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Process standardization for development of herbal buttermilk supplemented with *Aloe vera* juice

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Abstract
Cultured buttermilk is a highly refreshing fermented dairy product consumed in regular diet and is claimed to be aid in to digestion, reduces acidity and constipation while *Aloe vera* is known for several herbal benefits. In our current project, buttermilk was fortified with *Aloe vera* juice in the range 10-20% in order to enhance the nutritional and therapeutic value of original product. The product was optimized based on sensory evaluation by expert panel of judges. The overall acceptability score of optimized product (15% *Aloe vera*) was 7.85. It also differs non-significantly with control sample. Higher concentration of *Aloe vera* increased stickiness, and gave pungent taste & slight greenish tinge. The product was further analyzed for compositional, physico-chemical and microbiological analysis. The protein, moisture, total solid content and viscosity differ significantly whereas fat and acidity showed non-significant difference. The absence of coliform and yeast & mold count indicates good hygiene processing. Product was containing sufficient amount of live lactic acid bacterial cell (>10^6 per mL) to provide positive health effect. The optimized product mask the typical taste of *Aloe vera* (15%) in popularly consumed beverage buttermilk. The newly developed product is good for health and can be considered as herbal product.

Keywords: *Aloe vera*, buttermilk, herbal beverage, shelf life, microbial quality, lactic count

Introduction
Since the functional foods contain bioactive components, they have the potential to improve human health more than basic nutrition. For ages, milk and milk products have been an vital part of the human diet. Cultured buttermilk is a well-known fermented dairy product with medicinal properties; it is also referred as Chhash in South Asian countries. It is one of the most popular drinks since ancient times and is believed excellent for gastric disorders and digestive issues. Cultured buttermilk is a nutritious and health-promoting beverage because it is rich in proteins, lipids, minerals like calcium, phosphorus, zinc, potassium, vitamins B2, B12 & pantothenic acid-vitamin B5 along with presence of good gut bacteria. Additionally, cultured buttermilk also possesses more phospholipids than skim milk (Conway *et al*., 2014; Kumar *et al*., 2015) [1, 7]. Among the several food items that can be regarded as novel herbal food ingredients is *Aloe vera* (*Aloe Barbadensis Millar*) that provides several therapeutic and health benefits (Srikanth *et al*., 2017) [23]. *Aloe vera* is well established medicinal plant rich in vitamins like vitamins C, A, E, B1&B6; minerals including trace elements; antioxidants; and other bioactive components those are beneficial for human health. *Aloe vera* believed to boost immune system, promotes hair growth, reduce arthritis pain, and delay the aging process. Its antioxidants, such as polyphenols, are particularly effective at preventing infections. It is commonly used in cosmetics but rarely used in food products because of its bitter/astringent taste.

Earlier studies have been conducted on the fortification of dairy products, bakery goods, processed foods with function ingredient like dietary fiber, polyphenols, etc (Mudgil *et al*., 2011; 2012, 2016a, 2016b; Raju & Pal, 2014; Shah *et al*., 2025) [9-14, 17, 20]. Although buttermilk is most popular throughout pan India, literature contains very little information about cultured buttermilk fortification or value addition. The impact of vitamin and mineral fortification on buttermilk properties was investigated by few researchers (Ziarno *et al*., 2009, Saviraite *et al*., 2012) [24, 19].

Conversely, *Aloe vera* gel or juice fortified flavored milk or fermented milk products such as dahi, lassi, buttermilk been formulated and described (Jothylingam & Pugazhenthi, 2013;Ramachandran & Srividya, 2014; Mudgil *et al*., 2016; Kumar *et al*., 2021) [6, 18, 9, 7]. However, the success on commercialization of *Aloe vera* supplemented fermented milk products is not achieved yet in particular due to its strong pungent flavor not acceptable in common.

So, in current study we have tried to create product utilizing valuable nutrients present in *Aloe vera* in regularly consumed beverage like cultured buttermilk without affecting its original taste and can also be regarded as a reasonably priced herbal product that is suitable for all age groups and, ideally, health-conscious consumers. The key objectives of the study include optimization of *Aloe vera* juice concentration followed by physico-chemical, microbiological and compositional analysis of the optimized product.

Materials and methods

Raw materials

Standardized pasteurized double toned milk containing 1.5% fat and 9.0% SNF was procured from DudhSagar Dairy, Mehsana, India and subsequently stored at 4°C until use. A DVS (direct vat set) Yoghurt starter culture namely RST-744 & CHN-11 comprised of mixed strain of mesophilic & thermophilic homofermentative bacterial culture was obtained from Chr. Hansen Inc. (Milwaukee, WI) and was maintained at -18 °C. *Aloe vera* juice with fiber (Patanjali, India), salt, rock salt (*sanchar*), and cumin powder of standard brand were purchased from local market. All microbiological media and chemicals were procured from Hi-Media (Mumbai, India).

Preparation of cultured buttermilk

All the glass wares used in study were pre-sterilized and the contact surfaces of the incubator and blender were alcohol sanitized. The control buttermilk sample was prepared by traditional method in that double toned milk was heated at 80°C/20 min with continuous stirring and then immediately cooled to room temperature. Subsequently, it was inoculated with starter cultures, RST-744 (0.1 unit/l) and CHN-11 (0.01 unit/l) blend and mixed thoroughly in the milk. The milk was transferred to the pre-sterilized beakers (1l capacity) with lids and incubated at 42°C for 6h till curd settling or development of uniform coagulum. The settled curd was agitated and broken using laboratory blender (Cello Blend-N-Mix 300, India) at a speed of 10,000 rpm, followed by addition of pasteurized chilled water (40%), rock salt (0.3%), salt (0.8%) and cumin powder (0.5%) (Shah and Prajapati, 2014; Nagesh *et al*., 2024) [22, 15]. Finally, the product was packed in close containers and transferred in refrigerator (5±2 ºC) until analyzed.

Optimization of the level of *Aloe vera* juice in the buttermilk

Aloe vera juice supplemented buttermilk samples were processed similarly as control cultured buttermilk sample except the step involving addition of only pasteurized chilled water, in that *Aloe vera* juice was added in pasteurized chilled water in three different ratios, *viz*. 10:30, 15:25, and 20:20 on weight basis to make buttermilk as shown in Figure 1. All the samples were packed and stored in refrigerator.

Physico - chemical and compositional analysis of the herbal buttermilk supplemented with *Aloe vera* Juice

The herbal buttermilk was analyzed for titratable acidity, viscosity total solids, protein, fat, and moisture for the optimized product. Titratable acidity of the product was measured by titrimetric method, and expressed as percent of lactic acid. The protein, fat and total solids content of the product was determined using the Kjeldahl method (Shah and Prajapati, 2014) [22], Gerber method (Indian Standards, 1981) [5] and procedure given in IS: 1479 (II) (1961) [4], respectively. Apparent viscosity of buttermilk samples was measured (expressed in cPs) using spindle viscometer (Brookfield, USA) at 5 rpm speed of spindle and the reading was recorded after one-minute rotation of the spindle (Mudgil *et al*., 2016) [9].

Microbiological evaluation of the herbal buttermilk supplemented with *Aloe vera* Juice

The suitable dilutions samples were poured with respective agar media in sterile petridishes in duplicates for evaluation of total plate count (TPC), yeast and mold count, and coliform count as per the standard methods (Shah *et al*., 2025) [20]. The lactic acid bacteria count was performed as described by Shah *et al*., (2025) [21]. The colony forming units (cfu) were counted from the respective plates following incubation under specific time- temperature combinations. The values were converted into log values before expressed.

Statistical analysis

The results of three individual experiments were gathered to generate the mean ± standard deviation (SD) throughout the study. The obtained results were analyzed through one-way analysis of variance (ANOVA) and t-test to determine the significance at $P<0.05$.

Result and Discussion

The aim of the current project was to develop functional beverage by combining buttermilk with *Aloe vera* which contains numerous health beneficial compound *i.e.* amino acids, phytosterols vitamins, minerals, and polysaccharides. It has also been observed that polysaccharide present in *Aloe vera* minimizes syneresis in product.

Optimization of level of *Aloe vera* juice

Aloe vera juice was added at three different levels *i.e.* 10%, 15%, and 20% on weight basis into pasteurized chilled water. Results of sensory evaluation of control and *Aloe vera* supplemented buttermilk samples are presented in Figure 2. The increase in score of all the sensory attributes *i.e.* flavor, body & texture, color & appearance, and overall acceptability was observed up to 15% *Aloe vera* juice addition. Score for body & texture was found non-significant between products with 10% and 15% *Aloe vera* juice and also while compared with control. Significantly ($p<0.05$) lower score obtained for product with 20% *Aloe vera* juice could be attributed to the increase in viscosity of herbal buttermilk which was unliked by the judges as the mouthfeel of buttermilk has been changed. The higher incorporation level of *Aloe vera* juice might have also produced the bland or flat taste in herbal buttermilk. Among all the samples, the highest body & texture score of 7.98 for control sample invariably indicates the effect of *Aloe vera*

juice on the characteristics of mouthfeel that gradually increases with increase in percentage of *Aloe vera* juice. It is believed that addition of *Aloe vera* juice had masking effect on the natural flavor of cultured buttermilk. Therefore, the control sample has achieved highest score of 7.76 followed by 7.7, 7.58 and 7.2 for 15, 10 and 20% *Aloe vera* juice containing samples, respectively. Nevertheless, non-significant difference was found among control and all test samples. Flavor of cultured buttermilk with 20% *Aloe vera* juice has obtained the least score (7.2) by judges among all sensory attributes due to generation of high intensity of blend flavor in product. The greenish color of *Aloe vera* juice has been found to affect the visual appearance of cultured buttermilk significantly ($p<0.05$) beyond 15% *Aloe vera* juice addition as the product with 20% *Aloe vera* juice gained lowest score of 7.56 for color & appearance. The score of other two products (with 10 & 15% *Aloe vera* juice) observed in close proximity with control. It shows the compatibility of *Aloe vera* juice with cultured buttermilk up to 15%. Product with 15% *Aloe vera* juice scored highest (7.88) for overall acceptability characteristics. It differs non-significantly with control and product with 10% *Aloe vera* juice, respectively, whereas it differs significantly ($p<0.05$) with product containing 20% *Aloe vera* juice. Despite of non-significant difference for scores of all attributes between buttermilk with 10% and 15% *Aloe vera* juice, product with 15% *Aloe vera* juice was found much more sensorially appealing by Judges. Furthermore, cultured buttermilk was probably capable of masking the original flavor of *Aloe vera* juice up to 15%.

Overall, control sample differs significantly ($p<0.05$) only with buttermilk having 20% *Aloe vera* juice for all sensorial attributes except flavor. The results are in agreement with the findings of many research workers, Hussain *et al*., (2015) [3], Mudgil *et al*., (2016) [9], Mohanpriya *et al*., (2019) [8] and Kumar *et al*., (2021) [7] who had developed cultured buttermilk/ lassi/ or synbiotic lassi supplemented with *Aloe vera* juice in the range of 10-16%. Mohanpriya *et al*., (2019) [8] found optimum level of carrot extract & *Aloe vera* extract addition @ 15% to score highest points of 8.73 & 8.85 in lassi, respectively. Hussain *et al*., (2015) [3] have also recorded the almost similar sensory score for probiotic lassi containing 16% *Aloe vera* juice. On the contrary, cultured buttermilk with low concertation (10%) of *Aloe vera* juice prepared by Mudgil *et al*., (2016) [9] scored highest (7.5) while products with other concentrations (5, 15, & 20%) of *Aloe vera* juice observed with a gradual decrease in sensory score. Noticeably in present study, product with higher concentration (15%) of *Aloe vera* juice still score higher (7.88) than the study of Mudgil and co-workers (2016)[9] which represents the importance as well as compatibility of salt & spices in enhancing the taste of product. The various factors play a crucial role could be development of bland flavor, gel like consistency as viscosity increases above 54 cPs, stickiness, greenish color of whey, etc. Conversely, in another study conducted by Hingne *et al.,* (2020) [2], the results of sensory evaluation observed that the most acceptable quality flavored milk contained 6% *Aloe vera* juice in combination with 3% Tulsi juice.

Compositional and physico-chemical analysis of the Herbal Buttermilk supplemented with *Aloe vera* Juice

The values of proximate composition and physico-chemical parameters of control and optimized *Aloe vera* enriched buttermilk are demonstrated in Table 1. Though the difference between values are very narrow, incorporation of *Aloe vera* juice at 15% significantly ($p<0.05$) affect the protein, total solids and moisture content of cultured buttermilk. The results are in agreement with the findings of Nichal *et al*., (2022) [16] and Nagesh *et al*., (2024) [15]. However, in contrast to our results Nagesh and co-workers (2024) [15] reported significant difference ($p<0.05$) for acidity and fat, too. Reduced phase separation is usually observed in high viscosity buttermilk due to low possibilities of separation of whey from the network (Mudgil *et al.,* 2016) [9]. Titratable acidity of control and *Aloe vera* juice supplemented buttermilk ranged between 0.68 and 0.66% lactic acid (Table 1). It was noticed that increase in level of *Aloe vera* juice fortification did not have any significant effect on the titratable acidity of buttermilk. The results are in accordance with observations of Mudgil *et al*., (2016) [9].

Microbial evaluation of processed buttermilk

Microbiological analysis revealed the absence of total viable counts, coliform counts and yeast & mold counts in the fresh products indicating acceptable quality and the good hygienic condition during preparation of product whereas LAB count $>10^7$/g allows the product to be considered to deliver sufficient number of live bacterial cells. As it is evident from Table 2, interestingly the microbial (lactic) count was low in the buttermilk supplemented with *Aloe vera* as compared to control sample. Hussain *et al.*, (2015) [2] noticed viable probiotic cell count of 8.4 $\log_{10}$ cfu per g in *Aloe vera* supplemented lassi. Similarly, Kumar *et al*., (2021)[7] reported that the viable cell counts ranged from 7.98 to 8.35 and 8.18 to 8.36 $\log_{10}$ cfu per 100g in freshly prepared synbiotic lassi with various concentrations of stabilizers and copper, respectively. However, the difference in count was found non-significant.

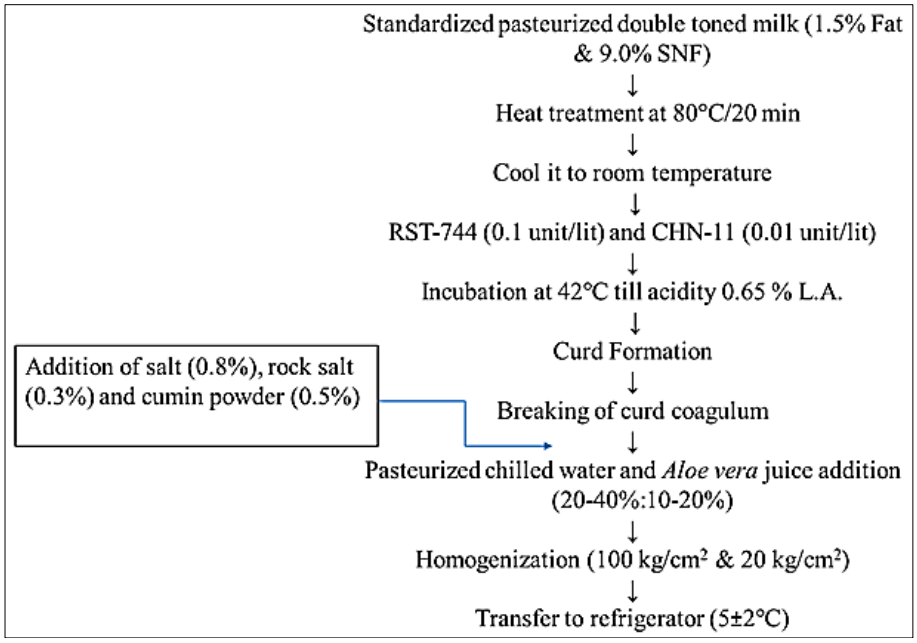

Fig 1: Flow diagram for preparation of control and *Aloe vera* supplemented cultured buttermilk

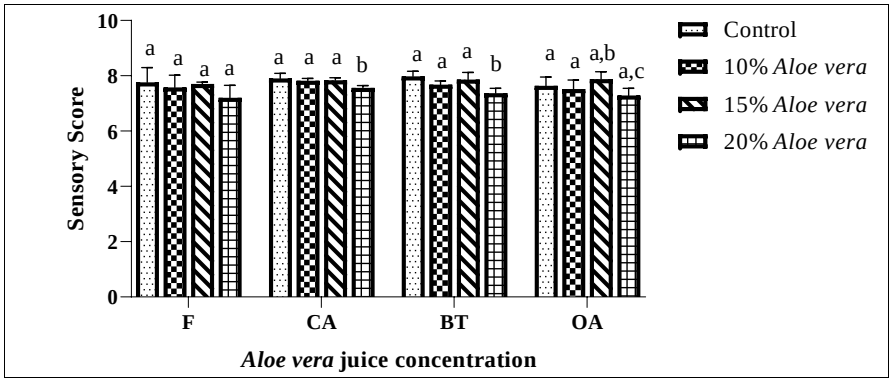

Fig 2: Effect of *Aloe vera* juice concentrations on sensory score of control and *Aloe vera* supplemented buttermilk. Means (n=5) with different small superscripts differs significantly (p<0.05). F= Flavor, CA= Color & Appearance, BT= Body & Texture, OA= Overall Acceptability.

Table 1: Proximate compositional and physico-chemical analysis of control and *Aloe vera* supplemented buttermilk

Parameters	Control sample	Optimized product (15% *Aloe vera*)
Fat	1.0 ±0.005[a]	1.0 ±0.004[a]
Protein	1.24 ± 0.02[a]	1.06 ± 0.03[b]
Total solids	8.25 ± 0.03[a]	7.59 ± 0.05[b]
Moisture	91.75 ±0.03[a]	92.40 ± 0.06[b]
Viscosity (cPs)	20.46 ± 0.52[a]	58.70 ± 0.37[b]
Acidity (% LA)	0.68 ± 0.02[a]	0.66 ± 0.03[a]

Values are Mean ± SD (n=3)

Conclusion

The development of herbal buttermilk supplemented with 15% *Aloe vera* juice was liked the most by expert panel of judges. Higher conc. of *Aloe vera* was effectively characterized by weak body due to high moisture content, dull appearance and pungent taste of *Aloe vera*. The incorporation of *Aloe vera* juice showed significant effect on the concentration of protein, moisture, total solid and viscosity of optimized product. The product contained sufficient viable lactic acid bacterial count to offer health benefits. On the other hand, the absence of coliform and yeast & mold indicated processing of product under hygienic condition. The future study would definitely focus on the stability of product under refrigeration storage. Overall, newly developed product can be considered as herbal product which masks and may offer natural health and nutritional benefits associated with ingredient in cost effective manner.

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