

Development, Quality Evaluation and Shelf Life Studies of Probiotic Beverages using Whey and *Aloe vera* Juice

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Abstract

The investigation was aimed to develop a probiotic beverage using whey and *Aloe vera* juice. *Bifidobacterium bifidus* (BB) was used as the probiotic organism, the level of whey and *Aloe vera* juice, addition of probiotic inoculums and fermentation time were optimized on the basis of sensory quality evaluation. The blend ratio 70:30 of whey and *Aloe vera* juice and whey fermented for 9 hrs using inoculums 1% BB resulted with highest sensory scores for overall acceptability. The developed probiotic beverage could be recommended for the large scale production at industrial level.

Keywords: Whey; Probiotic beverage; *Aloe vera* whey beverage; Sensory quality; Shelf life studies

Introduction

Milk whey drink can replace much of the lost organic and inorganic to the extracellular fluid. Whey, which is so rapidly assimilable, forms an ideal metabolic substrate. Whey is one of the highly nutritious by-products obtained from the dairy industry producing cheese, *chhanna* and *paneer*. It constitutes almost 45-50 percent of total milk solids, 70 percent of milk sugar mainly lactose, 20 percent of milk proteins, 70-90 percent of milk minerals and almost all the water soluble vitamins originally present in milk [1,2]. It resulted into unravelling the secrets of whey proteins and other components and established a sound basis for their nutritional and functional value [3]. Several authors have investigated the possibility of utilizing the milk whey in the fruit beverage preparation [4]. The manufacture of whey based beverage requires the mixing of appropriate fruit juices and minimally processed whey with selection of suitable stabilizers and acidulants to develop acceptable whey based fruit beverages [5].

The functional beverages covers probiotic beverages, dairy drinks, drinking yoghurts, functional and functionally fortified soft drinks, juices, energy drinks, sports drinks, functional waters and ready-to-drink tea. The global functional beverages market drives due to the growth in health and wellness concerns increase in disposable incomes, consumer awareness, introduction of new flavoured products meeting various nutritional and health requirements, obesity concerns, aging population and changing lifestyles [6]. Energy drinks is the fastest growing segment in functional drinks market followed by functional dairy products and nutraceutical drinks.

Functional Drinks Reassessing future potential, determining consumer targets, and delivering credible products provides a comprehensive overview of the functional drink landscape, analyzing the regulatory and consumer drivers to identify the best opportunities and strategies [7]. The global regulatory environment is examined and key hubs for innovation are highlighted. The report investigates the opportunities that the functional drinks market provides for beverage manufacturers as well as the challenges that diversification into functional beverages presents.

Fresh *Aloe vera* leaves used to obtain two components, firstly bitter yellow latex from peripheral bundle sheath of aloe, called *Aloe vera* sap and a mucilaginous gel from parenchymatous tissue. The interest and use of gel has increased dramatically in the field of health care and cosmetics [8]. It can be utilized as a valuable ingredient for food

application due to its biological activities and functional properties [9]. *Aloe vera* has a bitter taste which can be unpleasant in raw state and its palatability could be enhanced with addition of some other fruit juices.

The conception of probiotic came into focus in early 1900's but the term "probiotic" was suggested by Lilly and Stillwell. Probiotic cultures are live microbial food ingredients that are beneficial for human health [10], which includes improvement of intestinal microbial balance which results in the inhibition of bacterial pathogens, reducing the risk of colon cancer, improving the immune system, lowering serum cholesterol levels [11], alleviation of lactose intolerance and nutritional enhancement [12].

The probiotic culture like *Bifidobacterium bifidum* specific organism used as a probiotic and its health promoting effects [13,14] and in recent years, which has been considered important bacteria for human health; the main probiotic effects attributed to these bacteria include: improvement in lactose utilisation, prevention of diarrhoea, colon cancer, hypercholesterolemia, improvement of vitamin synthesis and calcium absorption [15], development of longer villi and significantly production of substances of low molecular mass with antimicrobial activity [16].

The manufacture of whey-based beverages requires the mixing of appropriate fruit juices and minimally processed whey with selection of suitable stabilizers and acidulants to develop acceptable whey based fruit beverages [17]. Recently, the key growth sector in utilization of whey has been probiotic drinks and their role in fermentation processes, some probiotic lactic acid bacteria have been studied as dietary sources of live microorganisms destined to promote a positive impact in the host by improving the properties of the indigenous beneficial microflora [18]. Based on these facts, the present investigation was conducted to develop a probiotic beverage using whey and *Aloe vera* juice and to study its storage stability.

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Materials and Methods

Sample preparation

Skim milk was heated in a stainless steel vessel to 95°C followed by cooling to 70°C. The hot milk was acidified by adding 2% of citric acid solution followed by continuous stirring which resulted in the complete coagulation of the milk protein (casein). The liquid (whey) was filtered using muslin cloth. The prepared whey was heated to 85°C before blending with *Aloe vera* juice.

The freeze dried probiotic culture *Bifidobacterium bifidus* NCDC 255 was obtained from National Dairy Research Institute, Karnal, India. Culture was cultivated in 10% non-fat dry milk, autoclaved at 115°C for 15 min, inoculated under sterile conditions, incubated at 42 ± 1°C and kept in refrigerator at 4 ± 1°C until used.

***Aloe vera* juice:** *Aloe vera* juice, chemical composition of juices depends majorly upon the method of juice extraction [19]. *Aloe vera* juice was extracted using cold extraction method and processed into juice as per the method reported by Williams [20]. Freshly harvested *Aloe vera* leaves were dipped into 500 ppm of potassium meta bisulphite (KMS) solution and washed thoroughly with tap water and kept for flash cooling to 5°C for juice stabilization. Further leaves were cut vertically into two half and juice was separated using stainless steel knife, it was allowed to settle for 12 hrs and then homogenized using mixer grinder and enzymatically treated with 1% pectolytic enzyme at 50°C for 20 minutes. Then it was filtered and pH was adjusted to 3.0 by adding citric acid and ascorbic acid to control browning while high heat treatment. Further it was deaerated, pasteurized, flash cooled and stored. During the pasteurization pectolytic enzyme was inactivated. The obtained juice was stored refrigerated temperature until further use.

Probiotic beverage process: The combination of whey and *Aloe vera* juice were optimized with different blends ratio, such that sample A (65wy:35Av), sample B (70wy:30Av), sample C (75wy:25Av) and sample D (80wy:20Av). The beverage incubation period was optimized by inoculating 1% of probiotic culture (*Bifidobacterium bifidus*) to whey and sample with addition of sugar level 15% and fermented for 5, 9, 13, 17 and 21 and 24 hrs then the beverages were evaluated for sensory characteristics, total viable count, pH, titratable acidity, storage stability and shelf life studies carried out for sample with different blend and fermented periods.

Analytical methods

Bio-chemical and microbiological methods: Total soluble solid was measured using a hand refractometer of 0-32°B (ERMA make). The pH of the beverages was determined using the digital pH meter (Model No. 5633, Electronics Corporation of India Ltd., Hyderabad). Titratable acidity was determined according to the AOAC [21] method. Viable counts in the samples were determined according to A.P.H.A. [22] procedure using lactic agar [23].

Statistical analysis

Statistical procedures as described by Snedecor and Cochran [24] were used to analyze the data for the interpretation of results. Mean, standard deviation and analysis of variance (ANOVA) were used to describe the results.

Sensory quality evaluation

The beverage samples were evaluated as described by Larmond [25] for their sensory characteristics namely colour and appearance, taste and flavour, body or consistency and overall acceptability by a trained

panels. The panellists were asked to record their observations on the sensory sheet based on a 9 points hedonic scale (9 and 1 points showing like extremely and dislike extremely).

Results and Discussion

Optimization of whey and *Aloe vera* juice blend ratio levels for beverage preparation

Whey and *Aloe vera* juice were blended in five different proportions i.e. A (65wy:35Av), B (70wy:30Av), C (75wy:25Av) and D (80wy:20Av) and evaluated for sensory attributes namely colour and appearance, consistency, flavour and overall acceptability. The sample B was highest score for flavour, consistency, colour and appearance to beverage preparation. The mean scores of this blend for colour and appearance, consistency, flavour and overall acceptability were 7.93, 7.81, 8.37 and 8.93 respectively. Therefore, sample B was chosen for the further investigation and differed significantly ($P < 0.05$) and rated best among others samples.

Optimization of growth conditions for *Bifidobacterium bifidus* (BB) in beverage

Whey and sample B were supplemented with 15% sucrose and fermented for different time intervals. Variations in fermentation time were studied in terms of overall acceptability, pH, titratable acidity and total viable counts of the beverage. The effect of *Bifidobacterium bifidus* (BB) on sensory attributes of whey with or without addition of *Aloe vera* juice during fermentation has been shown in Table 1. Colour and appearance of the beverage was significantly ($P \leq 0.05$) affected by the incubation period. The mean scores for colour and appearance ranged from 7.56 to 5.87 for whey. The mean score was highest (7.56) for whey fermented for 9 hrs. The sensory scores for consistency reduced significantly ($P \leq 0.05$) with increase in fermentation time for both whey and sample B.

The sensory score for consistency of fermented whey ranged from 7.55 to 5.97 but the sensory scores for consistency of sample B was ranged from 8.12 to 6.15. The sensory scores for flavour ranged from 7.21 to 5.17 for whey fermented alone and from 7.91 to 6.97 for sample B. The mean score for flavour decreased significantly with increasing fermentation time irrespective of the medium. The mean score for overall acceptability ranged from 7.31 to 5.80 for whey and 8.10 to 6.34 for sample B. Highest score for overall acceptability was seen in case of sample B fermented for 9 hrs.

Enumeration of total viable counts

The viable counts were assessed after 5, 9, 13, 17, 21 and 24 hrs of incubation at 37°C. It was observed that whey gave higher viable counts (9.54×10^8 cfu/ml) up to 24 hrs of fermentation. But after 24 hrs, the sample B fermented alone, gave higher viable counts ranging from 3.35×10^7 to 8.10×10^8 cfu/ml. Both whey and sample B attained a total viable count of more than 5.0×10^8 cfu/ml within 13 hrs of fermentation (Table 2). The total viable count increased significantly with increase in fermentation time from 5 to 24 hrs. The total viable count for whey ranged from 2.23×10^7 to 9.54×10^8 cfu/ml. Sample B showed the stable increase total viable count with increasing fermentation time as controlled way but the whey alone showed uncontrolled fermentation with increasing fermentation time.

Effect of probiotic on pH and titratable acidity

Table 3 shows the effect of incubation period on the pH of whey and sample B, pH of the samples (whey and sample B) reduced significantly

Sr.No	Name of the sample	Sensory Scores *			
		Colour and Appearance	Consistency	Flavour	Overall Acceptability
1	Sample A	7.15 ^B	7.21 ^{AB}	7.38 ^B	7.23 ^B
2	Sample B	7.91 ^A	7.86 ^A	8.21 ^A	8.54 ^A
3	Sample C	6.95 ^B	6.81 ^B	7.11 ^C	7.12 ^C
4	Sample D	6.72 ^C	6.68 ^C	6.92 ^D	6.96 ^D
5	S.E.M	0.189	0.186	0.153	0.123
6	CD at 5%	0.533	0.525	0.452	0.361

Sample A (65wy:35Av), Sample B (70wy:30Av), Sample C (75wy:25Av) and Sample D (80wy:20Av)

*Average of four trials

* Means by different letters (A, B, C, D) as superscripts in a column differ significantly at 5% level

Table 1: Effect of different blends of whey and *Aloe vera* juice on the sensory characteristics of the beverage.

Fermentation Time (hrs)	Sensory Scores							
	Colour and Appearance		Consistency		Flavour		Overall Acceptability	
	Wy	B	Wy	B	Wy	B	Wy	B
5	7.15	7.66	7.18	7.55	7.10	7.55	7.11	7.60
9	7.56	8.12	7.55	8.11	7.21	7.91	7.31	8.10
13	6.95	7.25	7.10	7.25	6.75	7.45	6.87	7.50
17	6.34	7.10	6.30	7.14	6.14	7.24	6.31	7.20
21	6.21	6.95	6.11	6.21	5.43	7.13	5.91	6.98
24	5.87	6.17	5.97	6.15	5.17	6.97	5.80	6.34
	S.E.M	CD at 5%	S.E.M	CD at 5%	S.E.M	CD at 5%	S.E.M	CD at 5%
Medium (A)	0.043	0.123	0.045	0.093	0.053	0.135	0.53	0.135
Fermentation Time (B)	0.069	0.187	0.053	0.156	0.087	0.213	0.073	0.198
AXB	0.081	0.267	0.076	0.213	0.123	0.293	0.121	0.287

*Wy-Whey, Sample B (70wy:30Av)

Table 2: Effect of *Bifidobacterium bifidus*(BB) on sensory characteristics of whey and sample B at 37 ± 1°C.

($P < 0.05$) with increasing fermentation time. The mean value of pH obtained in case of whey fermented alone ranged from 4.85 to 3.43. The mean value of pH obtained in case of sample B ranged from 4.65 to 3.44. Higher viable counts during the last stage of fermentation (24 hrs) resulted in comparative lowering of pH for whey and sample B. The sample B showed gradual decrease pH value with increase of fermentation time because of controlled fermentation during entire 24 hrs, but whey alone showed uncontrolled fermentation process for 24 hrs.

Table 3 shows the effect of incubation period on the titratable acidity of whey and sample B. Titratable acidity increased significantly ($P \leq 0.05$) with increasing fermentation time irrespective of the medium. The mean values obtained for whey ranged from 0.390 to 1.293. The mean values obtained for sample B ranged from 0.453 to 0.945. Sample B showed balanced titratable acidity from 5 to 24 hrs of fermentation process, because of *Aloe vera* juice blend with whey, whereas there was a significant increase in the titratable acidity with respect of increasing fermentation time (5 to 24 hrs) for whey and sample B. The commercial probiotic beverage should possess a minimum viable count of 4.11×10^7 cfu/ml with optimum fermentation time (9 hrs) cfu/ml and should also have an acceptable flavour. The commercial probiotic beverage should possess a minimum viable count of 106 cfu/ml [26] and should also have an acceptable flavour.

Keeping in view these aspects, experiments were carried out to appraise the suitability of whey with or without the addition of *Aloe vera* juice as a growth medium for *Bifidobacterium bifidus* (BB) for the preparation of probiotic beverage. It was observed that sample B for 9 hrs gave the highest scores for overall acceptability (8.10) as compared to whey fermented alone (7.31). The scores for overall acceptability gradually declined with increasing fermentation time irrespective of the

medium. Table 4 shows that the pH and titratable acidity affects colour and appearance, consistency, flavour profile and overall acceptability of a beverage. In the present study, it was observed that sample B having a pH of 4.50 and titratable acidity of 0.525% gave the best colour and appearance, consistency, flavour profile and overall acceptability to the probiotic beverage. These observations exhibited an acceptable beverage by fermenting a blend of whey and *Aloe vera* juice (70:30) that is sample B for a period of 9 hrs. The prepared product had desired health benefits due to the probiotic organisms.

Storage studies of probiotic beverage

The changes in sensory attributes on storage studies of the probiotic beverage have been shown in Table 4 for refrigerated and ambient storage temperatures. The whey and *Aloe vera* based probiotic beverage did not show sensory differences for the first 15 days at refrigerated storage. But after the 15 days, difference was perceived in colour and appearance and flavour. According to a consensus made with the panelists during sensory evaluation, it was determined that the main descriptors that characterized the product were acidity and sweetness, with acidity being the attribute responsible for the sensory difference perceived by the panelists. Even though a slight acidification was detected by the sensory panels and agreed that the beverage was acceptable for a period of 30 days at $4^\circ\text{C} \pm 1^\circ\text{C}$ and 6 days at $35^\circ\text{C} \pm 1^\circ\text{C}$.

The pH of the fresh beverage prepared from blend of whey and *Aloe vera* juice fermented for 5 hr was 4.40. The pH of the samples gradually declined during 30 days at refrigerated storage. The pH ranged from 4.40 to 4.00 after 30 days of storage. During storage of the samples at ambient temperature, pH lowered significantly ($P \leq 0.05$) after 6 days. The initial acidity of the fermented beverage prepared

Sr.No	Fermentation Time (hrs) @ 37°C	Medium		
		Whey (cfu/ml)	Sample B (cfu/ml)	
1	5	2.23×10 ⁷	3.35×10 ⁷	
2	9	3.23×10 ⁷	4.11×10 ⁷	
3	13	5.90×10 ⁸	5.18×10 ⁸	
4	17	8.23×10 ⁸	6.88×10 ⁸	
5	21	9.10×10 ⁸	7.64×10 ⁸	
6	24	9.54×10 ⁸	8.10×10 ⁸	
		Medium (A)	Fermentation Time (B)	AXB
	SEM	3.11×10 ⁴	4.31×10 ⁴	6.55×10 ⁴
	CD at 5%	8.91×10 ⁴	1.22×10 ⁵	2.14×10 ⁵

Table 3: Effect of fermentation period on the total viable count of whey with and without *Aloe vera* juice.

Sr.No	Fermentation Time	Medium				
		Whey		Sample B		
		pH	T.A %	pH	T.A %	
1	5	4.85	0.390	4.65	0.453	
2	9	4.55	0.496	4.50	0.525	
3	13	4.13	0.915	4.25	0.680	
4	17	3.98	1.023	4.00	0.796	
5	21	3.60	1.165	3.87	0.815	
6	24	3.43	1.293	3.44	0.945	
		Medium (A)		Fermentation Time (B)		AXB
	SEM	0.0250		0.00051		0.0716
	CD at 5%	0.0564		0.0219		0.0303

* Incubation temperature: 37 ± 1°C.

Table 4: Effect of fermentation period on the pH and titratable acidity of whey with and without *Aloe vera* juice.

Sr.No	S.P (d) at 4°C	pH	T.A. (%) La	TPC (cfu)	S.P (d) at 35°C	pH	T.A (%) La	TPC (cfu)
1	0	4.40	0.553	3.5×10 ⁷	0	4.40	0.553	3.5×10 ⁷
2	5	4.39	0.551	3.1×10 ⁷	1	4.35	0.610	5.2×10 ⁸
3	10	4.38	0.563	2.7×10 ⁷	2	4.30	0.723	8.7×10 ⁸
4	15	4.37	0.580	2.7×10 ⁷	3	4.26	0.793	6.1×10 ⁷
5	20	4.35	0.594	2.1×10 ⁷	4	4.10	0.843	4.4×10 ⁷
6	25	4.35	0.610	1.7×10 ⁷	5	4.00	0.910	3.1×10 ⁷
7	30	4.00	0.870	1.3×10 ⁷	6	3.83	1.120	2.3×10 ⁷

Table 5: Changes in pH, total viable count and titratable acidity of the sample B probiotic beverage during storage.

SP(d) at 4°C	C and A	Cons	Flav	OAA	SP(d) at 35°C	C and A	Cons	Flav	OAA
0	8.53 ^a	8.65 ^a	8.74 ^a	8.63 ^a	0	8.58 ^a	8.60 ^a	8.73 ^a	8.67 ^a
5	8.51 ^a	8.64 ^a	8.73 ^a	8.63 ^a	1	8.45 ^a	8.50 ^a	8.68 ^a	8.58 ^a
10	8.36 ^a	8.40 ^{b,c}	8.51 ^a	8.43 ^a	2	8.00 ^a	8.15 ^{a,b}	8.14 ^b	8.15 ^a
15	8.11 ^a	8.00 ^{b,c}	7.91 ^b	8.13 ^{b,c}	3	7.83 ^b	7.95 ^b	7.75 ^c	7.88 ^b
20	7.93 ^b	7.95 ^c	7.74 ^c	7.73 ^c	4	7.13 ^c	7.15 ^c	7.00 ^d	7.14 ^c
25	7.20 ^c	7.15 ^{d,e}	7.00 ^d	7.17 ^d	5	6.51 ^d	6.65 ^d	6.14 ^e	6.60 ^d
30	6.55 ^d	6.34 ^e	6.14 ^e	6.51 ^e	6	6.00 ^e	5.95 ^e	5.74	5.93 ^e
S.E.M	0.059	0.071	0.062	0.051	S.E.M	0.128	0.103	0.148	0.102
CD at 5%	0.171	0.027	0.184	0.157	CD at 5%	0.364	0.326	0.396	0.215

*Means by different superscript as letters (a,b,c,d,e) in a column differ significantly at 5% level SP(d) - Storage Period (day); C&A - Colour and Appearance; Cons.-Consistency; Flav-Flavour; OAA - Overall acceptability.

Table 6: Changes in sensory characteristics of the sample B probiotic beverage during storage

from whey and *Aloe vera* juice was 0.553% (La). The acidity increased during the refrigerated storage from 0.546 to 0.870% after 30 days. The increase in acidity was more prominent in case of storage at ambient temperature wherein the acidity reached 1.120% after 6 days of storage (Table 5). It showed that the titratable acidity values were significantly influenced by the days of storage of whey beverage both at refrigeration and room temperature. Rodas et al. [27] showed that the titratable

acidity values were significantly influenced by the day of storage whey beverage both at refrigerated and room temperature.

Total viable counts of probiotic beverage during storage

The initial total viable count of the beverage was 3.5×10⁷ cfu/ml which decreased to 1.3×10⁷ at refrigerated storage (4°C ± 1°C). Although the viability of *Biofidobacterium bifidum* (*B.p*) population decreased,

the viable count of the probiotic beverage did not fall below 10^7 cfu/ml (Table 6). During storage at $35^\circ\text{C} \pm 1^\circ\text{C}$ the total viable count first increased to 8.7×10^7 cfu/ml (in 2nd day) and then gradually declined to 2.3×10^7 cfu/ml after 6 days. Our results are in confirmation with other researchers [28] who also reported a decline in total viable count of *Lactobacillus acidophilus*, *Lactobacillus reuteri* and *Bifidobacterium bifidum* of whey based probiotic beverage stored at $4^\circ\text{C} \pm 1^\circ\text{C}$. Wang et al. [28], studied the survival *Bifidobacterium bifidum* (*B.p*) of in commercial yoghurt during refrigerated storage.

Conclusion

Above study has revealed satisfactorily good quality probiotic beverage with therapeutic value prepared by using a 70:30 blend of whey and *Aloe vera* juice inoculated with 1 percent inoculum of *Bifidobacterium bifidum* (*B.p*) with a shelf life of 30 days at $4^\circ\text{C} \pm 1^\circ\text{C}$ and 6 days at $35^\circ\text{C} \pm 1^\circ\text{C}$.

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