Intl. J. Ferment. Food. 7(1): 45-54, June 2018

DOI: 10.30954/2321-712X.01.2018.6

Development of Fermented Dairy Products from Lactic Acid Bacterial Biomass Grown in Whey based Medium

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Abstract

Whey is the liquid fraction that remains following manufacture of *cheese*, *chhana*, *paneer* and *casein*. It is utilized for the growth of lactic cultures, and scaled up to laboratory scale (500 ml), followed by pilot scale (3 liters) using whey based medium. The biomass of lactic cultures obtained from the whey based medium was tested for suitability in preparation of fermented dairy products like *dahi*, buttermilk, *yoghurt* and whey beverage. The fermented dairy products analyzed for physico-chemical properties and sensory attributes. The results obtained for the growth of lactic cultures showed that this medium can be a suitable low-cost alternate for producing high amount of cell biomass. Therefore, present study entailed to conclude that a novel use of these low-cost by-product of the dairy industry, offering an alternative way of its utilization, helping to minimize their negative impact on the environment.

Keywords: whey based medium, lactic acid bacteria, dairy products

Whey is transparent watery liquid which remains after removal of fat and casein from milk. Technically whey is termed as milk serum, however, practically it is greenish-yellow residual fluid obtained on coagulation of milk. In dairy industry whey is obtained as by-product during the manufacture of coagulated milk products such as *paneer, chhana, cheese*, *casein*, etc. (Agustriyanto and Fatmawati 2009). The high nutritional value of whey makes it an interesting substrate for the development of fermented foods (Pescuma *et al.*, 2012). This by-product is a rich substrate that has been suggested for many applications including solid enrichment in cheese manufacture, bacterial or yeast growth medium to produce biomass, animal nutrients supplement, source of added value proteins, among others (Aguirre-Ezkauriatza *et al.* 2010).

Most lactic acid bacteria (LAB) are facultatively anaerobic, catalase-negative, non-motile and non-spore forming. Lactic acid bacteria are recognised as 'generally regarded as safe' (GRAS) bacteria. This GRAS status underlines their increasing use in traditional foods and in an expanding range of novel foods and products designed to have specific nutritional or other health-enhancing benefits (nutriceuticals, prebiotics, probiotics, etc.). The genera that comprise LAB are at its core *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Teragenococcus*, *Vagococcus*, and *Weisella.* The key property in defining LAB is that these bacteria produce lactic acid as the major or sole fermentation product (Panesar *et al.* 2007).

Whey has mainly been used to grow LAB for purposes of lactic acid production, its potential as a growth medium for biomass production has not been explored. Therefore, looking into the nutritional value of whey proteins, the hydrolysed whey proteins can be a good ready to serve nutrient to enhance biomass production of the beneficial LAB.

MATERIALS AND METHODS

The pure strains of *Lactobacillus helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides*, *Lactococcus lactis* and *Lactobacillus bulgaricus* were acquired from the Dairy Microbiology Department, AAU, Anand, Gujarat. Before using for experiments, cultures were activated by 2-3 transfers in MRS, M17 broth at 42°C for 8-12 h, respectively.

Production of cell biomass at Laboratory Scale

500 ml of whey based optimized medium was prepared as per the composition standardized in our laboratory and sterilized by autoclaving (121°C for 20 min). *Lactobacillus helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis* were inoculated separately in whey based medium at the rate of 2% and incubated at 40 °C for 12 h. Biomass was collected by centrifugation method and the cells were harvested from each medium by centrifugation at 6000 x g for 20 min at 4 °C (REMI C30, India). Cell pellet was washed twice with saline water (0.85% w/v) and the wet yield was determined gravimetrically.

Production of cell Biomass at Pilot Scale

Batch experiments were conducted in a 5 lit fully automatic fermenter (Shree Biocare, India). Optimized whey based medium was inoculated with 2% (w/v) culture and fermented in batch fermenter in pH controlled condition. Sodium hydroxide solution (6 N) and hydrochloric acid solution (6 N) were automatically fed at 0.3 ml/min flow rate using peristaltic pump. The rpm of agitator was fixed at 80 rpm and the dissolved oxygen content was kept below 20%. After 12 h of fermentation. Samples were collected from 1lit of thoroughly mixed fermented media from bioreactor to determined viability and total biomass yield of bacteria.

Total Viable Counts

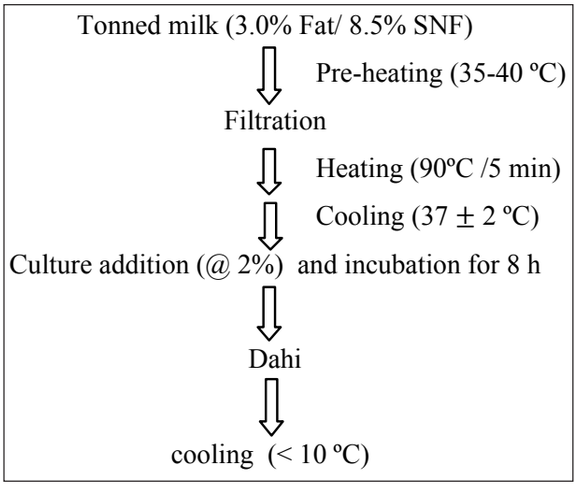
The samples were prepared aseptically serially diluted with normal saline (0.85% NaCl solution). Suitable dilutions were prepared and poured in a set of sterile MRS and M17 agar plate in duplicates. After setting of the agar, another layer of the same medium (5-7ml) was poured. The plates were then incubated at 37±2 °C for 48 h. After incubation, plates were removed for counting the colonies. The bacterial count was expressed as log cfu/ml.

Preparation of fermented milk products

Individual yield of lactic acid bacterial biomass was stored at -80°C. From that, biomass selected for the preparation of dairy products. *Dahi*, buttermilk, yoghurt and whey beverage were prepared. Biomass was thawed and depending upon the log cfu/g obtained for each culture, appropriate amount of biomass was added in sterilized normal saline to get 10^8 count per ml of saline. From this dissolved biomass, cultures were inoculated in sterilized skim milk and incubated at 37 °C for 12 h. These activated cultures were used for fermented dairy product preparation. Prepared product evaluated for sensory attributes such as color and appearance, body and texture, acidity, flavour and overall acceptability on nine-point hedonic scale and also analysed for viscosity, acidity, soluble nitrogen and free fatty acid content respectively.

Dahi

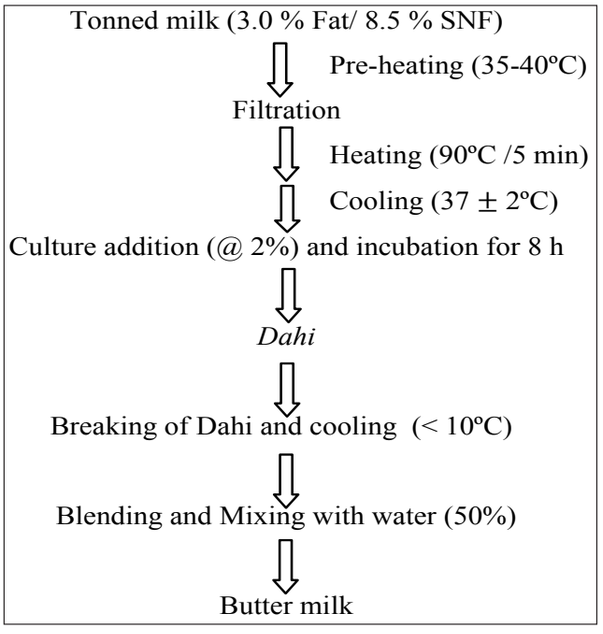
The tonned milk inoculated with combination of starter culture was filled in cleaned and sanitized glass beakers and the beakers were covered with aluminum foil. The milk was incubated at 37 2 °C for 8 h till desired acidity in the curd was obtained. Flow diagram for preparation of buttermilk in presented in follow:



Flow diagram for preparation of *Dahi*

Buttermilk

The tonned milk inoculated with combination of starter culture was filled in cleaned and sanitized glass beakers and the beakers were covered with aluminum foil. The milk was incubated at 37 2 ˚C for 8 h till desired acidity in the curd was obtained. Curd was blended thoroughly with water in 50:50 proportion. Flow diagram for preparation of buttermilk in presented in follow:

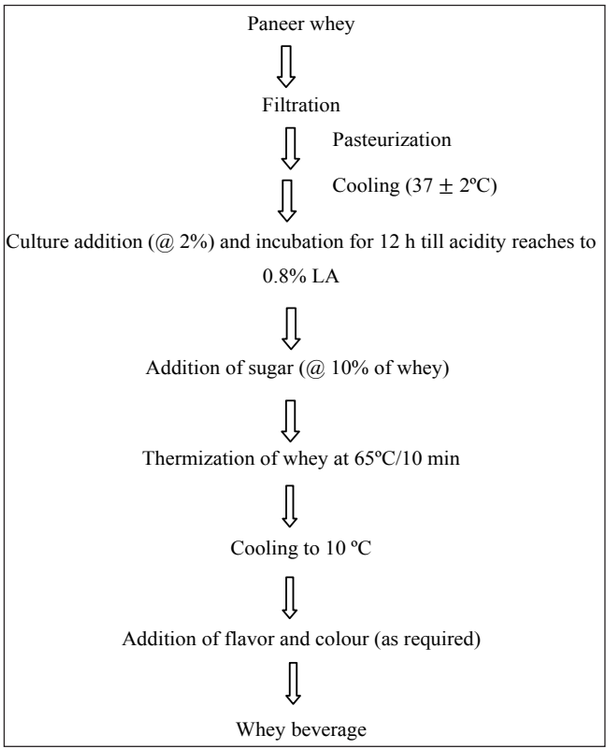


Yoghurt

Yoghurt was prepared using *lactobacillus helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *lactobacillus bulgaricus.* Remaining procedure was the same as described in *Dahi*.

Whey beverage

Procedure for preparation of whey beverage from *paneer* whey is described in the following flow diagram.



PHYSICO CHEMICAL ANALYSIS

Viscosity

The viscosities of the whey samples were determined using Ostwald viscometer as described in BIS Handbook (SP 18: part XI, 1981).

Titratable acidity (AOAC, 2007)

The titratable acidity of whey was determined by the method prescribed in BIS Handbook (IS: SP 18: part XI, 1981).

Free fatty acids (acid degree value)

A slightly modified version of the extraction - titration procedure of Frankel and Tarassuk (1955) was used. It consisted of reducing the whey sample size from 10 ml to 4 ml with proportionate reductions in prescribed reagents; mixing of sample and reagents was standardized by use of a vortex mixer instead of manual shaking. Accordingly, the procedure was: a 4 ml aliquot of milk in a screw-capped culture tube was mixed with 4 ml of 95 per cent ethanol for 15 s on a vortex mixer at a speed setting of 5. Six ml of a solvent mixture of 40 per cent diethyl ether and 60 per cent petroleum ether were added then, and the contents were mixed again for 30 s by the Vortex mixer at a speed setting of 9. After centrifuging at approximately 1500 rpm for 3 min, a 4 ml aliquot of the solvent layer in the tube was mixed with 12 ml of neutralized 95 per cent ethanol, and the mixture was titrated against 0.01 N alcoholic KOH solution with phenolphthalein as indicator. A blank was prepared for titration by following the same extraction procedure on 4 ml of distilled water in place of milk. The results were acid degree values (ADV), defined as the mill equivalents of alkali per 100 g of fat, and calculated:

$$\text{ADV} = \frac{T \times 1.5 \times 100}{4 \times 1.024 \times \text{fat (\%)}}$$

Where,

T is the net titration volume (sample titration value - blank)

1.5 is the factor to adjust the volume of alkali required to neutralize the total ether layer.

Density of whey at 20°C was taken as 1.024 (Determined using specific gravity bottle)

Fat was measured by Gerber Method.

Estimation of soluble nitrogen

Three grams of sample was taken in 100 ml volumetric flask, Sharp's extraction solution at 50 °C was added to make up the volume to 100 ml, the content was tempered at 50 1 °C for 1 h with intermittent shaking followed by filtration through whatman no 41 filter paper. From this 20 ml of filtrate was used for estimation of soluble N by Semi-Kjedahl method. Digestion, distillation and filtration were performed as per the method described earlier in Section 3.2.1.7 for total protein. The soluble nitrogen (in %) was obtained by multiplying corrected titration volume (burette reading -blank) in ml by 1.4 N/W, where, N is normality of H_2SO_4 and W is weight of sample in gram. (Maheta, 2012).

RESULTS AND DISCUSSION

Scaling up of the optimized process to a pilot scale for production of biomass

To scale up the optimized process two types of approaches were followed. In first scaling up was done at laboratory scale. Whereas in next stage of study scaling was done through fermenter experiment.

Laboratory Scale for Biomass Production

To scale up the optimized process, cultures were grown in laboratory scale. 500 ml of standardized whey based medium was prepared and cultures were added in their respective standardized whey medium. After 12 h of incubation, whole medium was centrifuged**.**

The yield of biomass is an important attribute for measuring the growth of probiotic bacteria, which ultimately determines industrial feasibility of the strain to be used for commercialization. The cell pellet obtained by centrifugation (6000 rpm for 20 min at 4 °C) of the ferment was washed once with phosphate buffer saline (0.85%). The cell pellet was dried up to constant moisture in an oven maintained at 102 ± 2 °C.

Table 1 showed that in laboratory scale experiment after 12 h of incubation, dry yield of *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and for *Lactococcus lactis* were 2.41, 1.12, 2.09 and 1.30 respectively.

Table 1: Dry yield of biomass at laboratory scale experiment

LAB Culture	Dry Yield of biomass per liter
Lb. helveticus MTCC 5463	2.41
Streptococcus thermophilus MTCC 5461	1.12
Leuconostoc mesenteroides	2.09
Lactococcus lactis	1.30

Scaling up of the Optimized Process at Pilot Scale for Biomass Production

After 12 h of incubation, dry yield was calculated and results showed in Table 2. Also Total viable count of both the medium and the pelleted biomass were counted. Results are presented in Table 3.

Table 2: Dry yield of biomass from fermenter experiment

LAB Culture	Dry Yield of biomass per liter
Lb. helveticus MTCC 5463	5.51
Streptococcus thermophilus MTCC 5461	2.56
Leuconostoc mesenteroides	4.98
Lactococcus lactis	3.11

Table 3: Total viable count in pelleted biomass

LAB Culture	Total Viable count in pelleted biomass (log cfu/g)
Lb. helveticus MTCC 5463	10.11
Streptococcus thermophilus MTCC 5461	10.07
Leuconostoc mesenteroides	10.10
Lactococcus lactis	9.91

Table showed that in fermenter experiment after 12 h of incubation, dry yield of *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and for *Lactococcus lactis* were 5.51, 2.56, 4.98 and 3.11 was respectively.

Khan (2014) found Biomass yield of the *Lb. helveticus* MTCC 5463 in cheese whey. Cheese whey inoculated (18 h fermentation time) with *Lb. helveticus* MTCC 5463 at 32 °C and 6.5 pH showed minimal dry biomass yield of 0.10 g/L whereas maximum dry biomass yield of 2.63 g/L was found in media fermented at 40°C at pH 6.5 for 28 hours.

Mondragón-Parada and co-workers (2006) worked to isolate and characterize lactic acid bacteria (LAB) strains to be used for biomass production using a whey-based medium supplemented with an ammonium salt and with very low levels of yeast extract (0.25 g/L). Five strains of LAB were isolated from naturally soured milk after enrichment in whey-based medium. One bacterial isolate, designated MNM2, exhibited a remarkable capability to utilize whey lactose and give a high biomass yield on lactose. This strain was identified as *Lactobacillus casei* by its 16S rDNA sequence. A kinetic study of cell growth, lactose consumption, and titratable acidity production of this bacterial strain was performed in a bioreactor. The biomass yield on lactose, the percentage of lactose consumption, and the maximum increase in cell mass obtained in the bioreactor were 0.165 g of biomass/g of lactose, 100%, and 2.0 g/L, respectively, which were 1.44, 1.11, and 2.35 times higher than those found in flask cultures.

Above reported study suggested that biomass yield increased by about two times in bioreactor experiment to that of in laboratory experiments. Therefore, results obtained in this study are in accordance with the literature cited above. Throughout the fermentation optimum pH, temperature and the amount of dissolved oxygen were kept constant. This might be the probable reason for getting higher biomass yield in fermenter compared to flask experiment.

Total viable count of the fermented whey not only measure the survivability of bacterial cell but also denotes the survivability against the metabolites produced by cell itself. Above Table showed data regarding the total viable count after 12 h in biomass obtained after pelleting the 12 h fermented optimized whey medium.

The total viable count obtained were 10.11 log cfu/g for *Lb. helveticus* MTCC 5463, 10.07 log cfu/g for *Streptococcus thermophilus* MTCC 5461, 10.10 log cfu/g for *Leuconostoc mesenteroides* and 9.91 log cfu/g for *Lactococcus lactis*.

Khan (2014) obtained an average TVC of *Lb. helveticus* MTCC 5463 varied from 13.21 to 15.98 log cfu/g biomass after 24 h of bioreactor fermentation. Total viable count was minimum (13.21 log cfu/g) when the strain was fermented in cheese whey supplemented with 0.03% YE and 0.75% PP at optimized growth parameter (39.89 °C, 6.259 pH for 24 h) whereas maximum TVC of 15.98 log cfu/g was obtained when cheese whey was supplemented with 0.6% YE and 0.75% PP. Thus, results obtained in present study are inlined with results obtained by this author.

Used of biomass for preparation of selected fermented dairy products

In order to test the performance of biomass, four different fermented dairy products were prepared *viz*. (i) *Dahi*, (ii) Buttermilk, (iii) yoghurt and (iv) whey beverage.

Dahi

Dahi is a tradition Indian fermented dairy product.To check performance of biomass in *dahi*, six different types of lactic culture combinations (Table 4) were selected. *Lb. helveticus* MTCC 5463 was added as a probiotic culture. Cultures were added at the rate of 2 percent individually in the milk and incubated at 37°C. After curd formation the beakers were taken out from the incubator and kept in refrigerator (7±1°C) for overnight. These *dahi* samples were evaluated sensorial and for selected physico-chemical characteristics.

Table 4: Different combinations of lactic cultures were used in fermented dairy products

Types of *dahi* prepared with different cultures	Combination of starter culture
D_1	*Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis*
D_2	*Lb. helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5461
D_3	*Lb. helveticus* MTCC 5463 and *Leuconostoc mesenteroides*
D_4	*Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Leuconostoc mesenteroides*
D_5	*Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Lactococcus lactis*
D_6	*Lb. helveticus* MTCC 5463 and *Lactococcus lactis*

Sensory evaluation of *dahi*

All the samples of *dahi* were subjected to evaluation for their sensory attributes viz. flavour, colour and appearance, body and texture and overall acceptability using 9 points hedonic scale. Results along are presented in Table 5.

Table 5: Scores of sensory attributes of *dahi*

Types of *dahi*	Score obtained for sensory attributes			
	Flavor	Colour and appearance	Body and texture	Overall acceptability
D_1	8.2	8.1	8.2	8.3
D_2	7.9	8.0	8.1	8.0
D_3	7.6	7.9	7.7	7.7
D_4	7.5	7.6	7.6	7.8
D_5	7.8	7.5	7.4	7.5
D_6	6.5	7.1	6.9	6.7

Looking to the scores of sensory attributes of *dahi*, it is evident that all the cultures have impact on flavour, colour and appearance, body and texture and overall acceptability of *dahi*. Out of six different combinations of four lactic cultures tried, D_1 type of *dahi* gave highest score in all the sensory attributes. Mean values of flavour scores for all six types of *dahi* (D_1, D_2, D_3, D_4, D_5 and D_6) were 8.2, 7.9, 7.6, 7.5, 7.8 and 6.5 respectively. Average values of colour and appearance scores for all six types of *dahi* were 8.1, 8.0, 7.9, 7.6, 7.5 and 7.1 respectively. Average values of body and texture scores for all six types of *dahi* were 8.2, 8.1, 7.7, 7.6, 7.4 and 6.9 respectively. Mean values of overall acceptability scores for all six types of *dahi* were 8.3, 8.0, 7.7, 7.8, 7.5 and 6.7 respectively.

Among all six types of *dahi*, scores of all sensory attributes of *dahi* (D_6) were the lowest. This effect was

masked when combination of *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Lactococcus lactis* was tried. Effect of *Lactococcus lactis* on overall acceptability was curbed by these two cultures.

The relatively better performance of *Lactobacillus helveticus* may be attributed to its ability to produce the aroma compound because the production of high quality fermented dairy products is dependent on proteolytic systems of starter bacteria, since peptidase and amino acids formed have a direct impact on flavour or serve as flavour precursors in these products as *Lactobacillus helveticus* high proteolytic activity (Shah, 2003). The relatively better colour and appearance scores of cultured buttermilk prepared using *Lactobacillus helveticus* may be attributed to its ability to produce exo-polysaccharide (EPS) and ability to give smooth appearance to the product (Pescuma *et al.* 2008).

Physico-chemical analysis of *dahi*

The average values of acidity, soluble nitrogen, free fatty acids and viscosity for different samples of *dahi* are shown in Table 6.

Table 6: Effect of lactic cultures on physico-chemical characteristics of *dahi*

Dahi	Physico-chemical characteristics			
	Titratable acidity (%)	Soluble nitrogen (%)	Free Fatty Acid (%)	Viscosity (cp)
D_1	0.66	0.08	8.71	2700
D_2	0.64	0.11	8.56	3100
D_3	0.54	0.07	8.57	230
D_4	0.54	0.09	8.58	2310
D_5	0.70	0.07	8.56	2220
D_6	0.70	0.07	8.62	289

Above data showed that titratable acidity for all six treatments (D_1, D_2, D_3, D_4, D_5 and D_6) tried were found to be 0.66, 0.64, 0.54, 0.54, 0.70 and 0.70 (% lactic acid) respectively. Development of acid from lactose by culture is the first factor of controlling in manufacture of fermented dairy products. Therefore, titratable acidity is one of the most important points of concerned for fermented dairy products. Soluble nitrogen contents of all six types of *dahi* were 0.08, 0.11, 0.07, 0.09, 0.07 and 0.07 respectively. Similarly, the free fatty acids values of all samples were found to be 8.71, 8.56, 8.57, 8.58, 8.56 and 8.62 respectively. Measurement of viscosity for *dahi* was done using two spindles, namely S64 and S62. S 64 spindle was used for *dahi* samples namely D_1, D_2, D_4 and D_5. For sample D_3 and D_6 spindle number S62 was used. Viscosity values for D_1, D_2, D_4 and D_5 were 2700 (at 44% torque), 3100 (at 51% torque), 2310 (at 33% torque) and 2220 (at 40% torque) respectively, whereas, for D_3 and D_6 viscosity were 230 (at 40% torque) and 289 (at 46% torque) respectively.

Buttermilk

Cultures were added at the rate of 2 percent individually in the milk and incubated at 37°C. After curd formation the beakers were taken out from the incubator and kept in refrigerator (7±1°C) for overnight. Water was added in 50:50 proportion to *dahi* and blended thoroughly to prepare buttermilk from *dahi*. To check performance of biomass in buttermilk six different types of lactic culture combinations (Table 7) were selected. *Lb. helveticus* MTCC 5463 was added as a probiotic culture.

Table 7: Different combinations of lactic cultures were used

Types of buttermilk prepared with different cultures	Combination of lactic cultures
B_1	*Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461, *Leuconostoc mesenteroides* and *Lactococcus lactis*
B_2	*Lb. helveticus* MTCC 5463 and *Streptococcus thermophilus* MTCC 5461
B_3	*Lb. helveticus* MTCC 5463 and *Leuconostoc mesenteroides*
B_4	*Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Leuconostoc mesenteroides*
B_5	*Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Lactococcus lactis*
B_6	*Lb. helveticus* MTCC 5463 and *Lactococcus lactis*

Sensory evaluation of buttermilk

All the samples of buttermilk were subjected to evaluation for their sensory attributes viz. flavor, colour and appearance, body and texture and over all acceptability using 9 points hedonic scale. Results are presented in Table 8.

Table 8: Scores of sensory attributes of buttermilk

Types of buttermilk	Score obtained for sensory attributes			
	Flavor	Colour and appearance	Body and texture	Overall acceptability
B_1	7.6	7.8	7.7	7.7
B_2	8.2	8.1	7.8	8.0
B_3	6.9	7.8	7.7	7.5
B_4	7.1	7.8	7.3	7.2
B_5	6.9	7.8	7.3	6.9
B_6	5	7.2	6.6	5.2

Looking to the scores of sensory attributes of buttermilk, it is evident that all the cultures have impact on flavor, colour and appearance, body and texture and overall acceptability of *dahi*. Out of six different combinations of four lactic cultures tried, B_2 type of buttermilk gave highest score in all the sensory attributes. Mean values of flavor scores for all six types of buttermilk (B_1, B_2, B_3, B_4, B_5 and B_6) were 7.6, 7.9, 6.9, 7.1, 6.9 and 5 respectively. Average values of colour and appearance scores for all six types of buttermilk were 7.8, 8.1, 7.8, 7.8, 7.8 and 7.2 respectively. Average values of body and texture scores for all six types of buttermilk were 7.7, 7.8, 7.7, 7.3, 7.3 and 6.6 respectively. Mean values of overall acceptability scores for all six types of buttermilk were 7.7, 7.8, 7.5, 7.2, 6.9 and 5.2 respectively.

Among all six types of buttermilk, scores of all sensory attributes of buttermilk (B_6) were the lowest. This effect was masked when combination of *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Lactococcus lactis* was tried. Effect of *lactococcus lactis* on overall acceptability was curbed by these two cultures.

The relatively better performance of *Lactobacillus helveticus* may be attributed to its ability to produce the aroma compound because the production of high quality fermented dairy products is dependent on proteolytic systems of starter bacteria, since peptidase and amino acids formed have a direct impact on flavor or serve as flavor precursors in these products as *Lactobacillus helveticus* high proteolytic activity. (Shah, 2003; Hassaine *et al*. 2008). The relatively better colour and appearance scores of cultured buttermilk prepared using *Lactobacillus helveticus* may be attributed to its ability to produce exo-polysaccharide (EPS) and ability to give smooth appearance to the product (Pescuma *et al.* 2008).

Physico-chemical analysis of buttermilk

The average values of acidity, soluble nitrogen, free fatty acid and viscosity for different samples of *dahi* are shown in Table 9.

Table 9: Effect of lactic culture on physico-chemical characteristics of buttermilk

Types of buttermilk	Physico-chemical characteristics			
	Titratable acidity (%)	Soluble nitrogen (%)	Free Fatty Acid (%)	Viscosity (cp)
B_1	0.66	0.06	6.11	71.1
B_2	0.64	0.05	6.14	73.3
B_3	0.54	0.07	7.36	61.1
B_4	0.54	0.06	6.13	79.5
B_5	0.70	0.06	7.34	36.6
B_6	0.70	0.05	7.35	43.6

Above data showed that titratable acidity for all six treatments (B_1, B_2, B_3, B_4, B_5 and B_6) tried are 0.66, 0.64, 0.54, 0.54, 0.70 and 0.70 (% lactic acid) respectively. Development of acid from lactose by culture is the first factor of controlling in manufacture of fermented dairy products. Therefore, titratable acidity is one of the most important points of concerned for fermented dairy products. Soluble nitrogen contents of all six types of buttermilk were 0.06, 0.05, 0.07, 0.06, 0.06 and 0.05 respectively. Similarly, the free fatty acids values of all samples were found to be 6.11, 6.14, 7.36, 6.13, 7.34 and 7.35 respectively. The viscosity values of all samples of buttermilk were 71.1 (at 42% torque), 73.3 (at 60% torque), 61.1 (at 50% torque), 79.5 (at 66%

torque), 36.6 (at 30% torque) and 43.6 (at 36% torque) respectively.

Yoghurt

Yoghurt is a *dahi* like fermented dairy product three types of lactic cultures were added. *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 and *Lb. bulgaricus.* The prepared yoghurt was found to be of acceptable quality. The mean score of overall acceptability of yoghurt was 7.7 when evaluated sensorially on a 9-point hedonic scale in Table 10.

Table 10: Scores of sensory attributes of yoghurt

Yoghurt	Score obtained for sensory attributes			
	Flavor	Colour and appearance	Body and texture	Overall acceptability
Y_1	7.6	7.8	7.7	7.7

The yoghurt was analyzed for titratable acidity (% LA), soluble nitrogen (%), free fatty acid (% oleic acid) and viscosity (cp) and found the mean values as 0.93%, 0.08%, 7.12% and 3210 cp respectively showed in Table 11.

Table 11: Effect of lactic culture on physico-chemical characteristics of yoghurt

Yoghurt	Physico-chemical characteristics			
	Titratable acidity (%)	Soluble nitrogen (%)	Free Fatty Acid (%)	Viscosity (cp)
Y_1	0.93	0.08	7.12	3210

Whey Beverage

Paneer whey was used to prepare acid whey beverage. Two lactic cultures were inoculated i.e., *Lb. helveticus* MTCC 5463, *Streptococcus thermophilus* MTCC 5461 to obtain desirable acidity.

Two types of flavors were tried in preparation of acid whey beverage, i.e. pineapple and mango. Both the beverages were evaluated on sensory attributes and scores are presented in the Table 12 and 13.

Both the types of beverages were acceptable on sensory attributes. However, the scores of all the sensory characteristics of pineapple beverages was higher as compared to mango beverage.

Table 12: Scores of sensory attributes of whey beverage

Flavour used in beverage	Score obtained for sensory attributes			
	Flavor	Colour and appearance	Body and texture	Overall acceptability
Pineapple	8.0	7.9	7.6	8.0
Mango	7.5	7.7	7.6	7.6

Table 13: Effect of lactic culture on physico-chemical characteristics of whey beverage

Flavour used in beverage	Physico-chemical characteristics			
	Titratable acidity (%)	Soluble nitrogen (%)	Free Fatty Acid (%)	Viscosity (cp)
Pineapple	0.8	0.04	5.27	1.4
Mango	0.8	0.04	5.26	1.4

Both the types of acid whey beverages were analyzed for titratable acidity (% LA), soluble nitrogen (%), free fatty acid (% oleic acid) and viscosity (cp). The mean values of titratable acidity (% LA), soluble nitrogen (%), free fatty acid (% oleic acid) and viscosity (cp) of pineapple beverage were found to be 0.8, 0.04, 5.27 and 1.4 whereas that of mango beverage were 0.8, 0.04, 5.26 and 1.4 respectively.

Thus, the biomass produced can be successfully used in preparation of selected fermented dairy products with acceptable quality.

CONCLUSION

The obtained biomass of lactic cultures from the whey based medium developed in the present study was tested for preparation of fermented dairy products. For testing the suitability of the biomass *dahi*, buttermilk, yoghurt and whey beverage were taken as model fermented dairy products. The results obtained in the present study suggests that use of whey based medium for the growth of lactic cultures showed that this medium can be a suitable low-cost alternate for producing high amount of cell biomass. In general, fermentation in this economical

medium under optimized growth condition could be used as a viable alternate to the high cost commercial media (MRS and M17 broth). Therefore, present study entailed to conclude that the use of whey as culture media for the production of biomass of lactic acid cultures implies a novel use of these low-cost by-product of the dairy industry, offering an alternative way of its utilization, helping to minimize their negative impact on the environment.

ACKNOWLEDGEMENTS

The author was highly thankful to Department of Dairy Microbiology, Anand Agricultural University Anand for providing laboratory facility for carrying out this work.

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