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Preparation of Fermented Beverage from Whey-Based Watermelon (*Citrullus lanatus*) Juice

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Abstract

The study was aimed to develop a probiotic beverage using *Chhana* whey and watermelon juice. The blends of whey and watermelon juice were optimized based on physicochemical and sensory evaluation. The bacterial culture was used as per 1% *Lactobacillus acidophilus* NIAI L-54 as a probiotic culture. The 75:25 blend of whey and watermelon fermented for 5 h at 37ºC obtained the highest sensory scores for overall acceptability. The shelf-life of the selected blend was examined using titratable acidity, pH, microbiological analysis as well as sensory evaluation. The results suggested that probiotic beverages can be preserved for 21 days in refrigerated conditions (4ºC) with higher acceptability along with probiotic bacterial count 4.00 Log_{10}CFU /mL. It could be concluded that the whey-based watermelon beverage enabled by-product utilization with excellent nutritional and functional value.

Key words: Beverage, *Chhana* whey, Probiotic, Shelf-life, Watermelon.

Introduction

In modern times, there has been a great concern in the production of fermented beverages especially probiotics owing to their beneficial effects on human health and nutritional wellbeing. This has directed to a surge in the production of food items which are renowned as a functional food. Probiotics can be described as foods containing live microorganisms which are ingested in adequate amounts, confer a health benefit on the host (Vasiljevic and Shah 2008). Whey is a fluid by-product resulting from the precipitation of proteins in the milk of the cheese industry. It is a greenish-yellow and semi-translucent liquid that recognized as a dilute nutrient stream accounting for 85-95% of the milk volume (Oliveira *et al*., 2018). However, cheese whey retains about 55% of milk nutrients (lactose, soluble proteins and minerals especially calcium and phosphorus). The main whey proteins are α-lactoglobulin and α-lactalbumin, which contain essential amino acids and branched-chain amino acids (leucine, isoleucine and valine) (Davoodi *et al*., 2016). Leucine can minimize muscle loss under conditions of increased protein degradation and stimulate muscle protein synthesis. Sulfur-containing amino acids, such as cysteine and methionine - precursors of glutathione, have shown anticarcinogenic and antioxidant properties in addition to enhancing the immune function (Davoodi, *et al*., 2016). Whey is being considered as one of the most polluting food by-product streams since it has a biochemical oxygen demand (BOD) >35,000 ppm and the chemical oxygen demand (COD) >60,000 ppm (Smithers *et al*., 2008). An amount of 4000 L of whey could cause high environmental damage equivalent to that caused by fecal waste produced by 1900 humans (Tunick 2008). Whey dumping on land creates severe pollution concerns for the surrounding environment by affecting the Physico-chemical characteristics of soil that results in decreased crop yields. Furthermore, when discharged into water bodies, it reduces the dissolved oxygen, hampers biodegradability and poses a major risk to aquatic life and to the environment and human health (Ghaly *et al*., 2007).

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Watermelon (*Citrullus lanatus*) is consumed worldwide and is a potential source of bioactive compounds, such as citrulline and lycopene, which may display beneficial effects on human health (Choudhary, Haldhar, Maheshwari, Bhargava and Sharma, 2015). In Bangladesh, watermelon is one of the abundant and cheap fruits compare to others. Watermelon production contributes to 6–7 % of overall fruit production in the world (Reddy *et al*., 2008). Usually, the production is highest in the summer but available throughout the year.

The uses of probiotics have been shown to turn many health benefits to the human and play a key role in maintaining normal digestive processes. Probiotic drinks are typically dairy-based beverages with a consistency similar to milk. They are consumed for digestive health. The addition of probiotics to whey will enhance the properties and benefits to several folds. However, some uncertainties concerning technological, microbiological and regulatory aspects exist. Various scientists contributed to studies of probiotic and probiotic beverages (Kumar *et al*., 2013; Shukla *et al*., 2013; Ryan *et al*., 2016; Amaral *et al*., 2018; Routray *et al*., 2019).

However, the medicinal and nutritive value of whey can be employed with watermelon juices in the development of probiotic whey-based watermelon (WBW) beverages that emerge to be the most reasonable and probable avenue for utilizing the nutrients of whey into the human food chain. The manufacture of whey-based beverages requires the mixing of suitable fruit juices and minimally processed whey with the selection of suitable stabilizers and acidulants to develop acceptable whey-based fruit beverages (Singh *et al*., 2014). The present studies were conducted to develop a probiotic beverage using whey and watermelon juice and evaluation of its shelf-life.

MATERIALS AND METHODS

The present research work was carried out at the Laboratory of Dairy Microbiology and Biotechnology, Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Preparation of *Chhana* whey and watermelon juice

Chhana whey was prepared according to the method of De (2005) with few modifications. In brief, skim milk was heated at 85°C for 5 minutes in a vat with the help of heater and previous whey (pH 3.89) was added slowly when milk temperature reached at 70°C. Thus, *Chhana* was prepared and then filtered through the muslin cloth for whey collection. Finally, whey was collected in a can and heated to 85ºC for removing the curd particles. In contrary, fresh watermelon (Patenga giant variety) were collected from the local market and sliced followed by cutting into small pieces. The fruit pieces were grinded in a mixer (Siemens, Germany) and the resulting pulp was passed through a muslin cloth to extract the juice. The juice was heated up to 72°C for 30 seconds in a hot air oven (OF-22, Jeio Tech, Korea) to destroy the microbes.

Preparation of probiotic whey-based watermelon (WBW) beverage

Five (05) different types of WBW beverages were prepared by mixing of different blends of whey and watermelon juice. In each blend, 10% sugar level was used as constant. The control (100% whey) was marked as A and samples with 65, 70, 75 and 80 % level of blending with *Chhana* whey were marked as B, C, D and E. The proportion of watermelon juice in the samples B, C, D and E was 35%, 30%, 25% and 20%. The probiotic starter culture was used as per 1% Lactobacillus acidophilus NIAI L-54 (Okayama, Japan). For the preparation of the fermented beverage, 1% probiotic bacterial culture (*Lactobacillus acidophilus* NIAI L-54) was added into different blends of whey and watermelon juice and incubated for 5 h at 37ºC (Shukla *et al*., 2013). The experimental layout can be presented as follows:

Sample Name	Whey (%)	Watermelon juice (%)	Sugar (%)	Starter culture (%)
A	100	0	10	1
B	65	35	10	1
C	70	30	10	1
D	75	25	10	1
E	80	20	10	1

Sensory quality evaluation

The fermented beverage samples were evaluated for their sensory characteristics *viz*. color and appearance, flavor, mouth feel, sweetness and overall acceptability by using a 5 point hedonic scale (5 and 1 points showing like very much and dislike very much, respectively) (Watts *et al*. 1989). Trained panels comprising of 10 panelists were drawn from the Department of Dairy Science, BAU, Mymensingh.

Physicochemical characterization

The proximate components total solids, protein, fat and ash as well as pH and titratable acidity were determined. Moisture and ash content were determined according to the AOAC (2000). Protein content was determined by the Micro-Kjeldahl method. Acidity percentage was also determined by titrating with 0.1N sodium hydroxide solution as described by AOAC (2000). The pH of the raw materials and the blends made was recorded by using a pH meter (Hanna, Romania) with the direct immersion of the glass electrode in the samples. The carbohydrates content was determined by the difference between total percentage (100) and the sum of the percentages found for moisture, protein, ash and fat (Brasil, 2003). The fat content was determined by the methodology recommended by Bligh and Dyer (1959) with little modifications.

Microbiological analysis

Total viable count (Log_{10}cfu/mL) was performed as a microbiological assay in the selected blends by standard plate count method according to APHA (1967). In short, plate count agar and sterile saline of 0.9% NaCl (w/v) were prepared. The samples (1 ml) were transferred into sterile saline and each serial dilution, then put into the plate and poured agar (10-15 mL) and allowed for solidification. After solidified, the plates were incubated at 32°C for 48 h. The colonies were enumerated which plate having within 30-300 colonies. In all cases, duplicate-counting plates were prepared for appropriate dilutions.

Statistical analysis

Completely randomized design (CRD) was performed to investigate the effect of fermented beverages on the parameters. Least significant difference (LSD) values were also determined to rank the samples by SPSS version 16.

RESULTS AND DISCUSSION

Sensory evaluation

The sensory attributes of A, B, C, D and E type beverage samples were found significantly different ($p<0.05$) which is depicted in Table 1. The highest color score was found in C type (70% whey and 30% watermelon juice) beverage ($p=0.005$) compared to others which may be due to the proper blend of whey and watermelon. Although, Shukla *et al.* (2013) found that color score 7.93 (9-point hedonic scale) using 65% whey and 35% pineapple juice. By increasing the quantity of watermelon juices the redness of the blends gradually increased although we observed that the color and appearance of C and D type were quite similar those were also shared the score of E type WBW beverage. The highest flavor score was also recorded C type beverage

Table 1: Sensory attributes of whey-based watermelon beverages.

Types of beverages	Color and appearance	Flavor	Mouth feel	Sweetness	Overall acceptability
A	$2.60^{b}\pm0.05$	$2.00^{c}\pm0.02$	$2.24^{b}\pm0.09$	$2.14^{b}\pm0.03$	$2.26^{c}\pm0.14$
B	$2.62^{b}\pm0.05$	$2.12^{c}\pm0.02$	$2.43^{b}\pm0.09$	$2.25^{b}\pm0.03$	$2.67^{c}\pm0.14$
C	$4.97^{a}\pm0.02$	$4.40^{a}\pm0.00$	$4.00^{a}\pm0.05$	$4.85^{a}\pm0.04$	$4.52^{a}\pm0.19$
D	$4.90^{a}\pm0.02$	$4.30^{a}\pm0.09$	$4.00^{a}\pm0.05$	$4.89^{a}\pm0.04$	$4.07^{a}\pm0.19$
E	$4.25^{ab}\pm0.02$	$4.0^{ab}\pm0.09$	$4.02^{a}\pm0.05$	$4.87^{a}\pm0.04$	$3.79^{ab}\pm0.19$
LSD	0.126	0.037	0.210	0.090	0.240
P-value	0.005	0.001	0.040	0.002	0.031

Mean ± Standard error. Values followed by different superscript letter in a column are significantly different ($p \leq 0.05$). A = Whey 100%, B = Whey 65%+ watermelon juice 35%, C = Whey 70%+ watermelon juice 30%, D = Whey 75%+ watermelon juice 25% and E = Whey 80%+ watermelon juice 20%.

Table 2: Physicochemical analysis of whey-based watermelon beverages.

Types of beverages	Acidity (%)	Total solids (%)	Total sugar (%)	Protein (%)	Fat (%)	Ash (%)
A	$0.39^{c}\pm0.01$	$8.17^{c}\pm0.98$	$5.05^{c}\pm0.53$	$2.01^{c}\pm0.25$	$0.22^{ab}\pm0.01$	$0.20^{c}\pm0.08$
B	$0.52^{b}\pm0.01$	$8.17^{c}\pm0.98$	$5.05^{c}\pm0.53$	$2.01^{c}\pm0.25$	$0.22^{ab}\pm0.01$	$0.20^{c}\pm0.08$
C	$0.60^{ab}\pm0.01$	$12.13^{b}\pm0.98$	$10.95^{b}\pm0.53$	$2.39^{a}\pm0.25$	$0.23^{a}\pm0.01$	$0.19^{b}\pm0.08$
D	$0.73^{a}\pm0.01$	$14.06^{a}\pm0.98$	$12.56^{a}\pm0.53$	$2.26^{b}\pm0.25$	$0.23^{a}\pm0.01$	$0.17^{b}\pm0.08$
E	$0.69^{a}\pm0.01$	$13.57^{a}\pm0.98$	$10.83^{b}\pm0.53$	$2.48^{a}\pm0.25$	$0.24^{a}\pm0.01$	$0.29^{a}\pm0.08$
LSD	0.003	2.096	1.060	0.076	0.007	0.025
Level of sig.	0.001	0.001	0.011	0.041	0.012	0.013

Mean ± Standard error. Values followed by different superscript letter in a column are significantly different ($p \leq 0.05$). A = Whey 100%, B = Whey 65%+ watermelon juice 35%, C = Whey 70%+ watermelon juice 30%, D = Whey 75%+ watermelon juice 25% and E = Whey 80%+ watermelon juice 20%.

whereas the lowest score was found in the control group (100% whey and 0% watermelon blend). Like the color and appearance score, the flavor was showed the similar result as increased the amount of fruit juices apparently increased the favor. This finding is in agreement with Devi *et al*., (2004) who stated that the addition of fruit improves the flavor of whey beverages. Further, The C type was found best for mouth feel score compared to other blends which were similar to Panesar *et al*. (2012) who reported that mouth feel of yogurt drink improved due to the addition of fruit juices. Besides, the highest sweetness score of the beverages was noted C, D and E type WBW beverage, the reason can be attributed to the amount of watermelon addition as the fruit juices increases the sweetness of finished products (Ritu *et al*., 2007). Finally, the overall acceptability implied that the best combination of whey and watermelon beverage was recorded for type C (75:25) WBW beverage.

Physicochemical analysis

Chemical parameters like titratable acidity, total solids (TS), carbohydrate, protein, fat and ash content of different types of whey and watermelon beverages were assessed (Table 2). The highest acidity was found in type D and E type WBW beverage samples compared to others and there was a significant difference (p=0.001) that existed among the samples. This variation in acidity level of different blends may be due to the effect of dilution of whey and watermelon juice in beverages at various combinations. Divya and Kumari (2009) found the total acidity of whey-guava beverage 1.27 to 1.45 percent and stated that pasteurization temperatures and timings did not affect the acidity of whey-guava beverage. In addition, Pakseresht *et al*., (2017) found the acidity of different yogurt drinks was 0.45-0.47% which was quite similar to the present research findings. Thus, we could support the level of acidity as the different fruit have various level of acidity that ultimately changed the results of the combinations that we reported. A maximum amount of TS were found in type D, although the other types blended with watermelon showed more or less close values but significantly differed from the A and B type (p=0.001). Kumar *et al*., (2003) also found the highest TS in whey: sweet orange beverage (65:35) followed by whey: sweet orange beverage (70:30). The total carbohydrate content was noted a greater amount for the type D beverage (13%) with was double in percentage compared to the control group. Although both types of C and E were found the same carbohydrate contents each with 11%. The reason behind this case may be attributed to the sugar content of the fruit juices and the whey because the additional cane sugar was constant for each sample. The highest protein content around 2.5% was observed in both C and E type and the lowest was found in the control group. The increasing trend of protein per cent evidenced that the watermelon added very few amounts of protein by increasing the blends ratio (Table 2). Having said that, the watermelon juices contains very little amount of protein 0.6% (data not shown elsewhere) (Wani *et al*., 2008). In addition, Pareek *et al*. (2014) found higher protein in orange juice carbonated whey drink in the

Table 3: Shelf-life assessment of the highest scoring blends (75:25) of whey-based watermelon beverage.

Storage period(day)	Acidity (% Lactic acid) 30 °C	Acidity (% Lactic acid) 4 °C	pH 30 °C	pH 4 °C	TVC (Log_{10}CFU /mL) 30 °C	TVC (Log_{10}CFU /mL) 4 °C	Overall acceptability 30 °C	Overall acceptability 4 °C
0	0.60^{ab}±0.06	0.55^{c}±0.04	4.45^{a}±0.02	4.45^{a}±0.01	3.55^{b}±0.27	3.52^{cd} ±0.27	4.50^{a}±0.08	4.80^{a}±0.08
4	0.57^{abc}±0.02	0.52^{d}±0.02	4.30^{ab}±0.02	4.40^{a}±0.02	4.50^{a}±0.47	3.80^{b}±0.27	4.07^{a} ±0.19	4.07^{ab} ±0.19
7	0.67^{a}±0.02	0.57^{c}±0.02	4.25^{ab}±0.01	4.39^{a}±0.01	4.75^{a}±0.47	3.80^{b}±0.27	3.10^{b} ±0.19	4.40^{b} ±0.19
10	0.69^{a}±0.02	0.66^{a}±0.02	4.20^{ab}±0.02	4.35^{a}±0.01	4.88^{a}±0.47	4.47^{a}±0.27	2.26^{c}±0.19	4.26^{ab}±0.19
14	0.72^{a}±0.02	0.62^{b}±0.02	4.00^{c}±0.02	4.30^{b}±0.01	3.08^{b}±0.47	4.82^{a}±0.27	2.02^{cd}±0.19	4.82^{a}±0.19
17	0.64^{ab}±0.02	0.53^{b}±0.02	3.90^{c}±0.02	4.30^{b}±0.02	2.39^{c}±0.47	4.01^{ab}±0.27	2.00^{cd}±0.19	4.55^{b}±0.19
21	0.60^{ab}±0.02	0.56^{b}±0.02	3.30^{cd}±0.01	4.20^{b}±0.02	2.39^{c}±0.47	3.90^{d}±0.27	2.00^{cd}±0.19	4.00^{ab}±0.19
28	0.55^{abc}±0.02	0.48^{c}±0.02	3.20^{cd}±0.02	3.90^{c}±0.02	2.20^{c}±0.47	3.30^{d}±0.27	1.88^{d}±0.19	2.88^{c}±0.19
LSD	0.088	0.092	0.078	0.094	0.065	1.094	0.046	0.061
p-value	0.001	0.001	0.003	0.012	0.021	0.040	0.002	0.011

Mean ± Standard error. Values followed by different superscript letter in a column are significantly different (p d” 0.05). Overall acceptability was determined on the basis of sensory evaluation using 5-point hedonic scale. TVC = Total Viable Count.

ratio of 70:30 which was 1.1%. Again, Holsinger *et al*. (1978) reported earlier in their study that an orange-flavored carbonated whey beverage contained 1.5% proteins obtained by reverse osmosis from whey. We observed a lower level of significance in the fat content among the types of beverages. The facts regarding fat per cent in the WBW beverage were found very low that might be attributed to the fat content of whey and watermelon. On the contrary, the ash content was recorded highest in the case of type E (0.29%) which owing to a higher level of juices because the ash content of whey might be diluted with fruit juice (Saeed *et al*., 2013).

Shelf-life assessment

Based on sensory evaluation and nutrient content from the proximate analysis the Type D (75:25=Whey: Watermelon) was found as the best blend. Thus, the shelf life of the selected blend was assessed. The data on overall acceptability (OA) showed that there was a significant ($p<0.01$) changes during both room temperature (30±1.5°C) and refrigerated conditions (5±1.0°C). The OA significantly differed throughout the storage period at the refrigerated condition and the highest value was noted at 14^{th} day (4.82) but the acceptability of the resultant WBW beverage was found up to 21 days (4.00). On the contrary, the score for room temperature beverage showed acceptability only up to day 2 (Table 3). This result was agreed with Shukla *et al*. (2013) who reported that the fruit-based whey beverage was acceptable for 24 days at refrigerated storage at 5°C and 48 h at room temperature condition. Furthermore, Hossain *et al*., (2017) stated that organoleptic score decreased gradually with an increase in storage period at room temperature (24.4-38.5°C). Likewise, Baljeet *et al*. (2013) reported that the whey-based pineapple beverage had better up to 15 days of refrigerated storage.

The total viable count (TVC) significantly differed during storage conditions are shown in Table 3. The initial TVC of the beverage was 3.50 Log_{10}CFU/mL in both storage conditions. We noticed that the TVC was primarily increased up to day 10 at room temperature storage and followed by decreasing the count in the advancement of the storage period. On the contrary, the refrigerated conditions showed that the TVC was increasing up to 14^{th} day but the count up to 21^{th} day was recognized as acceptable for use as a functional food. This finding is in agreement with the research work of Mendoza *et al*. (2017); Divya and Kumari (2009) who reported that decline in the TVC of *Lactobacillus reuteri* and *Biofidobacterium bifidum* whey-based probiotic beverage during storage at 4±1°C. Similarly, Shah *et al*. (1995) studied the survival of *Lactobacillus acidophilus* in commercial yogurt during refrigerated storage.

We found a significant difference in response to the acidity at both storage conditions. The results revealed a remarkable change in both conditions in which the acidity value in case of cold storage was found as a decreasing trend compared to room temperature storage conditions (Table 3). After the first week of storage, the acidity was gradually increased up to the third week of preservation. The highest acidity was recorded during the second week of storage in both cases. However, the average titratable acidity was 0.63 and 0.56% in terms of lactic acid at room temperature and refrigerated condition, respectively, which is almost similar to the results obtained for probiotic whey-pineapple beverages by Shukla *et al*. (2013). The causes behind the acidity spectrum might be due to the optimum temperature for enzymes or metabolic activities of microorganisms in the WBW beverage. On the contrary, changes in pH value during the preservation at two different conditions were found significantly different ($p<0.05$). The average pH value was observed for the WBW beverage was 3.95 and 4.28 at 30 and 4°C, respectively. Bensmira and Jiang (2011) reported a drop in the pH value of peanut-milk kefir during fermentation. They attributed this drop to a decrease in lactose amount and a consequent increase in lactic acid content. On the other hand, Magalhães *et al*. (2010) reported the production of lactic acid during fermentation of the beverage is of great importance due to its inhibitory effect on both spoilage and pathogenic microorganisms. So it can be said a reduction in pH value moreover leads to an increase in total acidity, causes inhibition of spoilage and pathogenic microorganism growths.

Conclusion

It can be summarized that whey bids a good option for the development of functional whey-based watermelon (WBW) beverages. Thus, the study was targeted to formulate a fermented WBW beverage using different blends of *Chhana* whey and watermelon juice with the addition of *Lactobacillus acidophilus* as the probiotic bacterial culture. The study has revealed competently good quality probiotic beverage by using a 75:25 blend of whey and watermelon juice with a shelf life of 21 days at refrigerated condition (4 ± 1°C) and 2 days at room temperature (30 ± 1°C) condition.

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