Estimation of Nutritional and Health-promoting Potential of Developed Whey Incorporated Functional Food: A Potential Sustainable Approach

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ABSTRACT

Background: Whey, a dairy by-product, hosts important nutrients and bioactives but is an environmental and economic concern when accumulated. The study developed low-cost functional food products (beverage and thepla) to promote sustainable whey utilization for improved health.

Methods: The nutritional and phytochemical compositions were determined using Association of Official Analytical Chemists methods and standard biochemical protocols. Antioxidant and prebiotic properties were assessed by DPPH and the growth kinetics of *Lactobacillus rhamnosus* GG ($p \leq 0.01$, 95% confidence interval) before and after *in vitro* human gastrointestinal digestion.

Result: Variation C of both the beverage (VBC) and thepla (VTC) was the most organoleptically accepted product that possessed substantial amounts of macronutrients, dietary fibre, vitamins (vitamin C and β carotene), minerals (calcium, magnesium, sodium, potassium, iron and phosphorus) and phytochemicals (polyphenols, flavonoids and alkaloids) accompanied by low anti-nutrients (phytates, tannins and oxalates) compared to their respective basic products (without whey). Furthermore, they could efficiently scavenge free radicals and accelerate *Lactobacillus rhamnosus* GG growth even post- *in vitro* human gastrointestinal digestion, indicating their antioxidant and prebiotic potential. Therefore, the developed products may provide sustainable options for limiting whey gathering-associated issues along with alleviating nutritional insecurity and health through their nutritional and functional potentials.

Key words: By-product, Functional food, Health, Nutrients, Sustainable, Whey.

INTRODUCTION

Rapid expansion of the dairy industry due to a rise in economic activity, per capita consumption of milk and its products and shifting dietary choices brought on by increased urbanization have heightened the problems associated with the accumulation and disposal of its by-products (Grout *et al.,* 2020). Raw milk is converted into various foods, releasing by-products like buttermilk and whey. Whey, a significant dairy residue accounting for 145 million tons annually, poses a significant environmental risk due to its high organic content and potential high biological (BOD) and chemical oxygen demand (COD) if not properly treated. (Giulianetti de Almeida *et al.,* 2023). Moreover, whey disposal, a crucial nutrient reservoir, leads to potential energy and resource waste, attracting attention from environmentalists and technologists (Kaur *et al.,* 2023, Kumar *et al*., 2018). Although marginally utilized as animal feed, fertilizer and protein supplement, extensive processing and treatment costs have led to its underutilization and subsequent accumulation as effluents. Despite being biodegradable, whey discharges are more polluting than domestic wastewater. Lactose, a key component of whey, has been known to encourage the growth of sewage fungus. The high nitrogen and phosphorus contents enhance eutrophication, proving lethal to aquatic life (Zandona *et al.,* 2021). Reusing and recycling whey therefore warrant renewed efforts to meet the 2030 sustainable development goals (Pugliese *et al*., 2022).

Milk whey is a good source of several nutrients and has been documented to have potential benefits. About 10% of the total dry solids in whey are made up of proteins including β-lactoglobulin (48-58%), alpha-lactalbumin (13-19%), bovine serum albumin (6%), lactoferrin (1-2%) and immunoglobulin. These constituents have been associated with high protein efficiency ratios and antioxidant properties, impacting viral infections, hypertension, cancer and hyperlipidemia (Krishnaprabha *et al.,* 2024; Tsermoula *et al.,* 2021). Whey is also a good source of carbohydrates, minerals and vitamins, with a low fat content, making it ideal

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for fat-restricted foods and supplements (Amirani *et al.,* 2020). However, most whey remains unutilized due to high costs for transformation and isolation, including biotechnological techniques (Kandasamy *et al.,* 2021).

Functional foods and beverages are increasingly popular due to their health benefits. However, commercially available products are expensive, making it crucial to develop affordable alternatives (Damián *et al.,* 2022). The focus on achieving the Sustainable Development Goals has led to a shift in whey's role as a raw material for new products along with alternative variations of traditional food items (Saha *et al.,* 2017, Zandona *et al.,* 2021). Whole whey can be used as a value-added ingredient in food items, providing an ecological and sustainable approach. This low-cost, underutilized by-product can be affordable for people of all economic backgrounds. Therefore, the present study focused on the development of functional and nutritious food products (beverage and thepla) as modified versions of non-functional traditional items using whey generated as a by-product in the dairy industry. This approach would address the issues regarding the disposal of dairy waste as well as utilization for catering to the nutritional and health-enhancing requirements of the population through economical and ready-to-consume options.

MATERIALS AND METHODS

The study was conducted for a year (2022-2023) at the laboratories of J.D. Birla Institute, Kolkata, West Bengal, India. Whey samples (n=6) from cottage cheese-based sweet manufacturing units at Hindmotor, West Bengal, India (22.68°N Latitude, 88.34°E Longitude), where most of the generated whey is discarded as effluent, were used to create functional, nutritious beverages and thepla, with additional ingredients for cost-effective and easy availability.

Development of whey-based functional food products and sensory acceptance studies

The study developed three variations each of whey-based beverages (VBA, VBB and VBC) and thepla (VTA, VTB and VTC) *via* a combination of whey with ingredients mentioned in Table 1. These variations were standardized for portion size and sensory characteristics and sensory evaluation was conducted by 100 panel members aged 19-25 years through a 9-point hedonic scale method (Pal *et al.,* 2024). The most accepted products were then analyzed for their nutritional and functional parameters.

Determination of nutrients and anti-nutrients

Nutrient analysis was performed through the Association of Analytical Chemists (AOAC) recommended procedures (Mc Cleary, 2023). Thermo-gravimetric, muffle-furnace incineration, anthrone, kjeldahl, folch, acid-alkali and enzymatic digestion and benedict's method were used for estimation of the product's moisture, ash, total carbohydrate, protein, fat, dietary fibre and lactose content respectively. OCPC (o-cresolpthaleincomplexone), calmagnite, molyddate-UV, β-galactosidase activity, turbidometric assay and ferrozine methods were used for spectrophometry-dependent estimations of calcium, magnesium, phosphorus, sodium, potassium and iron, correspondingly through biochemical kits (Coral Clinical System, India) followed by measurement of optical density (O.D.) at the recommended wavelengths in a spectrophotometer (Hitachi U2910, India). Ascorbic acid and β-carotene content were measured through iodometric-redox titration and colorimetric method at 453 nm, respectively. Anti-nutrients including tannins, oxalates and phytates were determined *via* potassium permanganate-based redox titration using the indigo carmine indicator and EDTA-dependent titration (Chen and Xu, 2023).

Analysis of phytochemicals

The study measured phytochemicals in developed products, including alkaloids, flavonoids and total phenols. Total phenols were estimated by Folin Ciocalteau's phenol reagent using UV-VIS spectrophotometer, while flavonoid concentrations were determined using aluminum chloride. Total alkaloids were calculated using titrimetric techniques. Alkaloids were solubilized with 0.1 N HCl and titrated against 0.1 N NaOH. Total alkaloid content was estimated by considering 1 mL (0.1 N) of NaOH to be equal to 0.0162 grams of alkaloid (Mahalle and Gupta, 2021).

Table 1: Variants of whey-based value-added products.

Ingredients (ml/100 ml)	Beverage				Thepla				
	Basic (BB)	Variation A (VBA)	Variation B (VBB)	Variation C (VBC)	Ingredients (g/100 g)	Basic (BT)	Variation A (VTA)	Variation B (VTB)	Variation C (VTC)
Water	88.0	40.0	28.0	24.0	Water	40.0	16.0	12.0	4.0
Whey	0.0	48.0	52.0	56.0	Whey	0.0	24.0	27.0	32.0
Ginger juice	7.6	7.6	7.6	7.6	Sorghum flour	46.0	46.0	46.0	46.0
Salt	0.4	0.4	0.4	0.4	Salt	3.0	3.0	3.0	3.0
Lemon juice	4.0	4.0	4.0	4.0	Vegetable oil	10.0	10.0	10.0	10.0
Molten Jaggery	0.0	0.0	8.0	0.0	Turmeric	1.0	1.0	1.0	1.0
Guava juice	0.0	0.0	0.0	8.0	Chilli powder	0.0	0.0	1.0	2.0
					Carom seeds	0.0	0.0	0.0	2.0

Determination of free radical scavenging capacity and prebiotic potential

The free radical scavenging potential of food products was determined using the 2,2-diphenylpicrylhydrazyl (DPPH) method. The absorbance of the samples was measured at 517 nm using a UV-visible spectrophotometer. *In vitro* prebiotic properties were evaluated using De Man Rogosa Sharpe (MRS) broth, which contained *Lactobacillus rhamnosus* GG and 2% product extract. The growth of *Lactobacillus* was positively correlated with the prebiotic potential. *L. rhamnosus* in the MRS medium without any product extract was taken as the control (Pal *et al.,* 2024).

Analysis of shelf-life and cost

Utilizing the microbiological spread plate approach, shelf life was examined on nutrient agar. Measured quantities of the sample extracts were plated onto the pre-solidified agar plates that were pre-sterilized by autoclaving (121°C, 15 psi), incubated at 37°C (24-48 hours) and estimated for growth. Results were represented as the mean of triplicate experiments as colony-forming units/mL (CFU/mL). The cost of the product was estimated by using the formula recommended by Rachman *et al*. (2020).

In vitro gastrointestinal stability

A gastric juice simulation was created using pepsin, glucose, NaCl, KCl, $CaCl_2$ and KH_2P0_4 in distilled water. A human intestinal digestion environment was created using NaCl, $CaCl_2$, $MgCl_2$, trypsin, α-chymotrypsin and pancreatic lipase. Membrane filtration (0.2 µm) was used to sterilize the intestinal and gastric digesting fluids. The *in vitro* gastrointestinal stability was determined by subjecting 5 mL of the control or sample solutions subsequently to the above solutions for 60 minutes followed by assessment of the desired antioxidant and prebiotic potentials through methods listed above (Pal and Bhowal, 2023).

Data analysis and statistical evaluation

Using microsoft office excel (version 15.0) software, the data was calculated and assessed. All results were given as Mean ± SEM on N≥3 experiments with p scores calculated using t-test for significance. $p \leq 0.0001$=***; $p \leq 0.005$=** and $p \leq 0.001$=* were considered significant with Confidence Interval (CI) = 95%.

RESULTS AND DISCUSSION

Whey was used to create beverage and thepla products as value-added functional alternatives to conventional snacks, focusing on popularity, economic cost and consumption patterns in the Indian population. A beverage, being liquid is easy to consume by all age groups, whereas, thepla is portable and craved by many. The products were created as improved nutritional and functional snack alternatives, while incorporating whey to minimize disposal and create value-added items.

Sensory acceptance of the whey-based value-added products

The developed products were assayed for their sensory properties owing to the importance of the former in determining customer acceptability. Results revealed that the variation C of both beverage (VBC) and thepla (VTC) displayed the greatest organoleptic acceptability as observed by average ratings of 8.2 and 8.8 compared to their respective basic products (5.9 and 6.1 for BB and BT) amongst the three developed variants, indicating that the panel members highly valued their appearance, texture, taste and consistency (Table 2). Optimum amounts of whey water in combination with the other ingredients may be responsible for the observed appeal of the products. Notably, the present study utilized sorghum instead of wheat in the thepla, thereby resulting in a gluten-free product that may be consumed even by patients suffering from gluten intolerance.

Nutrients, anti-nutrients and phytochemicals in the whey-based value-added products

Since VBC and VTC were the most accepted products based on sensory evaluation; these were thereafter analyzed for the content of important nutrients, anti-nutrients and phytochemicals to predict the nutritional and health benefits that may be obtained through their use. The moisture content, though higher in the beverage (VBC) than the thepla (VTC) was found to be lower than their respective basic recipes indicating an improved shelf-life versus the latter (Table 3). VBC and VTC also depicted a higher ash percentage with respect to BB and BT, indicating a greater presence of minerals. As denoted in Table 3, the

Table 2: Sensory analysis of the whey-based value-added products.

Parameters	Beverage				Thepla			
	Basic (BB)	Variation A (VBA)	Variation B (VBB)	Variation C (VBC)	Basic (BT)	Variation A (VTA)	Variation B (VTB)	Variation C (VTC)
Appearance	6.1±0.11	6.4±0.16*	6.9±0.19*	8.3±0.09**	6.2±0.19	6.6±0.12*	7.5±0.16*	8.9±0.17*
Colour	6.2±0.21	6.3±0.18*	6.8±0.17*	8.2±0.08**	6.1±0.17	6.5±0.10*	7.4±0.14*	8.8±0.16*
Taste	5.8±0.17	6.2±0.21*	6.6±0.21*	8.3±0.06**	6.1±0.10	6.3±0.09*	7.1±0.12*	8.9±0.08**
Texture	5.6±0.15	6.1±0.12*	6.4±0.18*	8.1±0.04**	5.9±0.14	6.3±0.12*	7.1±0.07**	8.8±0.10**
Odour	5.8±0.18	6.3±0.15*	6.6±0.19*	7.9±0.12*	5.9±0.12	6.5±0.13*	7.3±0.08**	8.9±0.06**
Overall ranking	5.9±0.18	6.3±0.16*	6.7±0.14*	8.2±0.11*	6.1±0.13	6.5±0.10*	7.3±0.05**	8.8±0.09**

$p \leq 0.0001$, $p \leq 0.005$ and $p \leq 0.01$ expressed as ***, **, * at 95% confidence interval.

Table 3: Nutrients, anti-nutrients and phytochemicals in the whey-based value-added products.

Parameter	Beverage		Thepla	
	Basic (BB)	Variation C (VBC)	Basic (BT)	Variation C (VTC)
Moisture content (%)	25±0.1	18±0.01*	14±0.05	9±0.07*
Ash content (%)	0.32±0.01	1.48±0.01*	2.75±0.001	4.01±0.001*
Energy (Kcal)	6.3	39.6	93.8	118.6
Carbohydrate (g/100 g)	1.26±0.01	6.4±0.01***	3.4±0.001	5.8±0.001**
Protein (g/100 g)	0.012±0.1	0.49±0.1***	1.6±0.1	8.4±0.1**
Fat (g/100 g)	0.00	0.1±0.001	6.01±0.05	7.02±0.07*
Dietary fibre (%)	0.00	20.12±2.04	8.04±1.02	18.32±1.65**
Lactose (g/100 g)	0.00	1.6±0.001	0.00	1.4±0.002
Calcium (mg/dL)	0.00	41.8±0.03	7.8±0.01	19.8±0.01***
Phosphorus (mg/dL)	40.2±0.12	47.4±0.03*	120±2.14	130±2.0 2**
Magnesium (meq/100 g)	0.00	14.4±0.01	0.00	2.5±0.05
Sodium (%)	44.26±1.06	46.37±1.19	75.17±0.03	77.28±0.01**
Potassium (mmol/L)	180±2.02	190±3.05**	150±2.11	160±1.12**
Iron (µg/dL)	4.5±0.01	7±0.01*	15.8±0.001	23.4±0.05*
β carotene (mol/L)	0	8±0.003	7.6±0.001	8.88±0.01*
Vitamin C (mg/ml)	128.5±0.001	180±0.001**	105±0.001	196±0.001**
Tannins (mg/ml)	0.0	0.0	0.03	0.01
Oxalates (mg/ml)	<0.01	<0.01	0.04	0.02
Phytates (mg/ml)	<0.01	<0.01	<0.01	<0.01
Alkaloids (mg/mL)	0.8±0.01	1.44±0.01*	0.22±0.01	0.34±0.01**
Flavanoids (µg/dL)	336.97±0.001	610.44±0.001***	165.33±0.01	230.07±0.01*
Polyphenols (mg/dL)	451.6±0.001	956.4±0.001***	824±0.001	940±0.001*

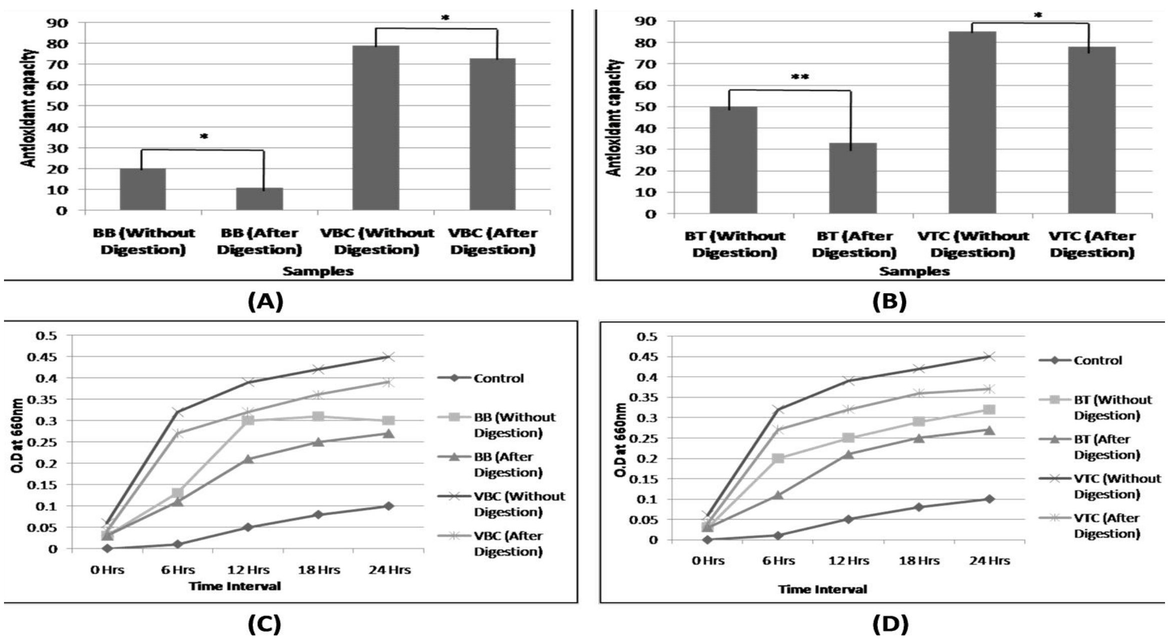
$p \leq 0.0001$, $p \leq 0.005$ and $p \leq 0.01$ expressed as ***, **, * at 95% confidence interval.

Table 4: Shelf life and cost of the whey-based value-added products.

Time (days)	Beverage				Thepla			
	Basic (BB)		Variation C (VBC)		Basic (BT)		Variation C (VTC)	
	30°C	4°C	30°C	4°C	30°C	4°C	30°C	4°C
0	0	0	0	0	0	0	0	0
7	13±2	7±2	11±1	<5	<5	<5	<5	<5
14	80±5	10±1	40±3*	15±2	45±7	13±5	7±1	<5
21	150±10	45±3	100±6*	25±2*	80±5	35±6	30±2	10±1*
Cost/100 g (Rupees)	4.0		4.5		8.5		10.0	

whey-based products displayed a greater content of carbohydrates, proteins, fibre and lactose mostly accounted by the proportion of whey present in them. The fat content though marginally higher in whey-based products, mainly VTC was within moderate limits. Micronutrient analysis found a significantly higher concentration of important minerals including calcium, phosphorus, magnesium, sodium, potassium and iron in both VBC (41.8 mg/dL, 47.4 mg/dL, 14.4 meq/100 g, 46.37%, 190 mmol/L and 7 µg/dL) and VTC (19.8 mg/dL, 130 mg/dL, 2.5 meq/100 g, 77.28%, 160 mmol/L and 23.4 µg/dL) as well as heightened vitamin C and β carotene versus BB and BT, which may be ascribed to the inclusion of whey in an optimum combination with other ingredients. Notably, the products were observed to contain low levels of anti-nutrients (phytates, tannins and oxalates), indicating limited restriction of the absorption of the above nutrients post-human intake (Table 3).

Variation C of both the whey-based beverage (VBC) and thepla (VTC) displayed an increased presence of phytochemicals versus their basic samples (Table 3). The higher content of alkaloids (1.44 mg/mL), flavonoids (610.44 µg/dL) and phenols (956.4 mg/dL) in VBC may be attributed to the presence of optimum amounts of ginger, lemon and guava extracts in addition to whey that have all been previously associated with a marked phytochemical profile. Additionally, the existence of turmeric, carom seeds and sorghum in VTC apart from significant amounts of



Antioxidant properties of whey-based beverages (A) and thepla (B). Prebiotic properties of whey-based beverages (C) and thepla (D).

Fig 1: Free radical scavenging and prebiotic potential of the products before and after *in-vitro* simulated gastrointestinal digestion.

whey may have resulted in an increased alkaloid (0.34 mg/mL), flavonoid (230.07 µg/dL) and polyphenol (940 mg/dL) content in the same versus BT. Consequently, VBC and VTC support a prominent supply of nutrients mainly, micronutrients and phytonutrients including polyphenols, flavonoids and alkaloids, which may not only satisfy a significant portion of recommended daily intake (RDI) but also offer significant protection against the progression of chronic diseases and improve overall health.

Free radical scavenging and prebiotic potential of the products

Given the presence of significant amounts of phytochemicals such as alkaloids, polyphenols and flavonoids as well as fibres in VBC and VTC that have been previously noted for antioxidant and gut enhancing abilities, the samples were tested for free radical scavenging and prebiotic capacity. As shown in Fig 1, VBC and VTC scavenged DPPH radicals at a higher rate (79.12% and 82%) than BB (20.26%) and BT (50%), indicating a significant antioxidant potential in the former. Moreover, both VBC (O.D600 nm 0.04 to 0.39) and VTC (O.D600 nm 0.06 to 0.45) demonstrated an enhanced prebiotic ability showcased by their propensity to elevate the growth of probiotic *Lactobacillus rhamnosus* GG evident by a longer logarithmic phase versus control (Fig 1C and 1D). Digestion of food in the human gastrointestinal tract has been reported to affect their bioavailability and functional quality. Nonetheless, both VBC and VTC were observed to display sufficient radical scavenging and prebiotic properties even beyond *in vitro* gastrointestinal digestion (Fig 1). As a result, whey-containing beverages (VBC) and thepla (VTC) may qualify as preferred functional food options versus their traditional counterparts due to their nutritional and functional properties.

Shelf life and cost of the products

The study found minimal microbial growth after 7 days of storage, with moderate growth at 14 days which increased rapidly beyond 21 days. Thepla had a greater shelf life and lower microbial colonies when stored at 4°C compared to 30°C (Table 4). Therefore, the results indicate that though the thepla (VTC) could be consumed after comparatively longer storage periods, it would be safe to consume the beverage (VBC) within a limited duration post preparation, especially by 7 days when kept at 4°C, to retain the desired quality and safety aspects. Note worthy, the shelf life may be increased through the addition of preservatives. Whey-containing beverage (Rupees 4.5/100 g) and thepla (Rupees 10.0/100 g) were affordable, accessible across various socio-economic strata and were more nutritious and functional than conventional options. Their low cost makes them attractive alternatives to commercially available ready-to-eat and functional food products.

CONCLUSION

The study aimed to develop low-cost, functional and value-added food products (beverage and thepla) using whey, a dairy by-product often discarded as effluent. The whey-containing beverages and thepla (VBC and VTC) were found to be the most preferred products due to their sensory attributes and high concentration of macronutrients, including carbohydrates and proteins. They also contained a significant concentration of micronutrients, with low anti-nutrients. Additionally, the products also contained bioactive components, including phytochemicals, free radical scavenging and prebiotic potential that were sufficiently retained after a simulated gastrointestinal digestion. Both the products were found to be low in cost and had an improved shelf-life when stored at a lower temperature. Hence, whey-containing beverages and thepla could be sustainable alternatives for better health, reducing issues associated with whey accumulation.

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Conflict of interest

The authors declare no conflict of interest.

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