chude *et al.*, 2023).

Chemical Analysis

Proximate analysis was carried out following (A.O.A.C., 2010). Instruments utilized in the Chemistry of Food laboratory, TMSL named Hot air oven (Instrumention India), muffle furnace (Instrumention India), Water bath, Heating mantle, pH Meter (Toledo), and Weighing machine (Mettler), and Spectrophotometer (Systronics).

Shelf-Life Study

A shelf-life study was done on the sample rated high by the panellist in sensory analysis. The shelf-life study was undergone by keeping the corked bottle in refrigerated condition at around 10±2°C for 4 weeks. The pH, brix flavour, haze, colour, appearance, consistency, and also the chemical analysis such as total sugar, total solids, titrable acidity (in terms of lactic acid), antioxidant (in terms of gallic acid equivalent), and soluble protein content were investigated between intervals of 7 days, of the total of the 4 week study was to get an idea for the shelf life of the prepared processed product (Khasanov & Matveeva, 2020).

Microbial Analysis

Microbial analysis was done by pour plate method with Nutrient Agar media for the bacterial count. The plates were kept in the incubator at 42°C for 48 hours maintaining the time temperature ratios for colonisation. Colonies were counted using the colony counter. By using Czapek Dox Media yeast and mould counts were taken. The plates were kept in the incubator at 25°C to 30° C for 48 hours maintaining the time temperature ratios for colonization and the colonies were counted using the colony counter. Overall work is done taking all the necessary precautions in the microbiology laboratory of TMSL, MOFPI (Chowdhury*et al.*,2023).

Antioxidant Analysis

1ml (1:20) diluted beverage sample has been taken in a conical flask followed by the addition of 1ml follin-ciocalteu reagent (1:5 with water) and kept aside for 5 minutes. Addition of 3ml 20% Na_2CO_3 was done and the sample was kept for 30 minutes in a dark environment and absorbance was recorded at 760nm in a Spectrophotometer (Systronics). The water-soluble antioxidants (bioactive components) were analyzed in terms of the gallic acid equivalent of the final beverage which is Trial-2 (Chowdhury *et al.*, 2023).

Thin Layer Chromatography (TLC) for Qualitative Analysis of Essential Amino Acids

Thin Layer Chromatography has been done using TLC Silica Gel 60 F254 25 Aluminium Sheets (Merck KGaA) and solution preparation, (Butanol: Glacial Acetic Acid: Water in the ratio of 4: 1: 1) has been taken. The known essential amino acids along with the beverage sample were been spotted on the aluminium TLC sheets and were kept aside to let the solution flow along with the essential amino acids and the beverage sample. Ninhydrin is used to spot the flow or the distance travelled by the essential amino acids (Bele & Khale, 2011).

RESULT AND DISCUSSION

Table 1 comprises the whey volume, along with the curdling temperature, brix, and pH; which are to be recorded for the generation of the final finish product

Table 1: Processing of whey

Total whey extracted from 1L of milk	700ml (Approx)
Curdling Temperature	80°C
Brix	7.1
pH	6

which may be the contributed fraction with the herbal extractives.

(All data shown in Table 1 and in all other Tables are the mean of the triplicate values).

Table 2 gives a view of the extractive preparation with the herbs (cinnamon, cumin, mint). Figure 1 shows the extractives that have been prepared in the Food Processing Laboratory of TMSL. The preparation of the extractives with the heterogeneous herbal fractions was observed with the values, almost the same for all

Table 2: Processing of whey

Cumin	Extractive Composition	Cinnamon	Extractive Composition	Mint	Extractive Composition
Water	250ml	Water	250ml	Water	250ml
Herb	10gm	Herb	10gm	Herb	10gm
Time	5minutes	Time	5minutes	Time	5minutes
Temperature	60°C	Temperature	60°C	Temperature	60°C
Brix	1°	Brix	1°	Brix	1°

fractions with respect to the temperature and time profile, kept constant for every extractive preparation step along with the curdled whey which has been developed in the laboratory of TMSL processing lab. Hygienic practices have been employed to handle the perishable items with a keen monitoring for production.

Figure 1: Extractives (Mint, Cumin, Cinnamon)

Table 3: Pasteurization time temperature of Final Beverage

Pasteurization	Amount	500ml
	Time	1minute
	Temperature	90℃

Table no. 3 contains the pasteurization time and temperature of 500ml final beverage which is 1 minute at 90°C. The beverage has been manufactured following Good Manufacturing Practices. Since all the protocols have been maintained the time for pasteurization has been kept low. The low time for pasteurization is also a measure taken to preserve the high-valued whey protein at its best condition. (Vajdi & Pereira, 1973) also reported almost the same time temperature profile with strawberry, lemon, chocolate, inserted whey drinks, the overall time temperature condition for the mixture was maintained at 82°C for 2 minutes, which is almost similar to our study.
Table no. 4 consists of the total bottling amount which is 200ml. The brix has been maintained to be 15° as per FSSAI beverage guidelines (Food Products Standards and Food Additives Regulations, 2011). The pH has been kept at 5.4 which inhibits microbial growth that is the parameter pH has been used as a hurdle for microbial growth. A

Table 4: Soluble solid vs pH of the Final Beverage

Bottling amount	200ml
Brix at 30℃	15°
pH at 30℃	5.4

report has been given with the strawberry-based drink where the pH was recorded as 6.5 to 6.7 with sugar and additional flavour notes, whereas for fermented products, sour milk, buttermilk, and kefir the pH was adjusted to an acidic range of 4.8 to 4.5 (Athanasiadis, I., *et al.*, 2004).
The pH value of the product of the current study has been recorded as 5.4 which may be corroborated with the previous report.
The combination of whey and extractives were continued with two trials which are reflected in Table 5 (Trails-1)

Figure 2: Final Beverage, (Whey with Herbs)

Table 5: Trial 1- Formulation with 70% whey and 30% Herbal Extractives

Ingredients	Fractions
Whey	70ml
Cumin extract	15ml
Cinnamon extract	10ml
Mint extract	5ml
Salt	40gm
Sugar	90gm

Table 6: Trial 2- Formulation with 60% whey and 40% Herbal Extractives

Ingredients	Fractions
Whey	60ml
Cumin extract	25ml
Cinnamon extract	10ml
Mint extract	5ml
Salt	40gm
Sugar	90gm

and Table no. 6 (Trial-2). The objective of the trial runs were to depict the idea about the acceptability of the finished product with respect to the sensory score. The sensory evaluation is done with respect to taste, flavour, mouthfeel, and appearance. These data reports are given in Tables No. 7 and 8.

The brix value of the final finished product was estimated at 15° brix (FSSAI Regulations, 2011) for preservation

Table 7: Hedonic Rating for Trial-1

Age Group	Taste	Flavour	Appearance	Colour	Mouthfeel	Overall Impact
	6.5	7	6	5.5	5	5.8
	7	7	5.5	5	5	
	7	6.5	5	5	5	
Mean	**6.8**	**6.8**	**5.5**	**5.2**	**5.0**	
20-40	6.5	6	6	6	6	6.2
	6	7	5.5	6	6	
	6	7.5	5.5	6	6	
Mean	**6.2**	**6.8**	**5.6**	**6.0**	**6.0**	
40-60	7	7	6	6	6	5.9
	6	6	6	5.5	5	
	6.5	5	5.5	6	6	
Mean	**6.5**	**5.9**	**5.8**	**5.8**	**5.6**	
Above 60	5	5	5	6	6	5.7
	6.5	5.5	6	6	6	
	7	6.5	5.5	5.5	6.5	
Mean	**6.1**	**5.6**	**5.5**	**5.8**	**6.2**	

Table 8: Hedonic Rating for Trial-1

Age Group	Taste	Flavour	Appearance	Colour	Mouthfeel	Overall Impact
Less than 20	7	6.5	6	7	6.5	6.6
	8	8	6.5	7	6	
	7	7	5.5	6.5	6	
Mean	**7.3**	**7.1**	**6**	**6.8**	**6.2**	
20-40	7	7.5	6	6.5	7	6.7
	8	8	6.5	5	7	
	7.5	7.5	5.5	5.6	6.5	
Mean	**7.5**	**7.6**	**6**	**5.8**	**6.8**	
40-60	5	6	5	6.5	7	6.4
	7	7.5	5.5	6	8	
	6.5	7	6	7	7	
Mean	**6.1**	**6.8**	**5.5**	**6.5**	**7.3**	
Above 60	6.5	7	5.5	7.5	7	6.6
	8	6.5	5	6	7.5	
	7	6.5	5	7	7	
Mean	**7.1**	**6.6**	**5.1**	**6.8**	**7.2**	

purposes and also for taste. The pasteurization time and temperature were adjusted for the finished product to 1 minute at 90℃ that data can be corroborated with (Vajdi & Pereira, 1973). The holding time for pasteurization temperature was 1 minute in a closed vessel to avoid the oxidative changes of the smaller peptides and also to minimize the loss of the volatiles from the herbal fractions. Further, the time-temperature profile might be affecting the composition of the finished product; the derivatized product of the sugar base, until we are not controlling the exposure to high pasteurization temperature for a longer period.

Figure 3 shows the sensory score data for both trials. The result shows that Trial 2 (60:40 ratios of whey: herbal extracts) had higher sensory scores among the people category of the age group of less than 20, 20-40,40-60, and above 60 years. The sensory score was pointed towards the high scale value with the sensory parameters

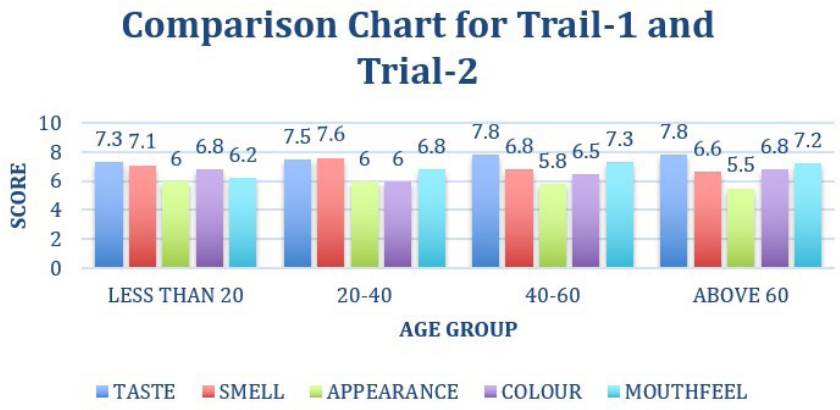

Figure 3: Best scores of the parameters from the comparison of each trial

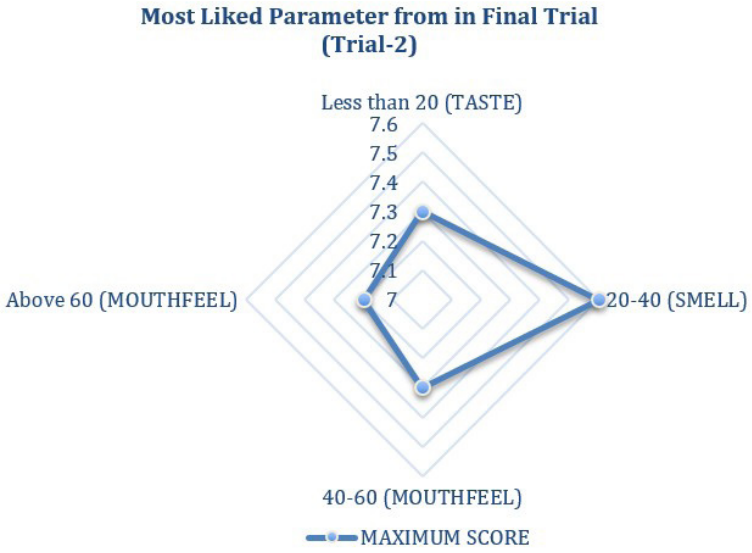

Figure 4: The most liked parameter from the final trial that is Trial-2

shown with respect to taste first for all categories. Whereas the colour effect for every group went down to a limit value of 5.8. When the sensory scores are evaluated the overall appearance of the product is almost the same. The overall acceptability score for Trial-2 was higher than Trial 1 shown in Table 7 and 8.

Figure 4 represents the most liked parameter in the final trial which is Trial 2. Among the panelists from the age group less than 20, taste is the most accepted parameter. Among the panelists from the age group 20-40, smell is the most accepted parameter whereas among the panelists from the age group 40-60 and above 60, mouthfeel is the most accepted parameter. The compositional variation of Trial 2 was higher with plant-based extract in comparison with Trial 1. The animal-based by-product (whey) has been mixed with plant-based extracts and was controlled to develop a designer food (whey-based herbal beverage) the final product's storage and its shelf life were judged. The chemical analysis report of the developed product was preserved for a longer period in refrigerated condition

Table 9: Chemical Analysis for shelf-life study

Chemical Analysis	0^{th} day	Week 1	Week 2	Week 3	Week 4
Temperature (±2)	10 °C	10 °C	11 °C	12 °C	12 °C
pH	5.4	5.4	5.4	5.4	5.5
Brix	15°	15°	15°	15°	15°
Total Sugar (After Inversion)	13.66%	13.66%	13.66%	12.9%	12.86%
Total Solid	15.1%	15.1%	15.2%	14.8%	14.8%
Titratable Acidity (in terms of lactic acid)	0.21%	0.21%	0.20%	0.21%	0.22%
Ash	0.26%	0.26%	0.25%	0.25%	0.24%
Soluble Protein mg/ml	6.75	6.75	6.70	6.65	6.66

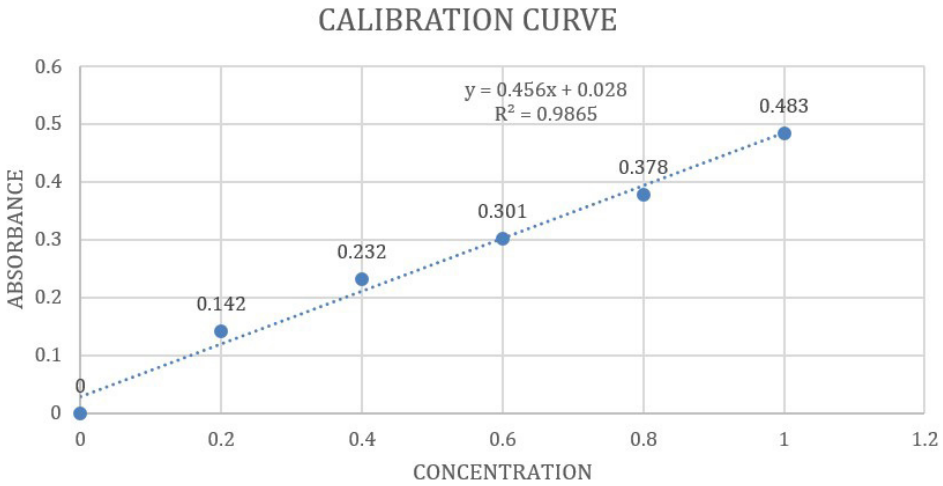

Figure 5: Calibration graph for soluble protein with BSA

kept at 10±2℃ in a corked glass bottle is depicted in Table 6. Upon storage for 4 weeks there were not any significant changes in pH value (5.4,5.4,5.4,5.5) starting from the 0th day, 7th day, 15th day, 21st day, and 30th day, and the recorded pH are given in Table 9, normal pH 5.4-5.5. This data can be corroborated with the data given by (Binsi *et al*, 2007), where we found no significant changes in pH with a marginal value reaching 6.58, 6.59, and 6.61 with the plant extract treatment starting from the initial day. As there is no increased value in pH observed upon storage, it expresses that loss of volatiles is being arrested with the effect of the plant extract which can control the pH values for up to 15 days on chill storage (Gao *et al.*, 2014). The investigated results showed that the present study depicts that pH is not a good indicator of spoilage for the early 15 days.

Table 9 suggests that the total solid content does not have any significant change within the established acceptable period starting from 0 days to the 30th day. The recorded soluble solid value was observed through a refractometer as a 15° brix (Brix has been considered as the soluble solid content). The result of the current study may suggest that the soluble solids (soluble proteins, soluble sugar, soluble metal ion, soluble plant extracts) do not show any effect upon the beverage consistency and also show some controlling effect on the bacterial growth during the storage in chilled condition. The total sugar was measured after doing inversion of the preserved sample and recorded weekly the total sugar in terms of glucose equivalent was recorded as 13.66%, 13.66%, 13.66%, 12.9%, 12.86% based on the 0th day, 7th day, 15th day, 21st day, and 30th day.

The developed product of the present work consists of different herbs and their extracts along with a certain volume of whey. Cumin contains essential oils that are well-recognized for antimicrobial effects, especially against *S. aureus* and *E. coli* (Monteiro-Neto *et al.*,2020). The extractives prepared from coarsely ground cumin powder have shown an inhibitory effect on microbial growth as said in (Škrinjar & Nemet, 2009), in the developed product, where cumin extract was the primary component in the herbal extracts that are admixed to develop the product (a whey-based herbal drink). The naturally occurring antimicrobial component present in all three extractives used in this current work that is Cumin, Cinnamon (Asimi *et al*,.2013), (Diniz do Nascimento *et al.*, 2020), and mint (Soleimani *et al.*, 2022) might have some controlling factor for giving a better shelf life for the developed product this can be exemplified by the use of natural bioactive components towards the conventional preservation mode of operation.

The recorded acidity was maintained for a 1-month tenure from 0th day as (0.21%, 0.21%, 0.20%, 0.21%, 0.22%) and the titrable acidity was measured in terms of lactic acid equivalent. The acidity will be the index for maintaining a well-preserved product. The current beverage which consists of whey along with herbal extracts may be a ready food source of microorganisms. The hurdles like acidity have to be given properly so that bacteria, fungi, or protozoa can be inhibited. The observed data for acidity for a month shows that it is more or less constant. And thereby preventing spoilage and maintaining the quality of the preserved product.

Mineral content was measured in terms of total ash of the developed products and recorded from the 0th day to 30th day as (0.26%, 0.26%, 0.25%, 0.25%, 0.24%) this indicates the product is a ready source of minerals as it is the ready source of natural extractives such as the phytochemicals which are coming from cinnamon (Khalisyaseen & Mohammed, 2021), cumin (Yessuf, 2015), and mint(Mimica-Dukic & Bozin, 2008) are mostly flavonoids, glycosides saponins, tannin vit. B complex vit. E Vit. A Vit. C, alkaloids, carotenes, zeaxanthin, resins. Ash content is quite good in cumin, mint, and also in cinnamon, which has been reflected in the total ash content of the developed product.

Soluble protein content was measured with respect to bovine serum assay which was set as a standard in drawing the calibration curve. The known concentrations were set as 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml, and 1mg/ml on the x-axis, and the corresponding absorbance were plotted against the y-axis which is depicted in figure 5. The

concentration of the soluble protein of the developed product was recorded as 6.75mg/ml value. The process whey-based beverage was filled in a 200ml capacity glass bottle where the measured soluble protein content was recorded as 1.35gm. This protein indicates the combination of soluble proteins which are identified as immunity proteins. Hence the developed product may be labelled as a rich source of functional proteins (Solak, B. B., & Akin, N. 2012). During the shelf-life study of up to 4 weeks, there is no significant changes occurred in soluble protein content. Shelf-life study of the developed product has been illustrated in Table 10. The preserved product was kept in a corked glass bottle of 200ml capacity at a temperature of 10℃ ±2 for a period of one month tenure. Smell, colour, taste clarity or haze, and consistency were measured with very little change. All the parameters except haze doesn't have any additional impact on the final product quality. In the case of haze up to 4th-week data, a small amount of haze was distinguishably observed throughout the 1-month period. The antioxidant content, pH value, titrable acidity, colour, smell, and taste, these were almost comparable with the changes found by the co-workers

Table 10: Shelf-Life Study

Week	Smell	Colour	Taste	Haze	Consistency
1st	No of flavour	No changes	No taste change	Little haze at the bottom of the bottle	Homogenous
2nd	No of flavour	No changes	No taste change	Little haze at the bottom of the bottle	No separation of water from the phase
3rd	No of flavour	No changes	No taste change	Little haze at the bottom of the bottle	No separation of water from the phase
4th	No of flavour	No changes	No taste change	Little haze at the bottom of the bottle	Consistency was as good as the first week

in the field of (Khasanov & Matveeva, 2020). Up to 30 days value there is a minimum decrease in the value of antioxidant content but when stored at a low degree temperature which is very similar to our observed result with respect to colour taste and flavour and antioxidant content when stored at cold temperature conditions.

Microbial analysis has been recorded in Table 11. The bacterial plate count was measured in two different serial dilutions,10^{-1} and 10^{-3} which gives a clear concept of mostly hygienic practices with the recorded count in a colony counter. The highest count up to the 4th week was recorded as 10 and 3 for 10-1 and 10-3 respectively. Whereas the plate counts for yeast and mould showed up to the 4th week were 2 and 3 for 10^{-2} and 10^{-4} respectively counted in a colony counter. Plate count for 10^{-1} bacterial plate count and 10^{-4} yeast and mould plate count are shown in Figures 6 and 7. As the cold preservation has been maintained for 4 weeks duration the growth of the

Table 11: Microbial Analysis

Weeks	Bacterial plate count at 10^{-1} dilution	Bacterial plate count at 10^{-3} dilution	Yeast and Mould plate count at 10^{-2} dilution	Yeast and Mould plate count at 10^{-4} dilution
Week 1	2	1	0	0
Week 2	3	1	0	0
Week 3	3	2	1	1
Week 4	10	3	2	3

organisms is arrested. The data can be corroborated with (Ismail, 2011).

Antioxidant Analysis

The report of spectrophotometric analysis of total polyphenolic content, in the developed product was measured with respect to the gallic acid equivalent is given in the given in Table 7. The standard calibration curve of gallic acid has been illustrated in Figure 8. On the 0th day of the production, the polyphenol content is recorded in the whey beverage as 3.25mg/ml (0.325gm per 100ml) which reflects a quite high value of polyphenol content of the developed beverage. As the preservation study was carried out in the current research, we find a less

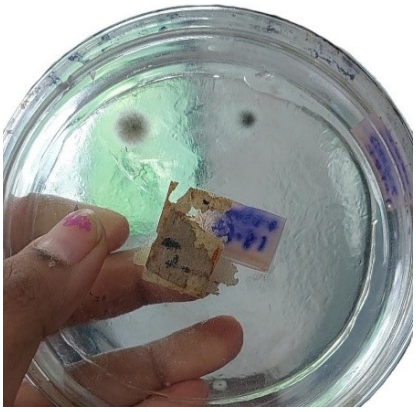

Figure 6: Bacterial plate count of 10^{-1} dilution

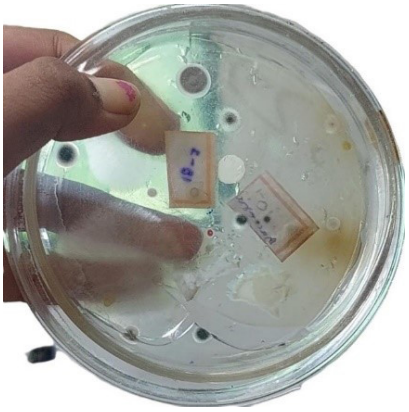

Figure 7: Yeast and Mould plate count for 10^{-4} dilution

depletion of antioxidant value up to 4th week of storage. This indicates that chilled storage might have some effect on the antioxidant capacity value. The developed beverage can be said as a healthy beverage keeping a good amount of soluble pigments, total polyphenolics.

The TLC value has been carried out with respect to the essential amino acid along with glutamic acid and aspartic acid with the prepared standard which are mentioned in Table 12. The image of the TLC sheets with the amino acids are given in Figures 9,10,11 and 12. Comparing the retardation factor of all amino acids with the sample analyte (whey-based beverage), the predominant amino acids which are found to be present are recorded as leucine, lysine, threonine, glutamic acid, aspartic acid, and proline.

The thin layer chromatography method could assess the nutrition aspects of the whey-based beverage in relation

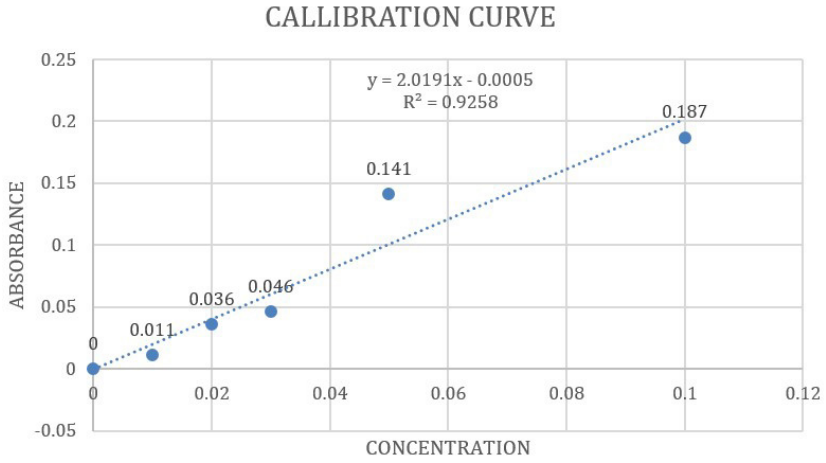

Figure 8: Calibration curve for Total Phenolic Content

Table 12: Total Polyphenolic/Antioxidant Content in the Final Beverage

Weeks/Days	Polyphenolic Content (In Terms of Gallic Acid Equivalent) mg/ml
0th day	3.375
Week-1	3.375
Week-2	3.375
Week-3	3.12
Week-4	3.06

Table 13: TLC Report

Amino Acid	Solute Front/Solvent Front (mm)	Experimental Retardation Factor (Rf)	Rf of beverage (u)
Histidine (A)	6/62	0.096	0.24
Isoleucine (B)	39/62	0.62	

Leucine (C)	20/62	0.32	
Lysine (D)	18/64	0.28	
Methionine (E)	35/64	0.54	0.23
Threonine (G)	20/64	0.31	
Phenylalanine (P)	34/64	0.53	
Tryptophan (H)	42/66	0.63	0.32
Valine (I)	34/66	0.51	
Glutamic Acid (G1)	20/62	0.32	0.22
Aspartic Acid (X)	13/62	0.20	
Proline (Y)	20/62	0.32	

to functional health drinks and some sensory attributes with respect to the presence of varied essential amino acid content. The qualitative test method has been adopted to find the nutritional aspect of the developed health beverage the result in concurrence with (Jain *et al.*, 2013)

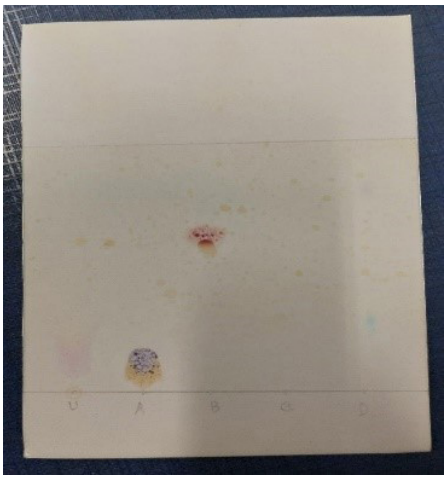

Figure 9: Amino acids A, B, C, D, and beverage sample U

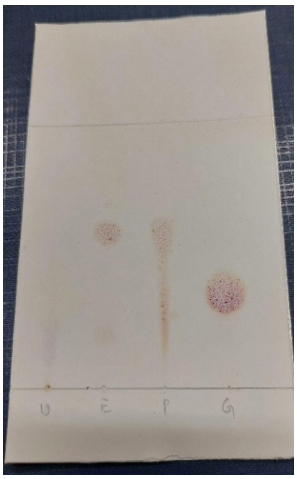

Figure 10: Amino acids E, P, G beverage sample U and Beverage Sample U

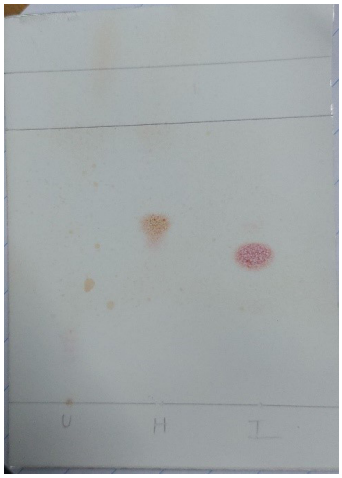

Figure 11: Amino acids H, I, and Beverage Sample U

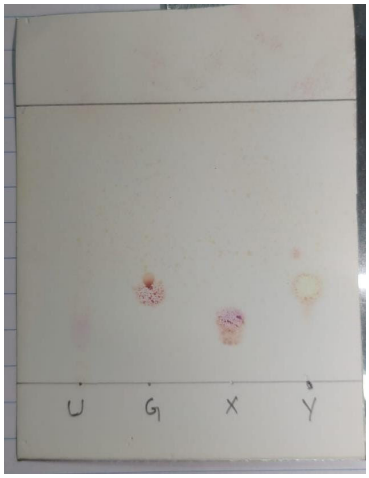

Figure 12: Amino acids G1, X, Y Beverage Sample U

CONCLUSION

Ready-to-serve beverages with the inclusion of herbs in dairy wastes (whey) can be a ready solution with some health benefits that have been identified through current research. The shelf-life study of the said product was observed for 4 weeks without major alteration of food concerning sensory, chemical, and microbial qualities. The developed beverage may be an alternative sustainable product without the addition of any chemical preservative, rather than preservation done

with the concept of bacteriostatic action of herbs like Cumin, Mint, and Cinnamon. In the conclusive part, the investigated result suggests that the presence of a good amount of polyphenols along with a high amount of soluble proteins may suggest the developed beverage could be mentioned as a functional beverage as per the chemical analysis. The developed beverage may also be utilized as a ready source for the supply of carbohydrates, soluble proteins, electrolytes, and antioxidants. For rehydration purposes, this beverage can effectively cater to all age groups including sports persons and elderly people for better fluid retention. Not only that that, developed beverages will satisfy some of the essential amino acids score within the human system and may be commercialized to formulate sports drinks. The best preservation method for storage and supply chain operations of the developed beverage is to be maintained at a low-temperature condition. Good hygienic practices are maintained to develop a value-added product with the herbal extracts during processing operations so as to create a temperature and time profile in the method of pasteurization. A hurdle technology has also been applied by maintaining proper acidity, brix value, and inclusion of herbal extractives for developing a sustainable Functional Nutritional Product (FNP).

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