Manufacture of probiotic dairy beverage using Buffalo milk's concentrated whey proteins and dried grape juice powder

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Abstract:

Given the recognized health benefits and high nutritional value of dairy products, this study aimed to develop a fermented dairy beverage fortified with a probiotic strain (Lactobacillus plantarum) using buffalo milk whey protein concentrate at varying concentrations, in combination with dried grape juice and sucrose. A control treatment (C) was prepared using buffalo milk protein concentrate only. Three treatments (G1, G2, G3) received protein concentrate at 1.0%, 2.0%, and 3.0%, respectively, without the addition of the probiotic. Three additional treatments (G4, G5, G6) received the same protein concentrate levels (1.0%, 2.0%, and 3.0%) with the probiotic added at a concentration of 10^8 CFU/mL. Chemical analyses included the determination of moisture, protein, fat, ash, carbohydrate, and titratable acidity. Physical assessments comprised viscosity, density, and pH, in addition to microbiological and sensory evaluations.

The results showed a marked decrease in moisture content with increasing levels of whey protein concentrate addition. In contrast, the percentages of protein, fat, carbohydrates, and ash increased in the treatments with added juice compared to the control treatment.

Titratable acidity increased, and pH decreased significantly in the treatments fortified with the probiotic during storage periods of 1, 7, 14, and 21 days.

Physical tests revealed that viscosity and density values increased in all treatments in direct proportion to the increasing levels of whey protein addition, compared to the control treatment, with the probiotic-containing treatments showing superior results.

Microbiological examinations indicated no contamination with E. coli, Staphylococcus aureus, yeasts, or molds in any of the treatments.

Furthermore, sensory properties were improved in the probiotic-fortified treatments compared to the control treatment.

Keywords:

Whey protein, probiotic, grape juice, dairy beverage, buffalo milk.

Introduction:

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Whey is a byproduct of cheese or casein production and contains approximately 93% water, 4.8% lactose, 0.8% protein, and 0.5% fat. Whey is classified into two types: sweet whey, produced during cheddar or other cheese manufacture using rennet at a pH ranging between 6 and 7, and acid whey, obtained during the production of fresh cheese, cream cheese, Greek yogurt, and caseins at a pH below 5. Whey protein can be prepared via quenching, ultrafiltration, nanofiltration, and drying. It is further classified into three main types: whey protein concentrate, whey protein isolate, and whey protein hydrolysates This is due to the differences in their composition and processing methods Ultrafiltration is employed to separate lactose from protein, yielding whey protein concentrate with a protein content ranging from 20% to 80%. Microfiltration is used to increase protein concentration and remove lipids. Due to the heat sensitivity of whey protein, particularly in concentrated solutions, nanofiltration is commonly applied as a cold processing technique to reduce protein denaturation, replacing vacuum evaporation in the production of 80% whey protein concentrate and whey protein isolatesIn order to concentrate the dry matter and remove salts [47].

The innovative use of whey in food production has expanded due to increasing emphasis on sustainability. Whey is increasingly utilized to produce healthy, environmentally friendly products, including fermented beverages and protein-rich snacks. In countries with advanced processing systems, over 90% of whey is recycled into valuable products. The development of functional beverages, such as whey mixed with fruit juices, represents a recent advancement in whey utilization. These products appeal to health-conscious consumers by extending the shelf life of whey while simultaneously improving its sensory properties. The composition of dairy waste, its nutritional properties, and processing methods have become significant topics in modern scientific and technological research. Although dairy waste was long considered a byproduct of manufacturing processes, technological advances and expanded biochemical research have transformed dairy processing waste into an important resource for food, pharmaceutical, and biotechnological production [34].

A wide range of whey-based beverages has been developed, utilizing sweetened, sweet, or sour whey, deproteinized whey, water-diluted sweetened whey, whey powder, or fermented whey. Non-alcoholic whey beverages include a wide variety of products prepared by mixing sweet, diluted, or acidified sweetened whey with various additives such as tropical fruits (as well as apples, pears, strawberries, and cranberries), cereal products (particularly bran), plant protein isolates, carbon dioxide, chocolate, cocoa, vanilla extracts, and other flavoring agents. Particular attention has been directed toward developing fermented whey beverages using probiotic bacteria. The selection of an appropriate bacterial culture is a critical step in producing a functional beverage with high nutritional value and acceptable sensory properties. Non-alcoholic whey drinks also include dietary beverages, lactose-hydrolyzed drinks, milk-like beverages, and powdered drinks [10].

Food fermentation is a widely practiced method of food preservation, wherein antimicrobial metabolites—organic acids, ethanol, and bacteriocins—produced during fermentation reduce the risk of contamination by pathogenic microorganisms. Fermentation also enhances sensory properties, taste, and texture, with applications in dairy products, bread, pickled vegetables, and meat products. Furthermore, the bioavailability of certain vitamins and minerals in cereals, milk, and fermented fruits can be increased by reducing phytic acid and other antinutrient content. Fermented foods may also exert beneficial effects on health and disease through various mechanisms, primarily related to bioactive enhancement, bioactive peptides, phenolic compounds, and other derived metabolites. Specifically, consumption of fermented foods can increase dietary microbial intake up to 10,000-fold, and the microorganisms

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associated with fermentation can modulate gut microbiota, immunity, metabolism, and neurological functions. Additionally, lactic acid fermentation can alter the structure and type of bioactive compounds and produce biologically active peptides that influence bodily functions by regulating immune responses, oxidative stress, microbial composition, hypertension, and inflammation. Fermentation also affects the levels of phenolic compounds, conjugated linoleic acid, and polysaccharides, leading to functional benefits such as antioxidant, antidiabetic, anti-inflammatory, and weight management properties [47].

Probiotics play a crucial role in modulating intestinal microbial composition, inhibiting pathogenic bacterial colonization in the gut, and supporting the development of a healthy intestinal mucosa. Probiotics are primarily found in the human intestine and function to balance gut microbes. Common probiotic preparations include Lactobacillus and Bifidobacterium species. Probiotics exhibit various beneficial roles, including immune enhancement, anticancer properties, anti-obesity effects, and antidiabetic activity in the treatment of chronic inflammatory and metabolic disorders. These nutritional factors exert regulatory effects on gut microbiome structure and composition, primarily influencing host immunity. The health benefits of probiotics are attributed to resistance to bacterial colonization, production of organic acids and short-chain fatty acids, competition with pathogens, normalization of gut microbial imbalance, regulation of bowel movements, direct antagonism, strengthening of the gut barrier, and neutralization of carcinogens. Gut microbial imbalance is associated with numerous metabolic, intestinal, and cardiovascular diseases. Probiotics have shown promising results as nutritional supplements or adjunctive therapies in mitigating these diseases, in addition to their benefits in boosting immunity and modifying the gut microbiome[13].

Consumption of probiotic foods is highly acceptable to consumers as a natural means of obtaining daily probiotic intake. Reports indicate that fruit juices fortified with probiotics represent a unique and suitable method for producing probiotic beverages. Fruit juices are widely accepted by consumers worldwide, regardless of age, gender, or geographic region, due to their content of essential nutrients. Probiotics exhibit a shorter shelf life in non-dairy foods compared to supplements, owing to the harsh conditions probiotics encounter in beverages. Manufacturers must consider multiple factors in probiotic juice production, including pH, temperature, anthocyanins, and, most importantly, the botanical form of the probiotics [27].

Grape juice possesses distinctive sensory properties, primarily attributed to its phenolic compounds, which are responsible for its color, flavor, and taste. Phenolic acids influence the sensory properties of dried grape juice and ensure a low pH, achieving a balance between sweet and sour tastes. Sensory properties are therefore an important factor in consumer choice of food products. Color is the most significant attribute in beverage selection. Furthermore, consumers use color as an indicator of juice quality, as a close relationship exists between color and flavor, enabling consumers to predict flavor from the color of food products. Replacing synthetic dyes with natural colorants is currently a challenge for the food industry. The attractive colors of anthocyanins—orange, red, and violet—and their water solubility allow their incorporation into aqueous food systems as natural colorants. Anthocyanins also participate in multiple chemical reactions, such as co-pigmentation and the formation of polymeric pigments that contribute to color changes. In grape juice, cyanidin, peonidin, delphinidin, petunidin, and malvidin are the most important anthocyanins responsible for red coloration. Their stability is affected by pH, temperature, oxygen, light, the presence of ascorbic acid and metal ions, as well as high sugar concentrations. On the other hand, anthocyanin consumption enhances health benefits by reducing the risk of cancer, inflammation, neurological and cardiovascular diseases, diabetes, obesity, and cognitive disorders due to their antioxidant, anti-cardiovascular, anticancer, anti-inflammatory, anticoagulant, anti-

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ulcer, antiallergic, immunomodulatory, vasodilatory, and analgesic activities [15].

The present study aimed to determine the develop and evaluate of manufacturing a healthy dairy beverage that enhances the viability of probiotic bacteria (*Lactobacillus plantarum*) using buffalo milk whey protein concentrate and grape juice, while evaluating the physicochemical, microbiological, and sensory quality characteristics of the final product.

Materials and Methods:

Materials

Buffalo milk was obtained from a farm near Al-Qasim Green University. Black grapes and table sugar were procured from a local market in Babil Governorate. The probiotic (*Lactobacillus plantarum*) was prepared by Swanson Health Products.

Methods

Preparation of Buffalo Milk Whey Protein Concentrate:

Whole buffalo milk was obtained from a farm near Al-Qasim Green University, and required tests were conducted. The milk was pasteurized, cooled to 38°C, and rennet was added. Cheese was manufactured using traditional methods as described by[8]. The resulting whey was used to produce whey protein concentrate via lyophilization according to the method of[33]. The buffalo milk protein concentrate powder was placed in polyethylene bags and preserved by freezing.

Juice Preparation:

Grape juice was prepared following the method described by[21]. Grapes were thoroughly washed, destoned, squeezed, and filtered to separate the juice from the peels. The juice was pasteurized at 65°C for 30 minutes, stored in polyethylene bags, and preserved by cryopreservation.

Manufacture of Dairy Products:

Dairy product samples were prepared according to the method described in reference [24]. Whey protein concentrate was added at concentrations of 1%, 2%, and 3%, respectively. Grape juice (2%) and sugar (2%) were also added to all samples. Additional samples were prepared with the same concentrations of whey protein (1%, 2%, and 3%), grape juice (2%), and sugar (2%). All samples were packaged in sterile glass bottles and pasteurized at 65°C for 30 minutes. The juice was then cooled, and the probiotic *Lactobacillus plantarum* was added to some of the samples. The samples were kept refrigerated at 4 ± 1°C for 21 days.

Physicochemical Tests:

Moisture, ash, fat, and protein were estimated according to the method described in[.and Carbohydrates were estimated mathematically according to the following equation:

% Carbohydrates = 100 – (% Ash + % Protein + % Fat + % Moisture)

pH Determination

The pH was determined following the procedure outlined in[5], using the sensor of a pH meter (Model 211, HANNA Instruments Microprocessor, made in Romania).

Titratable Acidity

Acidity percentage was estimated according to [5] by taking 10 g of the beverage, titrating with 0.1 M sodium hydroxide using phenolphthalein as an indicator, the monitoring continued until the pink color disappeared for 15 seconds. and calculating acidity as lactic acid (equivalent weight = 90) using the following equation:

Acidity (%) = Volume of base consumed (ml) × Normality of base × Equivalent weight of lactic acid) / Weight of sample (gm)× 100

Viscosity

Apparent viscosity of whey-based dairy product samples fortified with grape juice and probiotic was measured at 10°C after 1, 7, 14, and 21 days of refrigerated storage using a Brookfield DVII

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Viscometer (Brookfield Engineering Lab Inc., Stoughton, MA, USA) according to[13]. An axial spindle No. 4 was used at 10 revolutions (rounds) per minute with a sample volume of 150 mL. The spindle was rotated for 60 seconds inside the shattered sample (by spinning it 10 strokes clockwise and 10 strokes counterclockwise). Readings were taken three times and averaged in centipoise units.

Density

Density was estimated using a standard glass pycnometer according to the method of[16]. The pycnometer was calibrated with distilled water, then filled with the product sample and weighed using a digital balance. Measurements were performed in triplicate, and density was calculated using the following equation:

Product density (kg/m³) = (Product weight (kg) / Water weight (kg)) × Water density (kg/m³)

Microbiological Tests:

Enumeration of Probiotic Bacteria (*Lactobacillus plantarum*):

The count of probiotic bacteria was estimated according to [3]using solid MRS agar and the pour plate method after performing appropriate decimal dilutions in 0.1% peptone solution. Plates were incubated at 37°C for 48 hours under anaerobic conditions. Colony counts were performed using a colony counter.

Enumeration of Escherichia coli:

E. coli counts were estimated according to [9] using MacConkey agar, with incubation at 37°C for 24–48 hours.

Enumeration of Yeasts and Molds:

Yeast and mold counts were estimated according to [6] using Potato Dextrose Agar (PDA), incubated at 21±2°C for five days.

Enumeration of Staphylococcus aureus:

S. aureus counts were estimated according to [44]using Mannitol Salt Agar, incubated at 32°C for 48 hours. Golden colonies surrounded by a yellow halo, representative of S. aureus, were counted.

Sensory Evaluation:

Sensory evaluation of the dairy beverage treatments was conducted by a specialized panel of faculty members from the College of Food Sciences, Al-Qasim Green University. Evaluated criteria included flavor, color, texture, and acidity according to the sensory evaluation form described by[4]with minor modifications.

Statistical Analysis:

The Statistical Analysis System (SAS) software, User's Guide (2018), was used to analyze the data to study the effect of different treatments on the studied traits according to a Completely Randomized Design (CRD). Significant differences between means were compared using the Least Significant Difference (LSD) test.

Results and Discussion:

Chemical Composition:

It is evident from the results in Table (1), which presents the chemical analysis of the different dairy product treatments manufactured from buffalo milk whey protein concentrate, represented by the control treatment (C), treatments G1, G2, and G3 with added juice, and treatments G4, G5, and G6 with added juice and probiotic.

The moisture content of the control treatment (C) immediately after processing was 98.5%, while the moisture content of the dairy product treatments with added whey protein concentrate at different concentrations and grape juice (G1, G2, G3) on the first day were 97.6%, 96.3%, and 95.8%, respectively. For the dairy beverage treatments fortified with grape juice and probiotic (G4, G5, G6), the moisture contents were 97.4%, 95.8%, and 95.0%, respectively.

Furthermore, the results of the statistical analysis showed no significant differences ($P \leq 0.05$) between the treatments with added juice and the control treatment, except for treatment G6, which exhibited significant differences compared to the control treatment.

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The results also show a decrease in the moisture content of the treatments to which grape juice and higher protein concentrations were added, compared to the control treatment, in direct proportion to the increase in whey protein concentrate concentration. This may be attributed to the increase in total solids content, which is considered a fundamental quality indicator for new product development. This parameter represents the content of sugars, soluble proteins, amino acids, and other organic substances, leading to a reduction in the moisture percentage. This is consistent with the findings of[29], who indicated that the increase in total solids is a criterion justifying the decrease in moisture content.

It can also be observed from the results that there was a slight decrease in moisture content across all treatments during storage. On day 7, the moisture percentages for treatments C, G1, G2, G3, G4, G5, and G6 were 98.3%, 97.4%, 96.0%, 95.7%, 97.3%, 95.7%, and 94.8%, respectively. After 21 days, the moisture percentages for the same treatments were98.0, 96.9%, 95.7%, 95.4%, 97.0%, 95.5%, and 94.6%, respectively.

This may be attributed to the fact that the sugar and carbohydrate components undergo significant moisture absorption during storage. This is consistent with the findings of[43], who reported that fruit juice powders absorb moisture due to their high content of sugars and low molecular weight acids.

Furthermore, the results of the statistical analysis showed no significant differences ($P \leq 0.05$) between the treatments with added juice and the control treatment during storage periods, except for treatment G6, which exhibited significant differences compared to the control treatment.

As for the protein percentage of the control treatment (C) on the first day, it was 0.99%. For the dairy beverage treatments with added whey protein at different concentrations and grape juice without probiotic (G1, G2, G3), the protein percentages were 1.22%, 2.27%, and 3.48%, respectively. For the probiotic-fortified treatments (G4, G5, G6), the protein percentages were 1.35%, 2.30%, and 3.34%, respectively.

It can be observed from the results that the protein percentage increased in all treatments with added juice compared to the control treatment. This increase may be attributed to the fact that grape juice contains protein in its composition. This is consistent with the findings of[42], who indicated that grape juice contains protein.

It can also be observed from the results that there is a gradual increase in protein percentage that is directly proportional to the increasing amount of added buffalo milk whey protein concentrate. Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between the treatments with added juice and the control treatment, except for treatments G1 and G4, which showed no significant differences compared to the control treatment.

It can also be observed from the results that there was a slight increase in protein percentage across all treatments during storage. On day 7, the protein percentages for treatments C, G1, G2, G3, G4, G5, and G6 were 1.00%, 1.24%, 2.28%, 3.50%, 1.38%, 2.32%, and 3.44%, respectively. After 21 days, the percentages were 1.10%, 1.27%, 2.30%, 3.55%, 1.45%, 2.35%, and 3.46%, respectively. This may be attributed to the decrease in moisture content, which generally affects the total solid content.

Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between the treatments with added juice and the control treatment, except for treatments G1 and G4, which showed no significant differences compared to the control treatment.

Fat percentages for control C on day 1 was 0.02%, while those for G1–G6 ranged from 0.12,0.14,0.17,0.13,0.14,0.15% respectively. After 21 days, the percentages for the aforementioned treatments were 0.71%, 1.55%, 1.67%, 1.66%, 1.30%, 1.85%, and 1.60%, respectively.

It can also be observed from the results that the fat percentage increased in all treatments compared to the control treatment. This may be attributed to the fact that grape juice contains a certain percentage of fat in its composition. This is consistent with the findings of[42], who indicated

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that grape juice contains a certain percentage of fat. Additionally, the added whey protein contains an amount of fat that is directly proportional to the increasing amount added.

It can also be observed from the results that the fat percentage increased during storage across all treatments. On day 7, the fat percentages for treatments C, G1, G2, G3, G4, G5, and G6 were 0.04%, 0.13%, 0.15%, 0.17%, 0.13%, 0.14%, and 0.15%, respectively. After 21 days, the percentages were 0.07%, 0.15%, 0.16%, 0.19%, 0.14%, 0.15%, and 0.16%, respectively.

As for the carbohydrate percentage of the control treatment (C) on the first day, it was 0.38%. The carbohydrate percentages for the remaining treatments were 0.94%, 1.14%, 1.37%, 0.99%, 1.62%, and 1.29%, respectively. It can be observed from the results that the carbohydrate percentage increased in all treatments compared to the control treatment, and this may be attributed to the addition of grape juice and sugar.

It can also be observed from the results that the carbohydrate percentage increased during storage across all treatments. On day 7, the carbohydrate percentages for treatments C, G1, G2, G3, G4, G5, and G6 were 0.55%, 1.11%, 1.41%, 1.44%, 1.07%, 1.71%, and 1.46%, respectively. After 21 days, the percentages for the aforementioned treatments were 0.71%, 1.55%, 1.67%, 1.66%, 1.30%, 1.85%, and 1.60%, respectively.

Regarding the ash content on the first day, the control treatment (C) had a percentage of 0.11%, while the other treatments had percentages of 0.12%, 0.15%, 0.18%, 0.13%, 0.14%, and 0.15%, respectively. It can also be observed from the results that the ash percentage increased in all treatments compared to the control treatment. This is consistent with the findings of [25], who indicated that there was a noticeable increase in ash content in the prepared whey drink mixed with fruit juices.

It can also be observed from the results that the ash percentage did not change during the seven-day storage period for all treatments. However, a slight increase in ash percentage was observed after 21 days of storage, where the ash percentages for treatments C, G1, G2, G3, G4, G5, and G6 were 0.12%, 0.13%, 0.16%, 0.19%, 0.14%, 0.15%, and 0.16%, respectively. This may be attributed to the decrease in moisture content and the increase in total solids.

Regarding titratable acidity, on the first day, the control treatment (C) had an acidity percentage of 0.17%, while the acidity percentages for G1, G2, G3, G4, G5, and G6 were 0.20%, 0.39%, 0.37%, 0.41%, 0.52%, and 0.84%, respectively. It is also evident from the results of the statistical analysis that there were no significant differences ($P \leq 0.05$) between the treatments with added juice and the control treatment, except for treatment G6, which showed significant differences compared to the control treatment.

It can be observed from the results that acidity levels increased in all treatments compared to the control treatment. This may be attributed to the addition of grape juice, which contains a certain percentage of organic acids. This is consistent with the findings of[28], who indicated that grape juice contains organic acids that lead to increased acidity.

It is also observed from the results that acidity increased in treatments with higher concentrations of whey protein. This may be due to the fact that as protein content increases, there is a proportional increase in acidity. This is consistent with the findings of [41], who attributed this to the presence of amino acids with specific structures, i.e., positively charged side chains.

It can also be observed from the results that there was a slight increase in acidity for the control treatment and the treatments without added probiotic during storage periods. On day 7, the acidity percentages were 0.18%, 0.21%, 0.39%, and 0.40%, respectively. Meanwhile, the treatments with added probiotic had higher acidity levels, reaching 0.86%, 0.84%, and 0.86%, respectively. This may be attributed to the fact that the addition of the probiotic led to increased acidity through the fermentation of lactose sugar and the production of lactic acid. This is consistent with the findings of both [12] and [20].

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After 21 days, the values were 0.20%, 0.40%, 0.47%, 0.50%, 1.05%, 1.09%, and 0.93%, respectively. This may be attributed to the continued microbial activity of certain bacteria, such as lactic acid bacteria, which is consistent with the findings of[32]. Alternatively, it may be due to the interaction of components with each other, as the product contains natural acids that can lead to increased acidity. This agrees with the findings of both[18]and[39]. Furthermore, the development of acidity during storage may be attributed to the presence of proteins, which may cause increased enzyme activity leading to higher acidity. This is consistent with the findings of[7]. It is also in agreement with[29], who reported an increase in the acidity of both grape and apple juice on the 21st day of storage.

The results of the statistical analysis also show no significant differences ($P \leq 0.05$) between the juice-added treatments and the control treatment during storage periods. However, the treatments containing both juice and probiotic exhibited significant differences compared to the control treatment.

The pH of control C on day 1 was 6.6, while that of G1–G6 ranged from 6.0, 5.8, 5.7, 5.5, 5.3, 5.1 respectively. It can also be observed from the results that pH values decreased in all treatments compared to the control treatment. This may be attributed to the increased acidity in the juice-fortified treatments, where the concentration of organic acids rises, increasing hydrogen ions, which leads to higher acidity and lower pH. This is consistent with the findings of both [28]and[14], who indicated that the perception of acidity is related to hydrogen ion concentration. It is also in agreement with[46], who pointed out that acidity and pH are interrelated concepts in food analysis.

Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between all juice-added treatments and the control treatment.

It can also be observed from the results that pH values decreased during storage. On day 7, the pH values for all treatments were 6.4, 5.9, 5.7, 5.6, 4.9, 4.8 and 4.9, respectively. After 21 days of storage, the pH values for all treatments were 6.2, 5.5, 5.4, 5.2, 4.2, 4.3, and 4.5, respectively.

Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between all juice-added treatments and the control treatment during the storage periods.

Table (1): Chemical composition of dairy beverage treatments during refrigerated storage (1–21 days).

Treatments	Storage peroid (Day)	The Components%						
		Moisture	Protein	Fat	Carbohydrate	Ash	Acidity	PH
C	1	98.5	0.99	0.02	0.38	0.11	0.17	6.6
	7	98.3	1.00	0.04	0.55	0.11	0.18	6.5
	14	98.1	1.08	0.05	0.65	0.12	0.19	6.4

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		21	98.0	1.10	0.07	0.71	0.12	0.20	6.2
Dairy product treatments with added whey protein concentrate and grape juice	G_1	1	97.6	1.22	0.12	0.94	0.12	0.20	6
		7	97.4	1.24	0.13	1.11	0.12	0.21	5.9
		14	97.1	1.25	0.14	1.38	0.13	0.28	5.7
		21	96.9	1.27	0.15	1.55	0.13	0.40	5.5
	G_2	1	96.3	2.27	0.14	1.14	0.15	0.39	5.8
		7	96.0	2.28	0.15	1.41	0.16	0.36	5.7
		14	95.9	2.29	0.15	1.49	0.17	0.46	5.5
		21	95.7	2.30	0.16	1.67	0.17	0.47	5.4
	G_3	1	95.8	3.48	0.17	1.37	0.18	0.37	5.7
		7	95.7	3.50	0.18	1.44	0.18	0.40	5.6
		14	95.6	3.53	0.18	1.49	0.20	0.48	5.4
		21	95.4	3.55	0.19	1.66	0.20	0.50	5.2
Dairy product treatments with added buffalo whey protein concentrate, grape juice, and probiotic	G4	1	97.4	1.35	0.13	0.99	0.13	0.41	5.5
		7	97.3	1.38	0.13	1.07	0.12	0.86	4.9
		14	97.2	1.42	0.14	1.13	0.11	0.92	4.5
		21	97.0	1.45	0.14	1.30	0.11	1.05	4.2
	G5	1	95.8	2.30	0.14	1.62	0.14	0.52	5.3
		7	95.7	2.32	0.14	1.70	0.14	0.84	4.8
		14	95.6	2.34	0.15	1.76	0.15	0.91	4.5
		21	95.5	2.35	0.15	1.85	0.15	1.09	4.3
	G6	1	95.0	3.43	0.15	1.26	0.15	0.84	5.1
		7	94.8	3.44	0.15	1.45	0.16	0.86	4.9
		14	94.7	3.45	0.16	1.53	0.17	0.90	4.6
		21	94.6	3.46	0.16	1.60	0.18	0.93	4.5
LSD Value			3.29*	0.752*	0.084*	1.05*	0.077*	0.489*	0.502*

Physical Properties:

The results sown in Figure (1) present the viscosity values. On the first day, the viscosity value for the control treatment (C) was 20 centipoises. The viscosity values for the remaining treatments G1, G2, G3, G4, G5, and G6 were 24, 25, 31, 26, 32, and 33 centipoises, respectively. It is observed from the results that the viscosity values increased in all treatments with added grape juice compared to the control treatment. This may be attributed to the addition of grape juice as well as the addition of sugar, which may increase the total solids content, leading to higher viscosity. This is consistent with the findings of[26], who indicated that total solids are among the most important factors af It is also observed from the results that viscosity values increased in the treatments with added probiotic compared to the treatments without probiotic. This may be attributed to the ability of probiotic bacteria to increase viscosity, which is consistent with the findings of [32], who indicated that the increase in viscosity is associated with the addition of probiotics through their ability to produce exopolysaccharides capable of improving the consistency of the juice, increasing the viscosity of the final product, and interacting with other juice components such as proteins.

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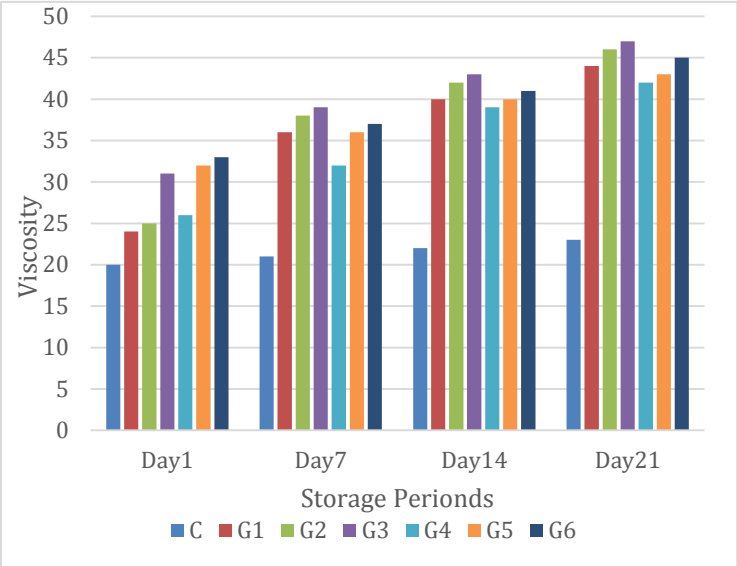


Figure (1) shows the viscosity of the product during periods of grief.

Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between all treatments compared to the control treatment.

It is also observed from the results that viscosity values increased during storage periods for all treatments. On the 7th day of storage, the viscosity values were 21, 36, 38, 39, 32, 36, and 37 centipoises, respectively. After 21 days, the values reached 23, 44, 46, 47, 42, 43, and 45 centipoises.

This may be attributed to the decrease in moisture content, which led to an increase in the total solids content of the beverage. Additionally, the addition of grape juice and sugar may have caused the increase in viscosity, which is consistent with the findings of [30], who indicated that apparent viscosity increases with increasing total solids content.

However, this differs from what was reported by[2], who found that viscosity values of fruit juice decreased during storage periods.

Furthermore, the results of the statistical analysis showed no significant differences between all treatments compared to the control treatment.

The results shown in Figure (2) present the density values. On the first day, the density value for the control treatment (C) was 1.02. The density values for the remaining treatments were 1.103, 1.102, 1.111, 1.106, 1.108, and 1.112, respectively. It is observed from the results that density values increased in all treatments compared to the control treatment. This may be attributed to the increase in total solids content upon the addition of grape juice, which is consistent with the findings of[23], who indicated that pineapple juice density increases with increasing total solids concentration. Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between all treatments compared to the control treatment.

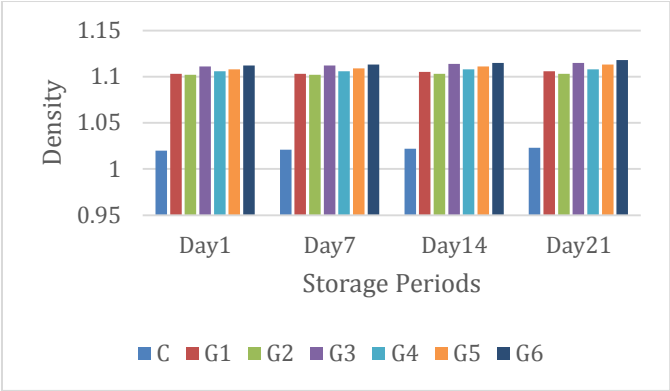


Figure (2) Product density during periods of grief.

It is also observed from the results that density values increased for all treatments during storage periods. On the 7th day, the values were 1.021, 1.103, 1.102, 1.112, 1.106, 1.109, and 1.113, respectively. After 21 days, the values reached 1.023, 1.106, 1.103, 1.115, 1.108, 1.113, and 1.118, respectively. The results of the statistical analysis also showed significant differences ($P \leq 0.05$) between all treatments compared to the control treatment during the storage periods.

Microbiological Characteristics:

The results presented in Table (2) show the microbiological analysis of the different dairy product treatments manufactured from buffalo milk whey protein concentrate, represented by the control treatment (C), treatments G1, G2, and G3 with added juice, and treatments G4, G5, and G6

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with added juice and probiotic. The analysis included the enumeration of probiotic bacteria, E. coli, yeasts and molds, and Staphylococcus aureus. These tests were conducted over storage periods of 1, 7, 14, and 21 days.

It is observed from the results that in the treatments with added probiotic (G4, G5, G6), the probiotic counts on the first day were 16.78×10^8 , 17.89×10^8 , and 18.72×10^8 CFU/ml, respectively. The probiotic counts were directly proportional to the increasing concentration of buffalo milk whey protein concentrate. This is consistent with the findings of[11], who indicated that proteins had an effect on increasing the growth of probiotic bacteria.

It is also observed from the results that probiotic counts decreased during storage periods. On the 7th day, the counts were 15.63×10^8 , 16.71×10^8 , and 17.65×10^8 CFU\ml, respectively. After 21 days, the probiotic counts reached 13.52×10^8 , 14.97×10^8 , and 15.85×10^8 CFU\ml, respectively. This is consistent with the findings of [37], who reported a decrease in probiotic bacterial counts during storage.

Furthermore, the results show no contamination with E. coli or Staphylococcus aureus in the control treatment or in any of the treatments during the storage periods. This may be attributed to the good hygienic conditions followed during product manufacturing and the fact that the beverage was subjected to a pasteurization process immediately after processing. This is consistent with the findings of[31], who indicated that pasteurization ensured the microbiological safety of juices and preserved them for a longer period. It is also in agreement with[1], who reported that no E. coli was detected in any samples of whey-mango juice beverages stored under refrigeration, attributing this to good hygienic conditions and the intensity of heat treatment (pasteurization).

It is also evident from the results of the statistical analysis that there were significant differences ($P \leq 0.05$) in the counts of probiotic bacteria in the treatments with added probiotic compared to the control treatment.

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Table (2): Microbiological counts of dairy beverage treatments during refrigerated storage (1–21 days).

Treatments		Storage period (day)	Probiotic Bacteria	*E.coli*	Yeast & Molds	*Staph.aureus*
C		1	Nil	Nil	Nil	Nil
		7	Nil	Nil	Nil	Nil
		14	Nil	Nil	Nil	Nil
		21	Nil	Nil	Nil	Nill
Dairy product treatments with added whey protein concentrate and grape juice	**G_1**	1	Nil	Nil	Nil	Nil
		7	Nil	Nil	Nil	Nil
		14	Nil	Nil	Nil	Nil
		21	Nil	Nil	Nil	Nil
	G_2	1	Nil	Nil	Nil	Nil
		7	Nil	Nil	Nil	Nil
		14	Nil	Nil	Nil	Nil
		21	Nil	Nil	Nil	Nil
	G_3	1	Nil	Nil	Nil	Nil
		7	Nil	Nil	Nil	Nil
		14	Nil	Nil	Nil	Nil
		21	Nil	Nil	Nil	Nil
Dairy product treatments with added buffalo whey protein concentrate, grape juice, and probiotic	**G_4**	1	16.78×10^8	Nil	Nil	Nil
		7	15.63×10^8	Nil	Nil	Nil
		14	14.56×10^8	Nil	Nil	Nil
		21	13.52×10^8	Nil	Nil	Nil
	G_5	1	17.89×10^8	Nil	Nil	Nil
		7	16.71×10^8	Nil	Nil	Nil
		14	15.58×10^8	Nil	Nil	Nil
		21	14.97×10^8	Nil	Nil	Nil
	G_6	1	18.72×10^8	Nil	Nil	Nil
		7	17.65×10^8	Nil	Nil	Nil
		14	16.58×10^8	Nil	Nil	Nil
		21	15.85×10^8	Nil	Nil	Nil
L.S.D			6.397*	00.0NS	00.0NS	00.0NS

Sensory Evaluation:

Table (3) presents the sensory evaluation of the aforementioned product treatments. The results show that the highest evaluation scores were obtained by the treatments with added probiotic compared to those without probiotic addition. Treatment G4 was superior to all other treatments in all sensory attributes, including flavor, consistency, acidity, and appearance. The results also showed that the control treatment (C) obtained low scores in all mentioned sensory attributes compared to the other treatments. This may be attributed to the fact that the addition of grape juice to the product improved flavor, consistency, acidity, and appearance. This is consistent with the findings of[17], who indicated that the addition of grape derivatives to dairy products improves their nutritional value and chemical, physical, and sensory properties.

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It is also observed from the results that flavor and consistency scores were higher in the probiotic-added treatments (G4, G5, G6) compared to the control treatment (C) and the treatments without probiotic addition (G1, G2, G3). This is consistent with the findings of [48], who indicated that probiotic addition improved and enhanced sensory properties and positively modified flavors and aromas.

Furthermore, it is observed from the results that acidity scores were higher in the probiotic-added treatments, indicating that probiotic addition increased consumer acceptance of the product. This agrees with[12], who stated that probiotic addition leads to increased acidity.

The results also show that sensory evaluation scores for consistency were higher than those of the control treatment and were similar across all other treatments. This may be due to the fact that grape juice addition improved consistency, which is consistent with the findings[45], who indicated that the addition of fruit juice to whey beverages led to improved taste and consistency of the product.

It is also observed that all sensory evaluation scores decreased in all treatments during storage. This may be attributed to color loss due to browning and oxidation reactions, increased acidity, and changes in taste and flavor during storage. This is consistent with the findings of both [36]and[1], who reported a decrease in sensory evaluation scores for whey-fruit juice beverages during storage.

Furthermore, the results of the statistical analysis showed significant differences ($P \leq 0.05$) between all treatments.

Table (3): Sensory evaluation scores of dairy beverage treatments during refrigerated storage (1–21 day

Treatments		Storage period (day)	Flavor -45	Body-35	Acidity-10	Appearance-10	Total -100
C		1	25	30	7	7	70
		7	24	28	6	7	65
		14	22	27	5	6	60
		21	20	25	4	5	54
Dairy product treatments with added whey protein concentrate and grape juice	G1	1	41	29	8	8	86
		7	40	28	7	8	83
		14	39	28	6	7	80
		21	37	27	7	6	77
	G2	1	40	30	7	7	84
		7	39	28	6	6	79
		14	38	27	5	6	76
		21	37	26	5	5	73
	G3	1	38	27	7	7	79
		7	37	26	7	6	76
		14	36	25	6	6	73
		21	35	25	6	6	72
added buffalo whey protein concentrate,	G4	1	44	34	10	10	98
		7	43	33	8	9	93
		14	42	31	8	8	89

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		21	40	30	7	8	85
	G5	1	44	33	9	8	94
		7	43	33	8	8	92
		14	43	32	7	7	90
		21	42	32	6	7	87
	G6	1	43	30	8	8	89
		7	42	28	7	7	84
		14	41	27	6	7	81
		21	40	25	5	6	76
L.S.D			5.02*	4.17*	2.26*	1.94*	8.15*

Conclusions:

Based on the findings of this study, which demonstrated the feasibility of manufacturing a healthy dairy product from buffalo milk whey protein concentrate at different

concentrations (1.0%, 2.0%, 3.0%) fortified with 2% grape juice and the probiotic Lactobacillus plantarum, the manufactured product exhibited good and acceptable chemical, physical, microbiological, and sensory properties.

The addition of grape juice to buffalo milk whey led to an improvement in the chemical properties of the product, including increased percentages of protein, fat, carbohydrates, and ash, as well as improved physical properties, including increased viscosity and density, and enhanced sensory properties, including flavor, consistency, acidity, and appearance, compared to the control treatment.

The manufactured product is considered a good source of proteins and probiotics, and its consumption may contribute to enhancing gastrointestinal health and modulating the gut microbiome, owing to its content of Lactobacillus plantarum and the phenolic compounds present in grapes.

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