



Development of Whey Choco Ball and Its Characterization

Kanika Mitra^{1*}, Md. Anwar Hossain² and Syeda Farhana Najnin¹

¹Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka-1205

²Planning and Development Division (P&D), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka-1205

Abstract

Dumping whey into sewage caused a substantial loss of nutrients, highlighting the failure to effectively utilize this valuable resource. The goal of this work was to use response surface modeling to determine the ideal conditions for alcalase-acylation of Whey Protein Concentrate (WPC) in order to generate protein hydrolysates with antioxidant and amino acid properties. In this work, we developed a whey-based ready-to-eat product called Whey Choco Ball (WCB) using the extracted WPC. Nutritional content, sensory assessment, and textural evaluation in addition to its microbiological examination and shelf life of newly developed product were assessed. The WCB formulation was high in protein, low in fat, and high in mineral content. Additionally, compared to locally available Brands 1, 2, and 3, WCB appears to have less fat (~10.0%), more protein (~10%), less total sugar (~5%) suggesting the product as one of the best sources of protein, and minerals. The product received a score of 9 for color ("liked extremely"), while taste, flavor, mouth feel, texture, and overall acceptance each scored 8 ("liked very much"), reflecting a generally positive sensory evaluation. From a microbiological viewpoint, WCB was considered appropriate up to 15 days. The sensory evaluation panel marked the product at a satisfactory level. Microbial analysis also showed the acceptable validity of that product. The texture analyzer showed different textural parameters that will be helpful for the consumer's approval. The analysis indicates that WCB can be recognized as a protein-rich food item due to its effective utilization of whey. The incorporation of WPC in WCB contributes significantly to its protein content, showcasing the product's ability to harness the nutritional benefits of this valuable by-product.

Received: 24/03/2024
Revised: 01/06/2025
Accepted: 18/06/2025

Keywords: WPC, Hydrolysis, Proximate, Sensory, Microbial Analysis, Texture.

Introduction

Whey is the primary by-product of the dairy industry, produced after milk coagulates during the process of making cheese and casein (Kareb and Aider, 2019). Whey proteins (WP) account for approximately 15 to 20% of all milk proteins (Camargo *et al.*, 2018; Patel, 2015). These proteins include α -lactalbumin (12% to 25%) and β -lactoglobulin (35% to 65%), along with smaller amounts of immunoglobulins (8%), albumin (5%), and lactoferrin (1%). It provides a lot of cysteine and branched chain

amino acids including leucine, isoleucine, and valine (Maqsood *et al.*, 2019; Patel, 2015). In this instance, 8 to 9 liters (L) of whey are produced from the 10 liters of milk used to make the 1 to 2 kg of cheese. Globally whey production exceeds 160 million tons annually, with a 2% yearly growth rate (Kareb and Aider, 2019). Therefore, whey presents a challenge to the dairy sector and can result in serious environmental issues if not treated properly. The biological oxygen demand (BOD), which is predicted to be >35,000 ppm, and chemical oxygen

*Corresponding author e-mail: mitra.kanika@gmail.com

demand (COD), which is >60,000 ppm, are both extremely high in whey in addition to its enormous volume (Kareb *et al.*, 2019). Whey was previously thought to be an environmental burden with limited use as an animal feed supplement (Mehra *et al.*, 2021). However, because of the large organic load (27 to 60 g/L) and high oxygen demand (50 to 102 g/L) for biodegradation, the residues were thrown in streams or left on wastelands, which had a detrimental effect on aquatic life and soil quality and productivity (Brandelli *et al.*, 2015; Smithers, 2015; Yadav *et al.*, 2015).

Whey disposal needs to be addressed in order to reduce environmental harm in addition to the loss of important nutrients and energy. Instead of discarding whey, it is crucial to concentrate on low-cost, long-term strategies for utilizing its potential nutrients and energy to produce new, useful goods (Zandona *et al.*, 2021). Numerous studies have been undertaken to explore viable and eco-friendly options for utilizing whey, rather than simply disposing of it in fields or other unsustainable ways (Valta *et al.*, 2017). The efficient use and conversion of liquid whey into a wide range of beneficial human food supplements has increased noticeably as a result of the imperative disposal (Mehra *et al.*, 2021).

Whey protein concentrate (WPC) has been employed as a functional component in numerous food applications because of its great functional, biological, and highly nutritious qualities (Mishra *et al.*, 2022). Whey-derived products are a great source of sulfur-linked amino acids, which are needed to execute a variety of metabolic activities, as well as a rich source of vitamins and minerals, highly digestible proteins, and important amino acids (Singh, 2016; Macwan *et al.*, 2016; Papademas and Kotsaki, 2019). Whey products, which are typically in dry form, are widely used as food ingredients in a variety of food products (such as confections, baked goods, health supplements, and sports nutrition). They have health beneficial nutritional (such as a high content of essential amino acids) and biological (such as antimicrobial, anticarcinogenic, and immunomodulatory activities) properties and also have functional (such as a gelation, foaming, and emulsifying agent) activities (Helkar *et al.*, 2016).

Whey protein isolates (WPI), whey protein hydrolysate (WPH), WPC, whey protein powder (WPP), and other metabolites facilitate the production of a wide range of valuable whey-derived products (Krunic *et al.*, 2019; Sharma, 2019; Yadav *et al.*, 2015). Whey protein powder, is produced from the whey that is obtained during the cheese-making process. The whey undergoes a series of steps, including clarification to remove impurities, heat-treatment to denature proteins, and drying to obtain a fine powder. This process concentrates the WP, resulting in a versatile and easily usable powder form. The WPP is widely utilized in the food and beverage industry, as well as in dietary supplements and sports nutrition products, due to its high protein content and beneficial nutritional properties.

The WPP can be combined with other food-grade compounds to increase or expand their technological functionality and/or biological activities (Setiowati *et al.*, 2020). The addition of WP improves the texture and sensory appeal of the dish. To enhance the quality and nutritional content of food like baked products, energy snacks, yogurt, and beverages (Baba *et al.*, 2021). Additionally, the WP-derived peptides have been associated with a number of biological activities that could be advantageous, including antihypertensive, antioxidant, and cholesterol-lowering effects, making them appropriate as constituents in foods that promote health (Baba *et al.*, 2021; Minj and Anand, 2020). In the study of (Mishra *et al.*, 2022), a goat milk rasogolla of acceptable grade was made using WPC as a functional ingredient. It has recently been revealed in that cheese whey is used in the manufacture of organic acids, including bioethanol and galactic acid (Zhou *et al.*, 2019). Abella *et al.* (2016) reported that *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures were used to ferment acid whey (3.32% lactose) in order to create a whey-based sports drink. According to the findings of (Ahmed *et al.*, 2023) whey can be used with fruits and vegetables to create whey-based beverages. Thus, this study aimed to develop a whey-based food product. For the first time in Bangladesh, WPC is used in a ready to eat food product- Whey Choco Ball (WCB). The present study also evaluated the

proximate analysis of this product and compare with other brands available in market. This study also examined the microbiological, sensory and other nutritional characteristics of prepared WCB, soon after manufacture and during the storage period in the refrigerator ($4\pm1^{\circ}\text{C}$) until 15 days.

Materials and Methods

Chemical reagents

Folin-Ciocalteu reagent, DPPH, ferric chloride, ascorbic acid, and tannic acid were purchased from Sigma Co. (St. Louis, MO, USA). Methanol, hydrochloric acid, sodium hydroxide, aluminum chloride, sodium carbonate was purchased from Merck, Darmstadt, Germany. Acetic acid, and ethanol was also obtained from Merck (Darmstadt, Germany). All the chemicals used in this study were of analytical grade.

Standardizing and preparing milk

Figure 1 shows the process steps for manufacturing cheese and preparing milk schematically. Pasteurized whole milk was used to standardize milk, aiming for nominal protein levels of 4, 5, or 6% while keeping a protein-to-fat ratio of roughly 1.0.

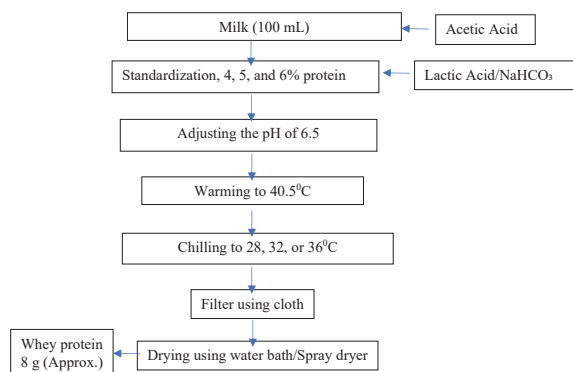


Figure 1. Schematic diagram of making curd and preparing milk (Panthi *et al.*, 2019)

Design of the WPC hydrolysis

The best conditions for hydrolysis are advised by the enzyme's manufacturer, although only ranges rather than exact values are given. The optimal circumstances are arbitrary and depend on numerous

variables (purpose of study, sample nature, and equipment availability). It is essential to carefully consider the ideal conditions on a case-by-case basis when conducting optimization research on WPC hydrolysis. The WPC hydrolysis parameters were optimized using a central composite design, in which each parameter has three levels coded as 1, 0, and +1, based on three independent variables: temperature (C , x_1), pH (x_2), and time (h , x_4). Due to its superior accuracy and suitability for the current investigation, which manipulates three independent variables and four dependent variables, CCD was chosen over an alternative Box-Behnken design (responses) (Hussein *et al.*, 2020).

Table 1. Hydrolysis process parameters have been coded, in accordance with the central composite design

Parameter	−1	0	+1
x_1 : pH	6	6.5	7
x_2 : Time (h)	4	5.5	7
x_3 : Temperature ($^{\circ}\text{C}$)	35	40.5	46

In a nutshell, Alcalase and WPC were combined in 100 mL of borate buffer at various temperatures (35 to 46°C), pH levels (6 to 7), and periods (4 to 7 hours), and then incubated in a water bath shaker with 150 rpm of agitation. Alcalase was then inactivated following hydrolysis by immersing the mixture in water that was boiling (100°C) for 10 minutes. After cooling, $10,000$ rpm centrifugation was completed for 15 min. Prior to the examination of protein, biological activities including amino acid, and DPPH, the supernatant was kept at or below -20°C .

To assess the regression coefficients and statistical significance of the model terms and to fit the expected mathematical models with the experimental data, analysis of variance (ANOVA) and the regression equation were used for data analysis. The obtained response variables (Alexopoulos 2010; Myers *et al.*, 1995) were used to generate the multiple regression coefficients for predicting quadratic and linear polynomial models using the least-squares method. The general polynomial model for determining the response (Equation 1) is as follows:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

Where, y stands for the response, β_0 denotes the offset term for the design, β_1 , β_2 and β_3 stand for the regression coefficients defining the linear effect terms, β_{11} , β_{22} and β_{33} stand for the quadratic effects, β_{12} , β_{13} and β_{23} stands the interaction effects and x_1 , x_2 , and x_3 stand for independent components in this model (Hussein *et al.*, 2020).

Raw materials

Whey protein concentrate is a necessary ingredient to prepare WCB. Oats, almonds, peanut butter, honey, cinnamon powder, chocolates, and peanut butter were among the other items purchased from a local market in Dhaka, Bangladesh

Formulation of whey choco ball

The WCB was made in accordance with the flowchart shown in Figure 2. The oats and almonds were roasted for 5 minutes, then blended using a blender. The other dry ingredients – WPP, choco chips, and cinnamon powder – were combined with the blended oats and almonds. Then, in order to prepare a smooth batter, honey and peanut butter were mixed together thoroughly. A small ball shape was made with that and it was placed in the refrigerator for 15 minutes. After that, they were covered with liquid chocolate and presented in small paper cups. Every choco ball has a weight of 15 to 17 g.

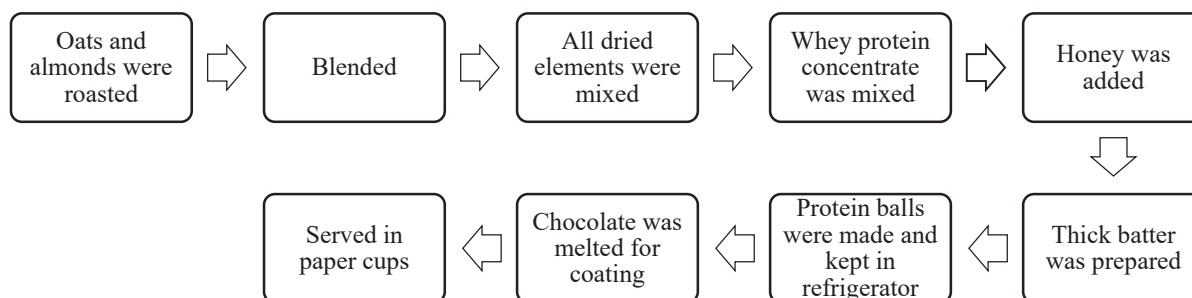


Figure 2. Schematic diagram of the Whey Choco Ball production process

Proximate analysis

A variety of procedures were employed to observe the nutritional profile of WCB. A manual of laboratory techniques was used to determine the moisture content (AOAC, 1995). The amount of ash was calculated using the procedure outlined by Kric and Sawyer. AOAC (1995) reported that organic Soxhlet extraction procedures were used to assess the total fat content. The Kjeldahl technique AOAC (2004) was used to estimate the total protein. The AOAC (1995) determined crude fiber. An known analytical method for quantifying reducing sugars, the Lane and Eynon titration method (Dunsmore *et al.*, 1980), was used to determine the sugar concentration. By titrating the sample against a standardized Fehling's solution, this technique made it possible to measure the sample's sugar concentration precisely.

Minerals content

As outlined in the Manual of Laboratory Techniques (AOAC, 2005), the mineral analyses for iron, sodium, calcium, phosphorus, potassium, magnesium, manganese, and zinc were carried out. A muffle furnace was used to ash a weighted sample for 6 hours at 600°C. After ashing, a stock solution was prepared using 6.0 M HCl, and the mineral concentration was measured with atomic absorption spectrometry (Thermo Scientific, ICE 3000 series spectrophotometer).

Sensory Analysis

An assessment of the WCB samples was carried out by eleven panelists, who were aged 25 to 45. Taste, mouthfeel, color, texture, flavor, and general acceptability were among the sensory attributes that were assessed. A 9-point hedonic scale was

employed to assess the senses (Wichchukit and O'Mahony, 2015). Every attribute's intensity was measured using a 9-point hedonic scale. Each attribute was scored based on its intensity scaled on a 9-point hedonic scale (1 = disliked extremely, 2 = disliked very much, 3 = disliked moderately, 4 = disliked slightly, 5 = neither liked or disliked, 6 = liked slightly, 7 = like moderately, 8 = liked very much, 9 = liked very extremely).

Microbial analysis

Following the guidelines provided by the Bacteriological Analytical Manual, a microbial quality analysis was conducted on this WCB (Maturin, 2001; Tournas *et al.*, 2001). A 25 g sample was carefully weighed using aseptic techniques and appropriately enriched using 250 mL of sterile Buffer Peptone Water (BPW, Hi-Media, India). The sample was diluted in 9 mL BNPW to obtain an aliquot of 1 mL, which was then placed on plate count agar (Hi-Media, India) and incubated at 37°C for 24 hours in order to determine the total viable count (TVC). Following the period of incubation, the number of colonies was counted and recorded as log CFU/g. EC broth, Brilliant green lactose bile broth (Hi-Media, India), and Lauryl sulfate tryptose broth (Hi-Media, India) were used in the most likely number (MPN) test for coliforms and *E. coli* using a screw-cap tube. Based on the number of tubes exhibiting gas generation, the most likely number of coliforms per milliliter or gram of material was computed. A table for the determination of most probable numbers was used according to (Feng *et al.*, 2002). Dichloran Rose Bengal Chloramphenicol (DRBC) and Dichloran 18% Glycerol (DG18) agar media were used in the plating procedure to

detect the counts of yeast and mold. The plates were incubated for five to seven days at 37°C. The CFU counts were made and reported as log CFU/g. Following the (Gdoura-Ben Amor *et al.*, 2018), the prevalence of *Bacillus sp.* in the cake sample was established. In accordance with Siala *et al.* (2017), *Salmonella sp.* was determined. Three duplicates of each analysis were performed.

Texture analysis

Using the 3-point bend fixture test method and a texture analyzer (FRTS, Texture Analyzer, IMADA), 10 repetitions of the texture profiles were evaluated to determine the texture qualities. The texture analyzer measured the firmness, adhesiveness, cohesion, springiness, gumminess and other parameters of the WCB.

Results and Discussion

Process optimization for the preparation of whey protein

The response surface approach was used to optimize the whey protein preparation procedure. Lactic acid and sodium bicarbonate were used to hydrolyze whey protein produced from pasteurized cow milk.

The three individual parameters were optimized by the small central composite design for a total of 15 runs, including 5 replicates. Table 2 lists the findings of 15 trials. For each treatment, the mean pertaining to triplicate measurements was considered as responses for yields (% y_1), protein (% y_2), DPPH inhibition (% y_3), and amino acids (% y_4). Randomization of experimental runs was performed to mitigate the impacts of unexpected variability on actual responses.

Table 2. Experimental design showing different hydrolysis conditions for the optimization of yield of whey protein concentrate in central composite design

No.	x_1	x_2	x_3	% Yield (y_1)	% Protein (y_2)	% DPPH inhibition (y_3)	(mg/mL) Amino Acid (y_4)
1	7	7	35	7.98	6.15	22.47	6.80
2	6	4	35	7.54	6.81	25.53	3.68
3	7	4	46	7.22	6.64	14.39	2.567
4	6.5	3.38	40.5	8.44	7.68	16.66	6.87

No.	x_1	x_2	x_3	% Yield (y_1)	% Protein (y_2)	% DPPH inhibition (y_3)	(mg/mL) Amino Acid (y_4)
5	6.5	5.5	40.5	8.21	8.79	16.64	5.19
6	6.5	5.5	40.5	8.21	8.79	16.64	5.19
7	6.5	5.5	32.72	7.30	6.88	6.85	3.47
8	6.5	7.62	40.5	8.22	6.81	3.05	5.54
9	5.79	5.5	40.5	7.38	8.95	34.84	1.85
10	6.5	5.5	48.28	8.02	8.67	31.95	5.19
11	6.5	5.5	40.5	8.21	8.79	16.64	5.19
12	7.21	5.5	40.5	7.50	6.48	19.25	3.99
13	6	7	46	7.81	8.19	18.92	4.08
14	6.5	5.5	40.5	8.21	8.79	16.64	5.19
15	6.5	5.5	40.5	8.21	8.79	16.64	5.19

Remark: Independent variables: x_1 = pH; x_2 = hydrolysis time; x_3 = Temperature.

The initial regression equations contain all linear effects, significant or not (reduced model). On the other hand, for reduced polynomial regression involving yields (y_1), protein (y_2), DPPH inhibition (y_3), and amino acids (y_4), only significant terms were taken into account for square and interaction effects.

Numerical optimization

One of the simplest ways to proceed is to superimpose the two contour plots corresponding to y_1 , y_2 , y_3 and y_4 to inspect the region of the factor space that meets the requirements, and from there choose possible optimal values.

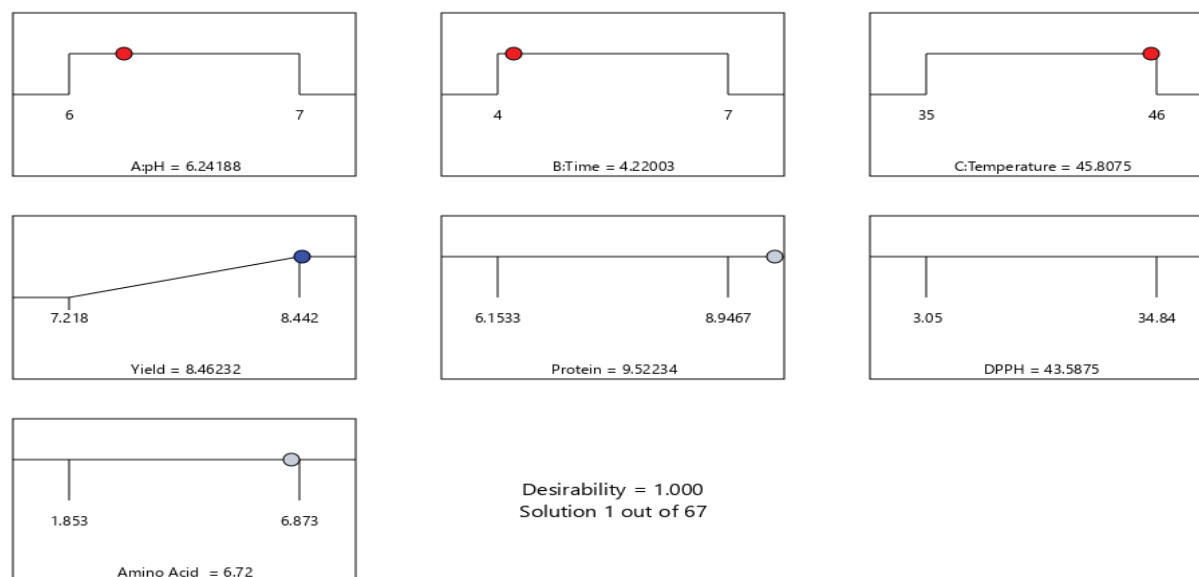


Figure 3. Numerical optimization of process variables

Optimized hydrolysis condition

Using Central Composite Design, an optimization process was carried out. The ideal estimated hydrolysis conditions for WPC are shown in Figure 3, which may theoretically result in the highest yields of protein, DPPH radical scavenging, and amino acid activities. The pH was projected to be 6.24, the hydrolysis time was predicted to be 4.22 hours, and the temperature was predicted to be 45.81°C. In order to validate the model, the predicted parameters were used in real experiments. The overall desirability (D-value) of 1.00 indicated a high level of confidence for the model to produce the responses as predicted. Additionally, the experimental values for yields (8.46), protein (9.52%), DPPH radical scavenging (43.59%), and

amino acid activity (6.72%) were the same as the corresponding values of the predictions.

Proximate analysis

Table 3 presents a comparison of the macronutrient composition of WCB with other brands that are currently available in the market. In terms of moisture, ash, fat, protein, and carbohydrate, there is a noticeable difference overall. The WCB offers less fat (~10.0%), less total sugar (~5%), and more protein (~10%) than brands (1, 2, and 3). Compared to brands, WCB has considerably more moisture and protein. In comparison with other brands, the protein content in WCB is approximately 10% higher, with Brand 3 having the lowest protein percentage.

Table 3. Comparative proximate analysis of developed WCB and other Brands (1, 2, and 3)

Parameters	WCB	Brand 1	Brand 2	Brand 3
Moisture (%±SD)	5.91±0.17 ^a	3.09±0.74 ^b	2.86±0.43 ^b	1.89±0.20 ^c
Ash (%±SD)	1.96±0.18 ^{ab}	1.42±0.39 ^b	1.22±0.14 ^b	3.19±1.59 ^a
Protein (%±SD)	17.27±1.83 ^a	8.37±0.98 ^b	8.79±0.48 ^b	5.08±2.51 ^c
Fat (%±SD)	27.47±1.25 ^d	43.41±1.16 ^a	36.83±0.80 ^b	33.75±0.32 ^c
Crude fiber (%±SD)	0.79±0.30 ^b	1.37±0.40 ^a	0.48±0.03 ^{bc}	0.04±0.06 ^c
Sugar (%±SD)	32.78±0.78 ^b	37±0.59 ^{ab}	35.83±3.30 ^{ab}	40.95±4.02 ^a
Carbohydrate (%±SD)	46.61±3.27 ^{bc}	42.33±0.87 ^c	49.82±0.94 ^b	56.06±4.46 ^a
Energy (%±SD)	502±0.01 ^a	593±1.04 ^c	565±2.12 ^b	548±5.83 ^c

Means with the same letter are not significantly different

According to previous (Mishra *et al.*, 2022), WPP products had a protein content that was about 10 to 12% higher than non-whey products. Our results are consistent with these findings. However, Table 3 clearly shows how WCB and other brands differ in terms of fat, total sugar, and carbohydrate amounts. The fat percentage in WCB is 27.47±1.25. In WCB, the fat percentage is considerably lower than that of the other brands, with Brand 1 exhibiting the highest level of fat. Table 2 additionally shown that Brand 3 has a larger percentage of total sugar than both WCB and (Brands 1 and 2). The effectiveness of WPC and its derivatives in the treatment and prevention

of a number of diseases has been documented in double-blind, placebo-controlled trials conducted on humans and animals in addition to the published meta-analysis (Mehra, 2021).

Mineral content

Figure 4 displays the minerals content of the newly prepared WCB. The mineral contents of WCB and Brands 1, 2, and 3 differ significantly. The WCB contains more sodium, more zinc, and less magnesium than Brands 1, 2, and 3. Furthermore, the only food that has a considerable amount of iron is WCB. Like iron, WCB has substantially

more sodium, copper, zinc, and manganese content than other brands. With regard to the content of calcium, potassium, and magnesium, this is not the case. Compared to WCB, Brand 1 has a higher concentration of magnesium, potassium, and calcium. According to earlier research, whey-

derived products are a great way to get vitamins and minerals (Singh and Geetanjali, 2016).

We may conclude that WCB has the potential to be an excellent supplier of certain minerals, such as iron, sodium, copper, manganese, and zinc, based on a comparison with Brands 1, 2, and 3.

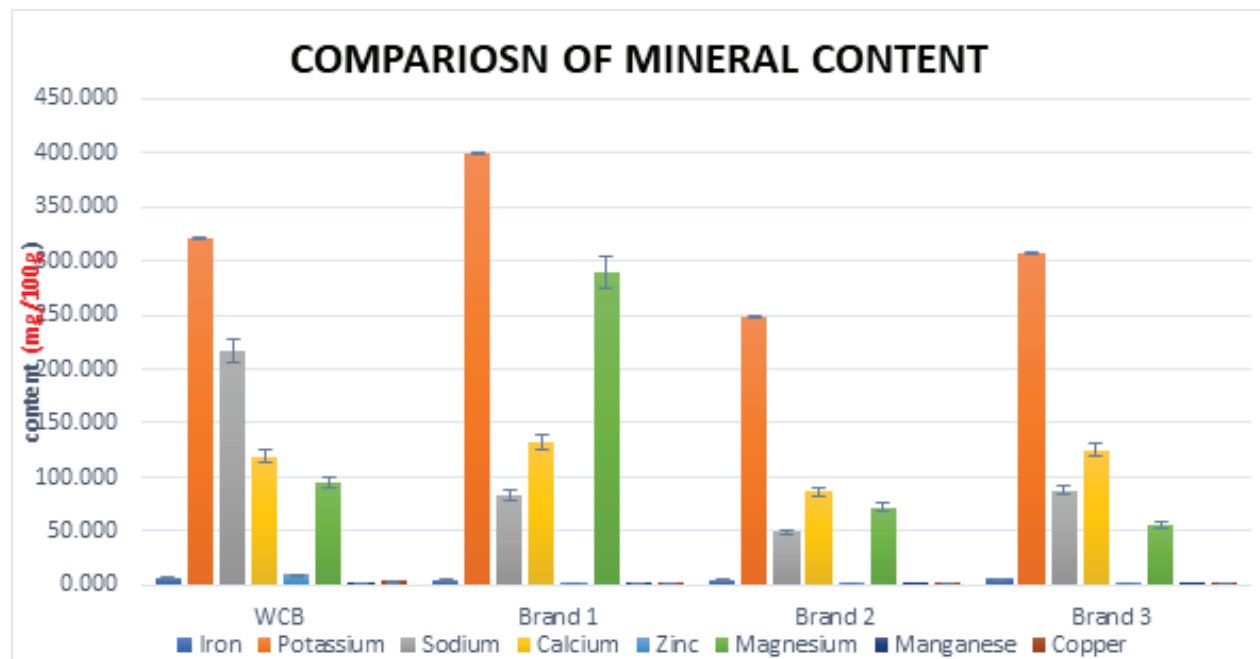


Figure 4. Comparison of mineral content of developed Whey Choco Ball and other Brands (1,2, and 3)

Sensory Evaluation

This study investigated the overall acceptability as well as the flavor, taste, color, and texture of WCB. A product's acceptance is mostly determined by its taste, which means that taste has the biggest influence on a product's commercial success. The product was rated highly in sensory evaluation, with color achieving a top score of 9, described as "liked extremely," showcasing its appealing visual quality. Additional attributes, including taste,

flavor, mouthfeel, and texture, all received scores of 8, indicating they were "liked very much" by the panel. This consistently high rating across multiple sensory aspects suggests that the product was well-received and met consumer expectations in terms of both appearance and overall sensory appeal. The findings indicate that WCB's sensory qualities and general acceptability are satisfactory. On the other hand, extensive sensory assessment is advised for future research. Table 4 shows the evaluation.

Table 4. Sensory evaluation of Whey Choco Ball

Quality Factors	Like extremely (9)	Like very much (8)	Like moderately (7)	Like slightly (6)	Neither like nor dislike (5)	Dislike slightly (4)	Dislike moderately (3)	Dislike very much (2)	Dislike extremely (1)
Color	✓								
Texture		✓							
Flavor		✓							
Mouth feel		✓							
Taste		✓							
Overall acceptance		✓							

Microbial Analysis

WHO guidelines from 1994 (WHO,1994) state that baked goods, such as cakes, bread, biscuits, and others should have a total viable bacterial count of no more than 2.0×10^5 cfu/g, no detectable *E. coli* or coliforms, and controlled levels of mold and

yeast. WCB sample testing revealed that the levels of mold and yeast matched WHO guidelines, while the total aerobic bacterial count remained within the allowed limits for up to 15 days. Furthermore, the WCB samples included no *Salmonella*, *Listeria*, or *Enterobacteriaceae* (Table 5).

Table 5. Microbial analysis of Whey Choco Ball on different time period

Sl No.	Parameters	Results (Preparation day)	Results (1 week later)	Results (15 days later)
1	TVC (Total Viable Count), cfu/g	5.7×10^2	8.9×10^2	9.2×10^2
2	Total Coliforms, MPN/g	<0.3**	<0.3**	<0.3**
3	<i>Escherichia coli</i> , MPN/g	<0.3**	<0.3**	<0.3**
4	Yeasts and Molds, cfu/g	<10*	<10*	<10*
5	<i>Salmonella</i> sp., cfu/g	Absent	Absent	Absent
6	<i>Staphylococcus</i> sp., cfu/g	<10*	<10*	<10*
7	<i>Listeria</i> sp.	Absent	Absent	Absent
8	<i>Enterobacteriaceae</i> sp.	Absent	Absent	Absent

*<10 indicate absence of test organisms in 1g of sample

**As per MPN (most probable number) chart, MPN <0.3 indicates absence of test organism in 1g

Textural analysis

Texture evaluation is considered to be critical for the consumer's approval of food as palatable and is a critical step in the development of new food products. The textural parameters viz., hardness, fracturability, stickiness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, probe diameter, threshold, and filtering range of WCB were presented in Table 6.

Customers value hardness above other textural attributes because it influences their perception of the quality and perceived value of things. The hardness value for WCB 3.240×10^5 . The value for fracturability, stickiness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, probe diameter, threshold, and filtering range found to be 7.925×10^4 , 9.230×10^3 , 0.1416, 3.9770, 0.1222, 4.589×10^4 , 5.608×10^3 , 20.000, 10 and 0.4, respectively.

Table 6. Textural analysis of Whey Choco Ball

	Texture Profile	Unit
Hardness	3.240×10^5	N/m ²
Fracturability	7.925×10^4	N/m ²
Stickiness	9.230×10^3	N/m ²
Cohesiveness	0.1416	
Adhesiveness	3.9770	J/m ³
Springiness	0.1222	
Gumminess	4.589×10^4	N/m ²
Chewiness	5.608×10^3	N/m ²
Probe Diameter	20.000	mm
Threshold	10	N
Filtering Range	0.4	N

N = Newton, mm = millimeter

In an innovative approach, whey protein powder was incorporated into a ready-to-eat food product named WCB, a pioneering initiative in Bangladesh. The study compares the nutritional profile of WCB with other commercially available brands (Brands 1, 2, and 3) in a comprehensive proximate analysis. Compared to the other group, WCB showed lower fat content (~10.0%) because of using peanut butter. Peanut butter contains about 50% fat (mostly unsaturated), while regular butter has around 81% fat, with a higher saturated fat content (Pickford *et al.*, 2022), lower total sugar content (~5%) as no extra sugar was added other than honey, and higher protein level (~10%) since using the Whey Protein Concentrate.

Furthermore, WCB exhibited the potential to serve as an excellent source of essential minerals, including iron, sodium, copper, manganese, and zinc, surpassing Brands 1, 2, and 3. Sensory evaluation and general acceptability assessments of WCB were favorable, indicating its potential appeal to consumers. Microbial analysis over a 15-day period confirmed the product's acceptable quality. Texture analysis revealed satisfactory hardness, fracturability, stickiness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, probe diameter, threshold, and filtering range of WCB.

Conclusions

The findings highlight WCB as a protein-rich food item, showcasing effective whey utilization. The incorporation of whey not only contributes significantly to the product's protein content but also emphasizes its potential to harness the nutritional benefits of this valuable dairy industry by-product. Whey Choco Ball emerges as a promising and innovative food product with a favorable nutritional profile and broad market potential.

Acknowledgments

This research was conducted by the financial support (SRG-221139, 2022–2023) from the Ministry of Science and Technology, Government of the People's Republic of Bangladesh.

Declaration

The authors declare that the research findings reported in this manuscript do not have any conflicting interest.

Authors' Contributions

KM conceptualized, resourced, supervise the experiments, reviewed and edited the manuscript. MAH analyzed and interpreted the data and SFN performed the experiments and drafted the manuscript.

References

- Abella M, Leano ML, Malig J, Martin G, Cruz CD and De Leon A 2016. Formulation of a sports drink from fermented whey. *CLSU International Journal of Science & Technology* **1**(1): 1–10.
- Ahmed T, Sabuz AA, Mohaldar A, Fardows HS, Inbaraj BS, Sharma M, Rana MR and Sridhar K 2023. Development of novel whey-mango based mixed beverage: effect of storage on physicochemical, microbiological, and sensory analysis. *Foods* **12**(2): 237.
- AOAC 2005. Official methods of analysis of the Association of Analytical Chemists International. *Official Methods: Gaithersburg, MD, USA*.
- Assembly UG 2015. UN General Assembly, Transforming Our World: The 2030 Agenda for Sustainable Development, 21 October 2015, A/RES/70/1.
- Alexopoulos EC. 2010. Introduction to multivariate regression analysis. *Hippokratia* **14**(Suppl 1): 23.
- Baba WN, McClements DJ and Maqsood S. 2021. Whey

- protein–polyphenol conjugates and complexes: Production, characterization, and applications. *Food Chemistry* **365**: 130455.
- Baba WN, Mudgil P, Kamal H, Kilari BP, Gan CY and Maqsood S 2021. Identification and characterization of novel α -amylase and α -glucosidase inhibitory peptides from camel whey proteins. *Journal of Dairy Science* **104**(2): 1364–1377.
- Brandelli A, Daroit DJ and Corrêa APF. 2015. Whey as a source of peptides with remarkable biological activities. *Food Research International* **73**: 149–161.
- Camargo LR, Silva LM, Komerowski MR, Kist TB, Rodrigues CE, Rios ADO, Silva MM, Doneda D, Schmidt HDO and Oliveira VR. 2018. Effect of whey protein addition on the nutritional, technological and sensory quality of banana cake. *International Journal of Food Science & Technology* **53**(11): 2617–2623.
- Deeth HC and Bansal N 2018 (Eds). *Whey proteins: From milk to medicine*. Academic Press.
- Feng P, Weagant SD, Grant MA, Burkhardt W, Shellfish M and Water B 2002. BAM: Enumeration of *Escherichia coli* and the coliform bacteria. *Bacteriological Analytical Manual* **13**(9): 1–13.
- Gdoura-Ben Amor M, Siala M, Zayani M, Grosset N, Smaoui S, Messadi-Akrouit F, Baron F, Jan S, Gautier M and Gdoura R. 2018. Isolation, identification, prevalence, and genetic diversity of *Bacillus cereus* group bacteria from different foodstuffs in Tunisia. *Frontiers in Microbiology* **9**: 447.
- Helkar PB, Sahoo AK and Patil NJ. 2016. Review: Food industry by-products used as a functional food ingredients. *International Journal of Waste Resources* **6**(3): 1–6.
- Hussein FA, Chay SY, Zarei M, Auwal SM, Hamid AA, Wan Ibadullah WZ and Saari N. 2020. Whey protein concentrate as a novel source of bifunctional peptides with angiotensin-I converting enzyme inhibitory and antioxidant properties: RSM study. *Foods* **9**(1): 64.
- Kareb O, Aider M 2019. Whey and its derivatives for probiotics, prebiotics, synbiotics and functional foods: a critical review. *Probiotics and Antimicrobial Proteins* **11**: 348–369.
- Krunić TŽ, Obradović NS, Rakin MB 2019. Application of whey protein and whey protein hydrolysate as protein based carrier for probiotic starter culture. *Food Chemistry* **293**: 74–82.
- Macwan SR, Dabhi BK, Parmar SC, Aparnathi KD 2016. Whey and its utilization. *International Journal of Current Microbiology and Applied Sciences* **5**(8): 134–155.
- Maqsood S, Al-Dowaila A, Mudgil P, Kamal H, Jobe B, Hassan HM. 2019. Comparative characterization of protein and lipid fractions from camel and cow milk, their functionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion. *Food Chemistry*, **279**: 328–338.
- Maturin LJ. 2001. Aerobic plate count. In: Bacteriological analytical manual online. <http://www.cfsan.fda.gov/~ebam/bam-3.html>.
- Mehra R, Kumar H, Kumar N, Ranvir S, Jana A, Buttar HS, Telesy IG, Awuchi CG, Okpala COR, Korzeniowska M and Guiné RP. 2021. Whey proteins processing and emergent derivatives: An insight perspective from constituents, bioactivities, functionalities to therapeutic applications. *Journal of Functional Foods* **87**: 104760.
- Minj S, Anand S 2020. Whey proteins and its derivatives: Bioactivity, functionality, and current applications. *Dairy* **1**(3): 233–258.
- Mishra K, Pinto S, Jambukiya H, Prajapati JP 2022. Evaluation of whey protein concentrate as a functional ingredient on quality of goat milk rasogolla-an Indian dessert. *Journal of Applied and Natural Science* **14**(1): 17–27.
- Myers RH, Montgomery DC, Anderson-Cook CM. 2016. *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. John Wiley & Sons.
- Pacheco VMM, Porras AOO, Velasco E, Morales-Valencia EM, Navarro A 2017. Effect of the milk-whey relation over physicochemical and rheological properties on a fermented milky drink. *Ingeniería y Competitividad* **19**(2): 83–91.
- Papademas P. and Kotsaki P 2019. Technological utilization of whey towards sustainable exploitation. *Journal of Advanced Dairy Research* **7**(4): 231.
- Panthi RR, Kelly AL, McMahon DJ, Dai X, Vollmer AH, Sheehan JJ 2019. Response surface methodology modeling of protein concentration, coagulum cut size, and set temperature on curd moisture loss kinetics during curd stirring. *Journal of Dairy Science* **102**(6): 4989–5004.
- Patel S. 2015. Emerging trends in nutraceutical applications of whey protein and its derivatives. *Journal of Food*

- Science and Technology* **52**: 6847–6858.
- Pickford C, McCormack L, Liu Y, Eicher-Miller HA. 2022. US Department of Agriculture Food Composition Databases, the Food and Nutrient Database for Dietary Studies 2013-2014, and the National Nutrient Database for Standard Reference Version 28 Yield Significantly Different Nutrient Totals of Food Items from Eight Midwestern Food Pantry Inventories. *Journal of the Academy of Nutrition and Dietetics* **22**(7): 1326-1335.e6.
- Setiowati AD, Wijaya W, Van der Meeren P 2020. Whey protein-polysaccharide conjugates obtained via dry heat treatment to improve the heat stability of whey protein stabilized emulsions. *Trends in Food Science & Technology* **98**: 150–161.
- Sharma R 2019. Whey proteins in functional foods. In: *Whey Proteins* (637–663). Academic Press.
- Siala M, Barbana A, Smaoui S, Hachicha S, Marouane C, Kammoun S, Gdoura R, Messadi-Akrout F 2017. Screening and detecting Salmonella in different food matrices in Southern Tunisia using a combined enrichment/real-time PCR method: Correlation with conventional culture method. *Frontiers in Microbiology* **8**: 2416.
- Singh R 2016. Whey proteins and their value-added applications. *Protein Byproducts*: 303–313.
- Smithers GW 2015. Whey-ing up the options—Yesterday, today and tomorrow. *International Dairy Journal* **48**: 2–14.
- Tournas V, Stack ME, Mislivec PB, Koch HA, Bandler R 2001. BAM: Yeasts, molds and mycotoxins. *Bacteriological Analytical Manual* **8**.
- Valta K, Damala P, Angeli E, Antonopoulou G, Malamis D, Haralambous KJ 2017. Current treatment technologies of cheese whey and wastewater by Greek cheese manufacturing units and potential valorization opportunities. *Waste and Biomass Valorization* **8**: 1649–1663.
- Wichchukit S, O'Mahony M 2015. The 9-point hedonic scale and hedonic ranking in food science: Some reappraisals and alternatives. *Journal of the Science of Food and Agriculture* **95**(11): 2167–2178.
- Yadav JSS, Yan S, Pilli S, Kumar L, Tyagi RD, Surampalli RY 2015. Cheese whey: A potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. *Biotechnology Advances* **33**(6): 756–774.
- Yiğit A, Bielska P, Cais-Sokolińska D, Samur G. 2023. Whey proteins as a functional food: Health effects, functional properties, and applications in food. *Journal of the American Nutrition Association*: 1–11.
- Zandona E, Blažić M, Režek Jambrak A 2021. Whey utilization: Sustainable uses and environmental approach. *Food Technology and Biotechnology* **59**(2): 147–161.
- Zhou X, Hua X, Huang L, Xu Y 2019. Bio-utilization of cheese manufacturing wastes (cheese whey powder) for bioethanol and specific product (galactonic acid) production via a two-step bioprocess. *Bioresource Technology* **272**: 70–76.