



DEVELOPMENT AND STANDARDISATION OF “DAIRY COOL”, WHEY BASED FERMENTED BEVERAGE

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Abstract

*Sambharam (spiced buttermilk) is a common summer refreshment beverage in southern states of India, made from cultured milk. The aim of the study was to develop a whey based sambharam as it is an excellent growth medium for lactic acid bacteria to ferment lactose in whey to form lactic acid. The effect of different starter culture, percent of inoculation, and concentration of spice mix on sensory aspects of product were studied. The spiced whey sambharam was packaged and cooled to 4°C and stored. Sensory evaluation of developed product “Dairy cool” was performed using 9 point hedonic scale and conventional sambharam as positive control. Data obtained from sensory analysis were subjected to statistical analysis. The sample containing starter culture of *Lactococcus lactis* ssp. *lactis* at 2 percent level with prepared spice mix at 2 percent addition was selected. Shelf life study conducted for optimized product showed that the beverage was acceptable for a period of upto 7 days without much deteriorating effects on the sensory attributes when stored at 4°C.*

Key words: Whey sambharam, Fermented beverage, *Zingiber officinale*, *Capsicum annuum*, *Murraya koenigii*.

Whey, the liquid remaining after the precipitation and removal of milk casein during paneer making, represents 85–95 percent of the milk volume. The high biochemical oxygen demand (BOD) of whey poses a major world-wide disposal and pollution problem for the dairy industry, for which an effective and permanent solution is urgently needed. Besides the environmental pollution aspect, dumping of whey constitutes a significant loss of potential food and energy, as whey retains about 55 percent of its total milk nutrients. The most abundant of these nutrients are lactose (45 g/l to 50 g/l), soluble proteins (6 g/l to 8 g/l), lipids (4 g/l to 5 g/l) and mineral salts (8–10% of dried extract). Whey also contains appreciable quantities of lactic (0.5 g/l) and citric acids, non-protein nitrogen compounds (urea and uric acid) and B group vitamins (Panesar *et al.*, 2007). Availability of lactose and presence of other essential nutrients for the growth of microorganisms make whey a potential raw material for the delivery of probiotic microorganisms to the human gastro intestine.

Ginger (*Zingiber officinale*) has been cultivated for thousands of years as a spice and for medicinal purposes (Park and Pizzuto, 2002). Chilli (*Capsicum annuum*) is a spice-cum-vegetable of commercial importance with

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distinctive aroma that makes nutritive foods more appetizing (Govindarajan *et al.*, 1987). *Murraya koenigii* is known as 'Curry leaves' and is widely used as a spice and condiment in India and other tropical countries. It stimulates digestive enzymes and helps break down food more easily.

About 3 million tonnes of whey is produced annually in India containing about 2 lakh tonnes of valuable milk nutrients (Naik *et al.*, 2009). Therefore, conversion of whey into fermented beverages is one of the most attractive avenues for utilization of whey for human consumption. The development of any process for its economical utilization would be of great benefit to the dairy industry. Hence the study was undertaken to develop and standardize a nutritious drink with improved aroma and taste for effective utilization of whey produced from paneer production unit in the University Dairy Plant.

Materials and Methods

Whey, the by-product during manufacture of paneer was collected from University Dairy Plant, Mannuthy, Kerala Veterinary and Animal Sciences University. The chemical analysis of fresh whey was done for the pH, acidity, fat, total solids, moisture, protein and lactose. A pH meter was used to measure the pH. Acidity of the whey sample was determined by titration method. Total solids content was determined by drying the samples at 105 °C up to a constant weight (AOAC, 1995). Protein content was determined by Lowery method and lactose by Lane- Eynon method. All the chemical analyses were performed in triplicate.

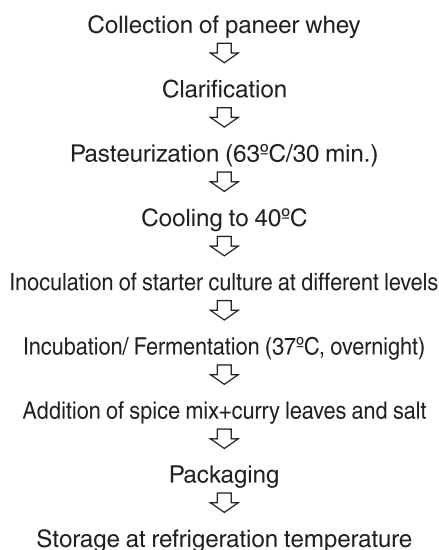
The whey was then pasteurized at 63°C for 30 minutes. After cooling the pasteurized whey to 40 °C, starter cultures were inoculated. Type of starter culture used for fermentation of whey was obtained from National Centre for Dairy Cultures, NDRI, Karnal. The type and the level of inoculation of starter cultures used in samples and respective sample codes (S1 to S9) are given below.

- Starter culture of *Lactococcus lactis* ssp. *lactis* was added at 1% (S1), 2% (S2), 3% (S3) levels.
- Starter culture of *Streptococcus thermophilus* was added at 1% (S4), 2% (S5), 3% (S6) levels.

- Mixed cultures of *Lactococcus lactis* ssp. *lactis* & *Streptococcus thermophilus* at 1:1 ratio was added at 1% (S7), 2% (S8), 3% (S9) levels.

All the nine samples were incubated at 37°C overnight. After fermentation sensory evaluation was performed for all the nine samples using 9 point hedonic scale, by an expert panel of six judges, for the sensory attributes such as colour and appearance, mouth feel, flavour as well as overall acceptability taking conventional *sambharam* as positive control. After selecting the sample containing suitable starter culture at optimum level of inoculation, spice mix was added at three different levels. Spices selected for the preparation were the same as that for conventional *sambharam*. Green chilli and ginger was taken at the ratio of 1:0.75 and made to paste by grinding. Prepared spice mix was added to the fermented whey sample at 1%, 1.5%, 2% levels. Salt was added to improve the taste. A total of three samples were then studied. All the sensory evaluation studies were conducted in triplicate.

Fig. 1. Process flow chart for preparation of whey sambharam



Data obtained from sensory analysis were subjected to statistical analysis using Analysis of variance for multiple comparisons (ANOVA) in order to confirm statistical significance of differences among the samples. Statistical analysis was done for all the samples for their sensory attributes. The optimized sample was subjected to shelf life study on the 0th, 3rd and

7th and 14th day of refrigerated storage.

Results and Discussion

The data pertaining to the various chemical characteristics of fresh whey and optimized product is given in Table 3. It clearly indicates that fresh whey has high lactose content of about 4.2 ± 0.03 gm/100gm. The titratable acidity of fresh whey was found as 1.25 ± 0.04 with a mean pH of 4.82 ± 0.14 . Total solids (TS) content in fresh whey base was found to be 7.86 ± 0.19 gm/100gm.

Sensory evaluation was done for a total of nine various samples in triplicate for the attributes like colour and appearance, mouth feel, flavour as well as overall acceptability. The flavor and overall acceptability scores were very poor for *Streptococcus thermophilus* and combination culture of *Lactococcus lactis* ssp. *lactis* & *Streptococcus thermophilus* at

1:1 ratio compared to *Lactococcus lactis* ssp. *lactis* alone when added at different levels. At 3 percent inoculum size all the samples scored poor for all the attributes evaluated. The scores for overall acceptability declined with increased inoculum size (3 percent) irrespective of the culture because of developed soreness in the product. This may be due to the increased acidity of the product on fermentation. While at 2 percent level of inoculation the overall acceptability of all the three culture samples were good with highest value for *Lactococcus lactis* ssp. *lactis* inoculated sample. At 1 percent level of starter culture *Lactococcus lactis* ssp. *lactis* addition flavour and mouthfeel of the product was poor. The study showed that fermentation of whey using *Lactococcus lactis* ssp. *lactis* enhanced flavor by producing various volatile aroma substances in the product. The sample with *Lactococcus lactis*

Table 1. Effect of type and levels of Starter cultures on various sensory attributes of Whey based Sambharam

Name of Starter Culture	Level of inoculation of starter culture (per cent by weight of whey)				CD _{0.01}
	Positive control	1.0	2.0	3.0	
Flavour score of Whey based <i>Sambharam</i>					0.66
<i>Streptococcus thermophilus</i>	6.21 ±0.14 ^{bx}	6.90 ±0.29 ^{ax}	7.08±0.8 ^{ax}	4.67 ±0.33 ^{cy}	
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	6.33 ±0.17 ^{cx}	7.33 ±0.33 ^{bx}	8.33 ±0.17 ^{ax}	7.08 ±0.08 ^{bx}	
ST+LL (1:1)	6.17 ±0.17 ^{bx}	5.17 ±0.17 ^{by}	7.08 ±0.08 ^{ax}	4.33 ±0.17 ^{cy}	
CD _{0.01}	0.59				
Mouthfeel Score of Whey based <i>Sambharam</i>					0.43
<i>Streptococcus thermophilus</i>	6.17 ± 0.17 ^{ax}	5.67 ± 0.17 ^{by}	7.42 ± 0.08 ^{ay}	5.58 ± 0.08 ^{by}	
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	6.33 ± 0.17 ^{cx}	8.21 ± 0.08 ^{bx}	8.67 ± 0.17 ^{ax}	6.08 ± 0.08 ^{dx}	
ST+LL (1:1)	6.18 ± 0.08 ^{ax}	5.50 ± 0.14 ^{bz}	6.83 ± 0.17 ^{az}	5.67 ± 0.17 ^{by}	
CD _{0.01}	0.38				
Colour and appearance score of Whey based <i>Sambharam</i>					0.40
<i>Streptococcus thermophilus</i>	7.07 ± 0.17 ^{bx}	7.42 ± 0.08 ^{ay}	7.48 ± 0.08 ^{ay}	6.25 ± 0.14 ^{cy}	
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	7.17 ± 0.08 ^{bx}	8.07 ± 0.08 ^{ax}	8.17 ± 0.08 ^{ax}	7.08 ± 0.08 ^{bx}	
ST+LL (1:1)	7.25 ± 0.14 ^{ax}	5.42 ± 0.08 ^{bz}	5.33 ± 0.08 ^{bz}	5.08 ± 0.08 ^{bz}	
CD _{0.01}	0.36				
Overall Acceptability score of Whey based <i>Sambharam</i>					0.32
<i>Streptococcus thermophilus</i>	6.33 ± 0.17 ^{cx}	7.17 ± 0.17 ^{by}	8.05 ± 0.08 ^{ax}	5.63 ± 0.08 ^{dy}	
<i>Lactococcus lactis</i> ssp. <i>lactis</i>	6.30 ± 0.10 ^{cx}	7.72 ± 0.08 ^{bx}	8.08 ± 0.05 ^{ax}	6.08 ± 0.08 ^{cx}	
ST+LL (1:1)	6.33 ± 0.05 ^{cx}	7.02 ± 0.08 ^{by}	7.17 ± 0.08 ^{ay}	5.17 ± 0.17 ^{dz}	
CD _{0.01}	0.28				

Figures are the average \pm SE of three replications

abc - mean with different superscripts within row differ significantly ($p \leq 0.01$)

xyz - mean with different superscripts within columns differ significantly ($p \leq 0.01$)

Table 2. Sensory profile of whey based *sambharam* (*Lactococcus lactis* ssp. *lactis*) prepared by adding different levels of Green chilli and Ginger blend

Sensory attributes	Level of addition of Green chilli and Ginger blend, per cent by weight of Whey				CD _{0.01}
	Positive control	1	1.5	2.0	
Flavour	5.40 ± 0.12 ^d	6.48 ± 0.13 ^c	6.96 ± 0.09 ^b	7.50 ± 0.05 ^a	0.39
Colour and appearance	5.20±0.09 ^d	5.74±0.13 ^c	6.27 ± 0.10 ^b	7.40 ± 0.07 ^a	0.52
Mouthfeel	6.73 ± 0.23	6.96 ± 0.17	7.23 ± 0.10	7.27 ± 0.10	0.68 ^{ns}
Overall acceptability	5.98 ± 0.10 ^c	6.48 ± 0.19 ^b	7.19 ± 0.06 ^a	7.44 ± 0.06 ^a	0.51

Figures are the average ± SE of three replications

Mean with different superscripts within a row differ significantly ($p < 0.01$)

ns = Non-Significant

Table 3. Chemical composition of fresh whey and optimized product

Sample	Titratable acidity (%)	pH	Chemical composition			
			Moisture (%)	Total solids g/100g	Protein g/100g	Lactose g/100g
Fresh whey	1.25±0.04	4.82±0.14	91.8±0.37	7.86±0.19	0.74±0.03	4.2±0.03
Optimized product	3.2±0.14	3.75±0.03	89.8±0.37	9.8±0.14	0.74±0.03	2.32±0.09

Figures are the average ± SE of six replications

Table 4. Sensory profile of optimized product on storage

Sensory attributes	Days of storage			
	0	3	7	14
Flavour	7.46 ± 0.05 ^a	6.96 ± 0.09 ^b	6.48 ± 0.13 ^c	5.62 ± 0.1 ^d
Colour and appearance	7.38 ± 0.07 ^a	6.23 ± 0.10 ^b	5.74 ± 0.13 ^c	4.83 ± 0.06 ^d
Mouthfeel	7.27 ± 0.10 ^a	7.13 ± 0.10 ^a	6.96 ± 0.17 ^{ab}	5.94 ± 0.09 ^b
Overall acceptability	7.42 ± 0.06 ^a	7.26 ± 0.06 ^a	6.48 ± 0.19 ^b	5.47 ± 0.11 ^c

Figures are the average ± SE of three replications.

Mean with different superscripts within a row differ significantly ($p < 0.01$)

ssp. *lactis* as type of culture at 2 percent level of addition scored maximum for sensory attributes like colour & appearance (8.17 ± 0.08), flavour (8.33 ± 0.17), mouth feel (8.67 ± 0.17) and overall acceptability (8.08 ± 0.05) compared to all other samples and positive control (conventional *sambharam* at 1 percent level of inoculation of starter culture). Hence, *Lactococcus lactis* ssp. *lactis* as starter culture at 2 percent level was selected as the type of culture best suiting for developing whey based fermented beverage. The sensory scores obtained with respect to color, flavor, taste and overall acceptability are presented in Table 1.

Sensory profile of whey based *sambharam* (*Lactococcus lactis* ssp. *lactis* added at 2 percent level) prepared by adding different levels of Green chilli and Ginger

blend is given in Table 2. Spice mix for *sambharam* prepared of green chilli and ginger blend added at 2 percent level scored maximum for flavour (7.50 ± 0.05), colour and appearance (7.40 ± 0.07), mouth feel (7.27 ± 0.10) and overall acceptability (7.44 ± 0.06) based on sensory evaluation and statistical analysis. *Lactococcus lactis* ssp. *lactis* is a mesophilic lactic acid bacterium which can tolerate a higher salt concentration upto 4% (Obis *et al.*, 2001). By suppressing the growth of other undesirable bacteria and mold, salt provides a slower fermentation process that is perfect for cultured soft drink. Hence, this particular sample was selected as best combination and further storage studies were carried out for the selected sample.

Average lactose content of fresh

optimized product was decreased to 2.32 g/100g upon fermentation. Protein content was same as fresh whey. Total solids (TS) showed significant increase (9.8g/100 g) when compared to fresh whey bases probably due to added spice mix in the product. Lactic acid bacteria (LAB) have been extensively used as starter cultures in the fermented food industry due to their metabolic activity on lactose (Mauriello *et al.*, 2001). Whey fermentation by LAB could decrease the high lactose content in whey, producing mainly lactic acid and other metabolites such as aroma compounds contributing to the flavor and texture and increasing carbohydrate solubility and sweetness of the end product and makes the product acceptable to lactose intolerant group of people.

The titratable acidity of whey on storage showed slight increase ie, 3.24 percent lactic acid on 3rd day and 3.91 percent lactic acid on 7th day of storage. pH of product on storage showed slight decrease ie, 3.67 on 3rd day and 3.64 on 7th day of refrigerated storage. Microbial species present in starter culture used for the fermentation might have utilized the lactose present in the whey and converted into lactic acid along with decreasing the pH of the final product. This might be the reason for increase in acidity of the product.

The changes in sensory attributes of the beverage at refrigerated storage have been shown in table 4. The whey based beverage (*Whey sambharam*) did not show much sensory differences for the first week at refrigerated storage. But on 14th day of storage, difference was perceived in color and flavor. The decrease in the flavor during storage could be possibly due to loss of volatile aromatic substances derived from both fermentation and added spices. 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 associated soreness, with acidity being the attribute responsible for the sensory difference perceived by the panelists. Even though a slight acidification was detected by the sensory panelists even at 7th day of storage, they agreed that the beverage was acceptable for a period of up to 7 days without much deteriorating effects on the sensory attributes when stored at 4°C.

It may be concluded that whey, a dairy by-product can be successfully utilized as fermented beverage because it contains many essential amino acids, easily digestible protein

and lactose. Hence its utilization increases the economic returns of a dairy plant besides reducing the cost on dairy waste disposal. It is also important to understand how varying aroma, taste and mouthfeel affect quality, so that such products can be formulated to maximize consumer acceptability. The flavour of the product was a highly appreciated characteristic that must be related to the consumer habits in South Indian states. Therefore this product can be commercialized by adopting simple technology for the betterment of Indian dairy farmers and as well as consumers.

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National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, from where freeze dried cultures were obtained.

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