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Studies on Quality Evaluation of Probiotic Custard Apple (*Annona Reticulata*) *Dahi*

Research Article

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Abstract

The probiotic *dahi* samples were prepared by using *Lactobacillus acidophilus* starter culture. The total solids content of cow milk were raised by addition of custard apple powder i. e. 1,2,3,4 and 5P% levels. Prepared Probiotic *dahi* samples were evaluated for its sensory quality for finalization of experimental treatments. On the basis of sensory parameters of *dahi* samples. Custard apple powder levels viz; 1, 2, 3 and 4% were finalized for experimental trials. Experimental *dahi* samples were evaluated for chemical, microbiological and sensory qualities.

Fat and protein contents of *dahi* samples were ranged from 3.35 to 3.51% and 3.23 to 3.31%, respectively. The lactic acidity and total solids of *dahi* samples were 0.65 to 0.83% and 12.00 to 15.03% , respectively. Total solids content increased significantly ($P < 0.05$) due to addition of various levels of custard apple powder in the *dahi*. The lactic acidity of the *dahi* samples ranged from 0.65 to 0.83%. Total viable counts of *L. acidophilus* of *dahi* samples were ranged from $22.5x10^6$ to $26.25\ 10^6$ cfu/gm whereas the coliform counts and Yeast and mould counts (YMC) were ranged from 0.25 to 0.50 and 0.33 to 1.50 cfu /gm in the samples. Organoleptic quality of *dahi* samples observed in the study was also significantly ($p < 0.05$) improved. Better quality probiotic custard apple *dahi* can be prepared by using cow milk containing 12% total solids, 2% custard apple powder and 1 % starter culture of *Lactobacillus acidophilus* strain.

Keywords: Probiotic dahi; Chemical quality; Microbiological quality; Organoleptic quality

Introduction

The process of souring of fermentation of milk is one of the oldest method known for preserving milk constituents. The fermented milk products have reputation due to their nutritional and therapeutic properties from the time immemorial. Fermented milk products are easily digested because of breaking down of protein into peptides and free amino acids. Fermentation of milk converts lactose into lactic acid and other biochemical which stimulate gastric secretion and speed up the transport of gastric contents into the intestinal tract. This lactic acid suppresses the growth of putrefactive bacteria which are associate with constipation [1].

Dahi is one of the most important fermented milk product. *Dahi* possesses refreshing, nutritional and therapeutic values. The property of *dahi* is not only due to its refreshing and palatability but also due to its scientifically proven role of nutritive and therapeutic values [2].

Besides its therapeutic values; *dahi* is one of the most soothing food items with a high nutritive value and as such it is included as one of the choicest item in Indian menu especially during summer season [3]. *Dahi* also constitutes the base for preparation of *lassi* and *shrikhand* [4].

Probiotic are live microbial food supplements that have beneficial effect on the intestinal flora of the host, there by leading to health, improvements. Probiotic must be safe (i.e. Generally Regarded As Safe, GRAS); they must be amenable to industrial processes necessary for commercial production, they must remain viable in the food product and during storage, they

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must persist in the gastrointestinal tract long enough to elicit an effect and they must improve host health [5].

In the Indian context, the basic cultured product from milk is *dahi* (curd), which is the base material for manufacturing a range of fermented products. The incorporation of probiotics in the fermented milk foods is an emerging sphere of research and many traditional fermented milk products such as *dahi,* mishti doi, shrikhand, lassi, buttermilk and chakka that have a large market in India and have the possibility of being transformed into a potential health foods [6].

Most of the research related to *dahi* has been centered around technology development. Recently, there has been an increasing trend of fortifying the milk products with fruit extracts. Value addition of milk and milk products enhances consumer acceptability, their nutritive quality and these products fetch higher prices in the market which ultimately leads to socio-economic development of producer and processors.

The product like *dahi* occupies a prominent place in the diet of large section of Indian population. Incorporation of fruits not only results in value addition but also improve nutritional and therapeutic value of such products.

Health and nutrition benefits of custard apple are a storehouse of Vitamin C, which is an antioxidant and helps in neutralizing free radicals. Vitamin A present in fruit is good for hair, eyes and healthy skin. Custard apple contains magnesium, which plays vital role in relaxing muscles and protecting heart against diseases. It is a rich source of dietary fiber, which helps in digestion. As it contains low fat levels, it is good for maintaining optimum health. The paste of the flesh of the fruit can be used for local application on ulcers, abscesses and piles. The fruit, in its unripe form, can be dried, crushed and used for treating diarrhea and dysentery. Custard apple serves as an expectorant, stimulant, coolant and haematinic and is even useful in treating anemia.

Considering the importance of probiotics, custard apple in human diet, the present investigation has been planned with objectives of to prepare custard apple probiotic *dahi* and to study its sensory, chemical and microbiological qualities.

Materials and Methods

The study entitled, " Studies on Quality evaluation of Probiotic Custard Apple *Dahi*" was carried out in the Department of Animal Husbandry and Dairy Science, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (MS).

Materials

Milk

The composite milk samples of crossbred cows were obtained from Research-Cum Development Project on Cattle, located at the Central Campus, MPKV, Rahuri.

Custard apple powder

The branded custard apple powder (Weikfield) was purchased from local market.

Media for microbiological examination

For enumeration of *Lactobacillus acidophilus counts*, Coliform counts, yeast and mould counts., De Man Rogosa and Sharpe (MRS) Agar, Violet Red Bile Agar (VRBA) and Potato Dextrose Agar (PDA) media were used.

Starter Culture, Its Maintenance and Propogation

Freeze dried pure culture of *Lactobacillus acidophilus* was procured from the National Collection of Dairy Cultures (NCDC), Division of Dairy Microbiology, National Dairy Research Institute, Karnal (Haryana). The culture was maintained separately in sterilized reconstituted skim milk test tubes.

The sterilized skim milk test tube was inoculated with Freeze dried culture and incubated at 37 °C up to setting of culture and subsequently inoculated in another set of sterilized skim milk test tube and incubated at 37 °C for 8 h and thereafter stored at 5 °C temperature. In order to keep the culture active, they were propagated once in a week.

Manufacture of *Dahi*

The *Dahi* samples under pre-experimental and experimental trials were prepared by using the procedure prescribed by De S [7] with some minor modifications.

Fresh good quality cow milk was preheated to 45 °C and subjected to filtration/clarification. It was then standardized to 3.5% fat. The total solids of milk were maintained to 12%. The *dahi* was prepared by using *Lb acidophilus* culture with different total solids i.e. 12% as control; 13%, 14%, 15%, 16%, and 17%. The total solids were adjusted by using 1,2,3,4 and 5% custard apple powder. The prepared *dahi* samples were evaluated for its sensory quality by five semi-trained judges. The sensory score was recorded by using 9 point Hedonic Scale [8]. On the basis of the results of sensory evaluation the *dahi* samples containing 12 % (T_0), 13% (T_1) ,14% (T_2) and 15% (T_3), total solids were selected for experimental trials [Figure 1].

Figure 1: Flow Diagram for Preparation of *Dahi*

Receiving milk

↓

Preheating (45 ˚C)

↓

Filtration/clarification

↓

Standardization (3.5%fat)

↓

Pasteurization (85 ˚C /15Min)

↓

Cooling (30-35 ˚C)

↓

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Addition of Custard apple powder

(0, 1, 2, 3%)

↓

Inoculation

(Starter culture *Lb. acidophilus* 21%)

↓

Mixing

↓

Packaging in polystyrene cup

↓

Incubation

(35-37 ˚C/6-8 h)

↓

Dahi

↓

Cooling and storage (5 ˚C)

Chemical Analysis of Milk

Fat: Fat content of milk samples was determined as per Gerber method described in IS : 1224, Part I, [9].

Protein: Protein content of milk samples was determined by Kjeldahl method as per the procedure recommended in IS: 1479 (part-II) [10].

Total solid: Total solids contents of milk samples was determined **as** per the method given in IS: 1479 Part-II [10].

Titratable acidity: Titratable acidity of milk samples was determined as per the method given in IS: 1479, Part -I [11].

Chemical *Analysis* of *Dahi*

Fat: The fat of *dahi* samples was determined by Gerber method as described in IS: 1224, Part-I [9].

Protein: The protein was determined by estimating the per cent nitrogen by Micro-Kjeldhal method as recommended in IS: 1479 Part-II [10]. The per cent nitrogen was multiplied by 6.38 to find out protein percentage.

Acidity (% lactic acid): The acidity of *dahi* samples was determined by using method prescribed in IS: 1479 (Part I), 1960 [11].

Total solids: The total solids of *dahi* samples was determined by using method prescribed in IS: 1479 (Part I), 1960 [11].

Microbiological quality

Preparation of Dilution Blanks

Phosphate buffer (stock solution): The potassium dihydrogen orthophosphate (KH_2PO_4) 3.4 g was dissolved in 100 ml of distilled water. The pH was adjusted with 1 N NaOH solution so that after sterilization it would be 7.2. Then total volume was made to 100 ml with distilled water (ISI, 1962).

Phosphate buffer (dilution banks): To prepare dilution blanks, 1.25 ml of stock phosphate buffer solution was added to 1 lit of distilled water and transferred in 9.3 ml quantities into glass test tubes (18 x 150 mm) and sterilized at 121 °C/15 lb/15 min.

Microbiological quality of *Dahi*

Enumeration of Viable *Lactobacilli* Counts: The MRS agar having pH 6.4 was used for enumeration of total viable *Lb acidophilus* counts of *dahi* samples.

10 g of *dahi* sample was taken separately in 250 ml conical flask containing 99 ml sterilized phosphate buffer solution. The serial dilutions were made. The 1 ml from 5^{th} to 7^{th} dilutions were taken in duplicate into Petri plates and then MRS agar was added and mixed well. The Petri plates were allowed to solidify. The Petri plates were incubated at 37 °C/48 h and then the *Lb acidophilus* counts were recorded as c.f.u/g. On the basis of colonies observed the 6^{th} dilution was considered.

Enumeration of coliforms: The first and second dilutions of *dahi* samples which were prepared, has been used for enumeration of coliforms. The 1 ml from first and second dilution were taken in duplicate into Petri plates and the violet red bile agar was added and mixed well. The plates were allowed to solidify. The plates were again over layered with the same agar and allowed to solidify. These plates were incubated at 37 °C for 24 h and numbers of coliform colonies developed were counted as colony forming units (c. f. u.) per gram.

Enumeration of yeast and mould (YMC): The 1^{st} and 2^{nd} dilutions of all *dahi* samples were taken in duplicate into Petri plates and then potato dextrose agar was added by adjusting pH 3.5 by adding 5 ml of 10% sterilized tartaric acid. The plates were allowed to solidify and incubated at 25 °C for 5 days. Numbers of yeast and mould colonies developed were counted as colony forming units/gm.

Organoleptic Quality of Dahi: *Dahi* samples were subjected to the organoleptic evaluation. The samples of *dahi* were provided to the panel of five trained judges for sensory evaluation. Each treatment was given code number, which was changed during each replication so as to avoid identity. The scoring was recorded by using 9 point Hedonic Scale.

Statistical analysis: Experiment was laid out in Completely Randomized Design (CRD) with four replications.

Results and Discussion

Chemical Composition of Cow Milk

The average fat, protein, acidity and total solids content of cow milk used in the present study were 3.53% ,3.25,0.13%, and 12.33%, respectively.

Chemical Composition of *Dahi*

Fat: The fat content of *dahi* samples was determined and

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results are depicted in Table 1. It is observed that the mean fat content of the *dahi* samples were in the range of 3.35 (T_3) to 3.51 (T_0) per cent and the results of the investigation were coincided with the results of Pawar, Patil and Kale. Average fat content was maximum in control sample T_0 (3.51%). The fat content of dahi sample of T_3 (3.35%) was lower than other samples of *dahi* studied in the investigation.

Protein: The protein content of *dahi* samples was determined and the results are presented in Table 1.The protein content of *dahi* samples were ranged from 3.23 (T_0) to 3.31(T_3) per cent. Maximum protein content was observed in dahi sample of T_3 (3.31 %) while minimum in the dahi sample of T_0 (3.23 %). Protein content of *dahi* samples were more or less similar to protein content of samples of milk used for *dahi* preparation. Protein content of *dahi* samples under the study are coincided with the results of Rangappa and Achaya [12], Laxminarayana and Shankar [13], De [7] and Adsul [14].

Acidity (% L. A.): The acidity content of *dahi* samples was in the range of 0.65 to 0.83 % LA. The maximum acidity was observed in *dahi* sample T_3 (0.83 % LA), while minimum in the *dahi* sample of T_0 (0.65 % LA). The acidity of all samples of *dahi* differed significantly ($P < 0.05$) among all treatment samples. The acidity content of *dahi* samples in the study increased with increase in the level of custard apple powder. Acidity content of *dahi* samples in the study is coincided with the results of Rangappa and Achaya [12] Laxminarayana and Shankar [13], De [7] and Adsul [14].

Total Solids (TS): The total solids content of *dahi* samples was determined and presented in Table 1. It is revealed that all treatment samples differed significantly ($P < 0.05$) from each other. The *dahi* sample T_3 (15.03 %) showed higher total solids content than other samples of *dahi* studied in the investigation. The custard apple powder contained more percent of total solids than the milk therefore as custard apple powder level increased, the total solids content also increased proportionately in *dahi* samples. The total solids content of *dahi* samples in the study are coincided with the results of Srinivasan and Anantakrishnan [15].

Microbiological quality of *dahi*.

Lactobacillus acidophilus counts of *dahi* samples: It is suggested that, to have maximum therapeutic value, the fermented milk product should contain population of viable cells of probiotic culture more than 10^6 cfu/ml at the time of consumption [16,17].

Keeping this fact in view, the product was studied for their viable counts of *Lactobacillus acidophilus.* The counts of *dahi* samples was enumerated and depicted in Table 2. It is seen that *dahi* sample T_2 showed higher *Lactobacillus acidophilus* counts (26.25 cfu/g x 10^6) as compare to the control (T_0) than the rest of *dahi* samples in the study. The counts of T_3, T_1 and T_0 *dahi* samples were on par with each other.

The viable counts of *Lactobacillus acidophilus* observed in the study were slightly higher than that reported by Sheikh et al. [18] (1.7 x 10^4 to 3 x 10^6 cfu/ml). It indicates that the *dahi* samples contained sufficient *Lactobacillus acidophilus* counts which are beneficial from the nutritional and therapeutic point of view.

Table 1: Chemical composition of *Dahi* Samples.

Treatment	Fat (%)	Protein (%)	Acidity (% LA)	Total solids (%)
T_0	3.51	3.23	0.65^a	12.00^a
T_1	3.35	3.27	0.72^b	13.07^b
T_2	3.37	3.30	0.75^c	14.07^c
T_3	3.35	3.31	0.83^d	15.03^d
S. E.±	0.03	0.01	0.006	0.02
C. D. at 5%	-	-	0.02	0.07
Result	NS	NS	*	*

* Significant at 5% level NS = Non significant

Table 2: Microbiological Quality of *Dahi* Samples.

Treatment	Lactobacilli count (cfu / gm)	coliform count (cfu /gm)	YMC (cfu/gm)
T_0	25.0^{bc} x 10^6	0.50	1.50
T_1	24.0^{ab} x 10^6	0.50	1.25
T_2	26.25^cx 10^6	0.25	1.25
T_3	22.5^ax 10^6	0.25	0.25
S. E.±	0.49	0.27	0.33
C. D. at 5%	1.52	-	-
Result	*	NS	NS

* Significant at 5% level NS= Non Significant

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Table 3: Organoleptic quality *dahi* samples.

Treatment	colour & appearance	Body & texture Sensory score (out of 9)	Flavour	Overall acceptability
T_0	7.25c	7.62[c]	7.14[c]	7.14[c]
T_1	6.47[b]	6.68[a]	6.65[b]	6.71[b]
T_2	8.43[d]	8.06[d]	8.12[d]	8.45[d]
T_3	6.20[a]	7.11[b]	6.57[a]	6.23[a]
S. E. ±	0.02	0.08	0.04	0.02
C. D. at 5% level	0.08	0.26	0.15	0.08
Result	*	*	*	*

* Significant at 5% level

Coliform counts: The presence of coliforms in dairy products are suggestive of insanitary conditions or practices followed during production, processing and inadequate care taken during post processing. The coliform counts of *dahi* samples are presented in Table 2.

It is revealed that the coliform counts in the product were in the range of 0 cfu/g to 1 cfu/g. It was below (maximum 10 cfu/g) the standard prescribed by IS-9617 (1980). These counts are more or less similar to the results observed by Mohanan et al. [19] (coliform count 0 to 30 cfu/ml) and Adsul [14].

Yeast and mould counts (YMC): The yeast and mould counts (YMC) is one of the most important groups of spoilage micro-organisms in acidified dairy products, capable of reducing the shelf-life, even under refrigerated storage. It was observed that the counts of YMC were in the range of 0 to 2 cfu/g and it was below the limits (maximum 100 cfu/g) prescribed by IS: 9617 [20].

Organoleptic Quality of *Dahi*

Colour and appearance: The colour and appearance is one of the important attributes of the sensory quality. The colour and appearance score of experimental samples of *dahi* are presented in Table 3. The mean sensory score for colour and appearance of the product under different treatments were significant ($P < 0.05$). It is revealed that *dahi* sample (T_2) had the highest sensory score (8.43) for its colour and appearance. The colour of the *dahi* sample T_2 was attractive and had uniform , smooth and glossy surface without any free wheying off. The *dahi* sample T_3 had significantly ($P < 0.05$) lower score (6.20) than the rest of *dahi* samples.

Body and texture: From the Table 3, it is seen that the addition of custard apple powder in *dahi* samples significantly ($P < 0.05$) influenced the body and texture of the product. The mean sensory score of experimental *dahi* samples under various treatments were ranged from 6.68 (T_1) to 8.06 (T_2).

The mean body and texture score for *dahi* sample T_2 was highest (8.0) than other *dahi* samples in the study. The body and texture of *dahi* sample T_2 was smooth and glossy and the surface was firm and free from crack and gas holes. *Dahi* sample T_1 had significantly ($P < 0.05$) lower score (7.62) than the rest of *dahi* samples. As it had weak body and there was wheying off from the surface. The sensory score for body and texture of *dahi* were also significantly ($P < 0.05$) differed from each other.

Flavour: The flavour which is the most important component of sensory quality. The mean score for flavour was 7.14, 6.65, 8.12, and 6.57 for the treatment samples T_0, T_1, T_2 and T_3 respectively [Table 3]. Which differed significantly ($P < 0.05$). It is revealed that the mean sensory score of the samples under different experimental treatments were ranged from 6.57 (T3) to 8.12 (T2). Flavour score of different dahi samples was categorized as "like slightly to extremely good". The *dahi* sample T_2 had maximum flavour score (8.12) and significantly ($P < 0.05$) higher than the rest of other treatment samples. The mean flavour score for T_3 *dahi sample* was significantly ($P < 0.05$) lower (6.57) than other samples of *dahi*. It implies that the addition of 2% custard apple powder significantly improved the flavour score of product but it was again reduced to the 3% level. It may be due to flavoring compounds associated with custard apple.

Overall acceptability: The sensory score for overall acceptability of different samples of *Dahi* are presented in Table 3. It is revealed that *dahi* sample T_2 had highest score (8.45) for its overall acceptability. The *dahi* sample treatment T_2 was attractive, possessing pleasant acidic flavor, smooth and firm body and texture and glossy surface appearance without any free wheying off. The mean score for overall acceptability of all samples of *dahi* were significantly ($P < 0.05$) differed from each other. The *dahi* samples T_3 had significantly ($P < 0.05$) lowest score (6.23) than the rest of *dahi* samples in the investigation for its overall acceptability.

Conclusion

From the present studies it is concluded that the better quality probiotic custard apple *dahi* can be prepared by using cow milk containing 12% total solids, 2% custard apple powder and 1% starter culture of *Lactobacillus acidophilus* strain. The custard apple *dahi* had *Lactobacillus acidophilus* counts in the range of 22.5 x 10^6 to 26.25 x 10^6 cfu/g) and sensory score 6.23 to 8.45 for its overall acceptability.

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