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Development of Probiotic Beverage from Whey and Pineapple Juice

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Abstract

The aim of this study was to develop a probiotic beverage using whey and pineapple juice. *Lactobacillus acidophilus* was used as the probiotic organism. The level of pineapple juice addition was optimized on the basis of sensory quality evaluation. Fermentation time using 1 per cent inoculum of *L. acidophilus* was optimized on the basis of sensory quality evaluation, growth and activity in terms of pH and acidity. The 65:35 blend ratio of whey and pineapple juice fermented for 5 hr gave desirable results with highest sensory scores for overall acceptability and a total viable count of more than 10^6 cfu.ml^{-1}.

Keywords: Whey; Probiotic beverage; Pineapple whey beverage; Sensory quality; Storage stability

Introduction

The loss of body fluids from exertion, temperature or age gives rise to thirst, which is offset by drinking. The fluids lost are also accompanied by a loss of electrolytes, vitamins, lactates, amino acids and other organic components. A whey drink can replace much of the lost organics and in organics to the extracellular fluid. Whey, which is so rapidly assimilable, forms an ideal metabolic substrate. Whey is a genuine thirst quencher, unlike most soft drinks. Whey drinks are light and refreshing but less acidic than fruit juices [1]. The medicinal and nutritive value of sweet and acidic whey can be utilized with fruit juices/pulp and concentrates in developing acceptable long life beverages which appear to be the most obvious and logical avenue for utilizing the nutrients of whey into the human food chain. 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 [2]. Recently, the key growth sector in utilization of whey has been Probiotic drinks. In addition to 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 micro biota [3]. Documented benefits of the ingestion of probiotics include: reduction of serum cholesterol, alleviation of lactose intolerance, reduction of cancer risk, resistance to enteric pathogens, among others [4]. Based on these facts, the present investigation was conducted to develop a probiotic beverage from whey and pineapple juice and to study its storage stability.

Materials and Methods

Material collection and sample preparation

Skim milk was procured from Student's Dairy Plant, Department of Food Science & Technology, G.B. Pant University of Agriculture and technology, Pantnagar. Pineapple fruits (Variety Queen) were procured from a local market in Pantnagar. The freeze dried culture of Lactobacillus acidophilus NCDC-015 was obtained from Culture Collection Centre, National Dairy Research Institute, Karnal (Haryana). 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 two per cent 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 fruit juice. Pineapples were de-crowned and peeled followed by cutting into small pieces. The fruit pieces were grinded in a mixer and the resulting pulp was passed through a double layered muslin cloth to extract the juice. The freeze dried culture was activated using a 3 percent (w/v) Litmus milk solution. The culture was maintained bi-weekly transfers and stored at 5 ± 1°C.

Probiotic beverage process

The combination of whey and pineapple juice was optimized by preparing a beverage with different levels of pineapple juice and subjecting to sensory evaluation by a trained sensory panels using a 9 point hedonic scale for color, consistency, flavor and overall acceptability. The various blends of whey-pineapple juice prepared using a constant sugar level of 10% were 80w:20P, 75w:25P, 70w:30P and 65w:35P. The blend that was rated best after sensory evaluation was selected for acidification with *Lactobacillus acidophilus*. The incubation period was optimized by inoculating whey with 1% inoculum of *Lactobacillus acidophilus* with or without the addition of pineapple juice. The beverage was evaluated for sensory characteristics, total viable count, pH and titratable acidity for samples fermented for 5, 10, 15, 20 and 24 h. For storage stability, the optimized beverage samples were stored at 5 ± 1°C and 30 ± 1°C. Changes in sensory characteristics, total viable counts, pH and titratable acidity were studied during storage.

Analytical methods

Chemical and microbiological methods: Total soluble solids 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 [5] method. Reducing sugar, Non reducing sugar and total sugars were determined by the

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method described by Ranganna [6]. Ash content and Moisture were determined according to the AOAC [5] methods. Protein estimation was done by Micro-Kjeldahl method [5]. Viable counts in the samples were determined according to A.P.H.A.[7] procedure using lactic agar [8].

Bio-chemical, microbiological and sensory quality evaluation of beverage during storage: The beverage samples prepared from an optimized 65:35 blend of whey and pineapple juice with addition of 10% sugar was fermented for 5 hrs using a 1% inoculum of *Lactobacillus acidophilus*. The beverage samples so obtained were stored at refrigeration temperature (5 ± 1°C) and ambient temperature (30 ± 1°C) and analyzed for biochemical, microbiological and sensory attributes at an interval of 4 d and 24 hrs, respectively.

Statistical analysis

Statistical procedures as described by Snedecor and Cochran [9] 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 [10] for their sensory characteristics namely color and appearance, taste and flavor, body or consistency and overall acceptability by a trained panels comprising of 25 panelists drawn from faculty members and post graduate students of the Department. The panelists were asked to record their observations on the sensory sheet based on a 9 point hedonic scale (9 and 1 points showing like extremely and dislike extremely).

Results and Discussion

Proximate analysis

The total solids in whey, pine apple juice and Probiotic whey based pine apple beverage were 6.40, 12.79 and 12.80%, respectively whereas titratable acidity was 0.18, 0.87 and 0.546% lactic acid. Probiotic beverage (65w:35P) had 0.546% acidity, protein (0.23%), ash (0.51%), total soluble solids (12.2B), reducing sugars (1.48%) and non reducing sugars (9.96%) respectively.

Optimization of pineapple juice level in whey for beverage preparation

Whey and pineapple juice were blended in four different proportions i.e. 80:20, 75:25, 70:30 and 65:35 and evaluated for sensory attributes namely color and appearance, consistency, flavor and overall acceptability. The highest score for flavor, consistency, color and appearance was given to the beverage containing pineapple juice and whey in the ratio of 35:65.The mean scores of this blend for color and appearance, consistency, flavor and overall acceptability were 7.93, 7.81, 8.37 and 8.93 respectively. Therefore, the 65w:35P blend of whey and pineapple juice was chosen for the further course of investigation. The blend (65w:35P) differed significantly ($P<0.05$) and rated best among others blends (Table1).

Optimization of growth conditions for *Lactobacillus acidophilus* in probiotic beverage development

Variations in fermentation time and medium were studied in terms of Overall acceptability of the beverage, total viable counts, pH and titratable acidity. Whey supplemented with 10% sucrose was fermented for different time intervals with or without the addition of pineapple juice. The effect of *Lactobacillus acidophilus* on sensory attributes of whey with or without pineapple juice during fermentation has been shown in table 2. Color and appearance of the beverage was significantly ($P\leq0.05$) affected by the incubation period. The mean scores for color and appearance ranged from 8.83 to 5.99 for whey and from 8.92 to 6.53 for whey with pineapple juice. The mean score was highest (8.92) for whey fermented along with pineapple juice for 5 h. The sensory scores for consistency reduced significantly ($P\leq0.05$) with increase in fermentation time for both whey and whey-pineapple juice blend. The sensory score for consistency of fermented whey ranged from

Whey : pineapple ratio	Color and appearance	Consistency	Flavor	Overall acceptability
80 : 20	6.31^{C}	6.37^{C}	6.18^{D}	6.18^{D}
75 : 25	6.93^{B}	6.87^{B}	6.75^{C}	6.93^{C}
70 : 30	7.18^{B}	$7.25^{A,B}$	7.37^{B}	7.37^{B}
65 : 35	7.93^{A}	7.81^{A}	8.37^{A}	8.93^{A}
S.E.M.	0.187	0.185	0.159	0.121
CD at 5%	0.543	0.537	0.462	0.35

*Average of four trials

[1] Means followed 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 pineapple juice on the sensory characteristics of the beverage*.

Fermentation time (h)	Color and Appearance		Consistency		Flavor		Overall acceptability	
	Whey	Whey-pine-apple juice blend (65:35)	Whey	Whey-pine-apple juice blend (65:35)	Whey	Whey-pine-apple juice blend (65:35)	Whey	Whey-pine-apple juice blend (65:35)
5	8.83	8.92	8.74	8.69	8.58	8.88	8.65	8.87
10	8.63	8.39	8.29	8.21	8.33	8.42	8.26	8.3
15	8.08	8.07	8.09	8.03	7.88	7.16	7.92	7.57
20	7.48	7.90	6.36	7.62	6.52	5.87	7.00	6.41
24	5.99	6.53	5.76	6.04	4.34	4.39	4.82	4.99
	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)	0.035	0.099	0.03	0.086	0.045	0.129	0.044	0.124
Fermentation time (B)	0.055	0.156	0.048	0.136	0.072	0.204	0.069	0.196
A x B	0.078	0.221	0.068	0.193	0.102	0.288	0.098	0.277

Table 2: Effect of *Lactobacillus acidophilus* on sensory characteristics of whey with and without pineapple juice at 37 ± 1°C.

8.74 to 5.76. The sensory scores for consistency of fermented whey-pineapple juice blend ranged from 8.69 to 6.04. The sensory scores for flavor ranged from 8.58 to 4.34 for whey fermented alone and from 8.88 to 4.39 for fermented whey-pineapple juice blend. The mean score for flavor decreased significantly with increasing fermentation time irrespective of the medium. The mean score for overall acceptability ranged from 8.87 to 4.99 in case of whey-pineapple juice blend and 8.65 to 4.82 in case of whey fermented alone. Highest score for overall acceptability was seen in case of whey-pineapple juice blend fermented for 5 h.

Enumeration of total viable counts

The viable counts were assessed after 5, 10, 15, 20 and 24 h of incubation at 37°C. It was observed that whey with pineapple juice gave higher viable counts (4.7×10^{7} cfu.ml^{-1}) up to 10 h of fermentation. But after 15 h, the whey fermented alone, gave higher viable counts ranging from 6.89×10^{8} to 9.69×10^{8} cfu/ml. Both whey and whey-pineapple juice blend attained a total viable count of more than 10^{6} cfu/ml within five h of fermentation (Table 3). The total viable count increased significantly with increase in fermentation time from 5 to 24 h. The total viable count for whey ranged from 2.21×10^{7} to 9.69×10^{8} cfu/ml. Whey-pineapple juice blend showed higher counts during initial stages of fermentation ranging from 3.78×10^{7} to 8.38×10^{8} cfu/ml.

Effect of probiotic on pH and titratable acidity

Table 4 shows the effect of incubation period on the pH of whey and whey-pineapple juice blend. pH of the samples reduced significantly ($P<0.05$) with increasing fermentation time for both whey and whey-pineapple juice blend. The mean value of pH obtained in case of whey fermented alone ranged from 4.82-3.30. The mean value of pH obtained in case of whey-pineapple juice blend ranged from 4.36-3.87. Higher viable counts during the initial period of fermentation resulted in comparative lowering of pH for whey fermented along with pineapple juice. But, after 15 h of fermentation, pH was significantly lowered for whey fermented alone. Table 4 shows the effect of incubation period on the titratable acidity of whey with or without pineapple juice. Titratable acidity increased significantly ($P\leq0.05$) with increasing fermentation time irrespective of the medium. The mean values obtained for whey ranged from 0.394 to 1.353. The mean values obtained for whey-pineapple juice blend ranged from 0.546 to 0.926. Whey-pineapple juice blend gave higher titratable acidity for 5 and 10 h of fermentation whereas there was a significant increase in the titratable acidity for whey fermented alone after 15 h of incubation. The commercial probiotic beverage should possess a minimum viable count of 10^{6} cfu/ml [11] and should also have an acceptable flavor.

Keeping in view these aspects, experiments were carried out to appraise the suitability of whey with or without the addition of pineapple juice as a growth medium for *Lactobacillus acidophilus* for the preparation of probiotic beverage. It was observed that whey fermented along with pineapple juice for 5 h gave the highest scores for overall acceptability (8.76) as compared to whey fermented alone. The scores for overall acceptability gradually declined with increasing fermentation time irrespective of the medium. Table 4 shows that the pH and titratable acidity affect the flavor profile of a beverage. In the present study, it was observed that whey-pineapple blend having a pH of 4.36 and titratable acidity of 0.546% gave the best flavor profile to the probiotic beverage. These observations exhibited an acceptable beverage by fermenting a blend of whey and pineapple juice (65:35) for a period of 5 hrs. The prepared product had desired health benefits due to the probiotic organisms.

Fermentation time (h)	Medium		
	Whey	Whey-pineapple juice blend (65:35)	
5	2.21×10^{7}	3.78×10^{7}	
10	3.5×10^{7}	4.71×10^{7}	
15	6.89×10^{8}	5.20×10^{8}	
20	9.20×10^{8}	7.78×10^{8}	
24	9.69×10^{8}	8.38×10^{8}	
	Medium (A)	Fermentation time (B)	A X B
S.E.M.	3.10×10^{4}	4.91×10^{4}	6.94×10^{4}
CD at 5%	9.16×10^{4}	1.44×10^{5}	2.04×10^{5}

* Incubation temperature: 37 ± 1°C

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

Fermentation time (h)	Medium			
	Whey		Whey-pineapple juice blend (65w:35P)	
	pH	T.A%	pH	T.A.%
5	4.82	0.394	4.36	0.546
10	4.42	0.511	4.32	0.605
15	4.00	1.077	4.20	0.806
20	3.61	1.265	3.78	0.896
24	3.30	1.353	3.87	0.926
	Medium (A)		Fermentation time (B)	A x B
S.E.M.	0.0207	0.00043	0.0328	0.0668
CD at 5%	0.0612	0.0127	0.0968	0.0201

* Incubation temperature: 37 ± 1°C

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

Sensory characteristics of probiotic beverage during storage

The changes in sensory attributes of the probiotic beverage have been shown in table 5 for refrigerated and ambient storage temperatures. The whey based probiotic beverage did not show sensory differences for the first two weeks at refrigerated storage. But after the second week, difference was perceived in color and flavor. 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 24 d at 5 ± 1°C and 48 hrs at 30 ± 1°C. Srivastava et al. [12] developed a fermented whey beverage using a mixed culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus*.

The pH of the fresh beverage prepared from whey and pineapple juice fermented for 5 hr was 4.38. The pH of the samples gradually declined after 12 d at refrigerated storage. The pH ranged from 4.38 to 3.98 after 28 days of storage. During storage of the samples at ambient temperature, pH lowered significantly ($P\leq0.05$) after 48 hr (Table 6). Kumar et al. [13] reported a decline in pH of whey based pineapple juice RTS after 90 d at refrigerated storage. Kumar et al. [14] showed that there was a gradual decline in pH of Soy milk and whey blended papaya RTS. Similar results were reported by Devi et al. [15], for whey based fruit beverages stored at refrigeration temp. The initial acidity of the fermented beverage prepared from whey and pineapple juice was 0.546%. The acidity increased during the refrigerated storage from 0.546 to 0.89% after 28 d. The increase in acidity was more prominent in case of storage at ambient temperature wherein the acidity reached 0.89% after 120 hr of storage (Table 6). Reddy et al. [16] showed that that the titratable acidity values were significantly influenced by

SP (d) at 5 ± 1°C	C&A	Con.	Flavor	OAA	SP (h) at 30 ± 1°C	C&A	Con.	Flavor	OAA
0	8.74[a]	8.80[a]	8.82[a]	8.68[a]	0	8.40[a]	7.75[a]	8.75[a]	8.40[a]
4	8.77[a]	8.65[a]	8.82[a]	8.68[a]	24	8.05[a]	7.55[a,b]	8.36[a]	7.90[b]
8	8.67[a]	8.30[b,c]	8.64[a]	8.63[a]	48	7.05[b]	7.22[b]	6.85[b]	7.12[c]
12	8.71[a]	8.40[b,c]	8.68[a]	8.33[b,c]	72	6.34[c]	6.24[c]	5.84[c]	5.83[d]
16	7.92[b]	8.19[c]	8.26[b]	8.21[c]	96	4.59[d]	4.44[d]	3.51[d]	4.09[e]
20	7.72[c]	7.95[d,e]	7.69[c]	7.89[d]	120	4.17[e]	3.62[e]	3.04[e]	3.81[f]
24	7.11[d]	7.79[e]	6.53[d]	6.89[e]	--------	------	------	------	-----
28	6.77[e]	7.05[f]	5.73[e]	5.89[f]	------	-----	-----	------	-----
S.E.M.	0.060	0.078	0.067	0.057	S.E.M	0.131	0.116	0.154	0.110
CD at 5%	0.169	0.220	0.190	0.163	CD at 5%	0.374	0.330	0.439	0.311

[1]Means followed by different superscript as letters (a, b, c, d, e, f) in a column differ significantly at 5% level SP - Storage Period; C&A - Color & Appearance; Con. - Consistency; OAA - Overall acceptability

Table 5: Changes in sensory characteristics[1] of whey-pineapple (65:35) probiotic beverage during storage.

Storage period (d) at 5 ± 1°C	pH	Total viable count (cfu/ml)	Titratable acidity (% lactic acid)	Storage period (h) at 30±1°C	pH	Total viable count (cfu/ml)	Titratable acidity (% lactic acid)
0	4.38	3.8×10^7	0.546	0	4.38	3.8×10^7	0.546
4	4.37	2.9×10^7	0.56	24	4.34	8.2×10^8	0.59
8	4.37	4.1×10^7	0.56	48	4.28	9.5×10^8	0.71
12	4.35	3.6×10^7	0.58	72	4.19	5.6×10^7	0.80
16	4.34	2.8×10^7	0.59	96	4.10	4.8×10^7	0.82
20	4.32	1.9×10^7	0.60	120	3.90	2.9×10^7	0.89
24	4.32	1.8×10^7	0.60	-----	------	-----	-----
28	3.98	1.1×10^7	0.89	------	-------	-----	-----

Table 6: Changes in pH, total viable count and titratable acidity of the whey-pineapple (65:35) probiotic beverage during storage.

the days of storage of whey beverage both at refrigeration and room temperature.

Total viable counts of probiotic beverage during storage

The initial total viable count of the beverage was 3.8×10^7 cfu/ml which decreased to 1.8×10^7 at refrigerated storage. Although the viability of *Lactobacillus acidophilus* population decreased, the viable count of the probiotic beverage did not fall below 10^6 cfu/ml (Table 6). During storage at 30 ± 1°C, the total viable count first increased to 9.5×10^8 (in 48 hr) and then gradually declined to 2.9×10^7 cfu/ml after 120 hr. Our results are in confirmation with other researchers [17,18] who also reported a decline in total viable count of *Lactobacillus reuteri* and *Biofidobacterium bifidum* of whey based probiotic beverage stored at 4 ± 1°C. Shah et al. [19], studied the survival of *Lactobacillus acidophilus* in commercial yoghurt during refrigerated storage.

Conclusion

Above study has revealed satisfactorily good quality probiotic beverage with therapeutic value prepared by using a 65:35 blend of whey and pineapple juice inoculated with 1 percent inoculum of *Lactobacillus acidophilus* with a shelf life of 24 d at 5 ± 1°C and 48 hrs at 30 ± 1°C.

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