



## OPTIMISATION OF PREBIOTIC HONEY AND OLIGOFRUCTOSE FOR DEVELOPMENT OF CULTURED LOW FAT SYNBIOTIC BUTTERMILK

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### ABSTRACT

Buttermilk is one of the most important functional dairy products leftover after the churning of cream, that have excellent health and disease curing potentials and it is now receiving high interest from consumers all over the world. The churning of curd into buttermilk degrades the milk proteins which help in their easy digestion. In cultured low fat synbiotic buttermilk samples with optimum combination of prebiotics and probiotics will help to improve its nutritional qualities and deliciousness. Different levels of prebiotics such as honey and oligofructose at 2, 3, 4 and 5 per cent level were added in the preparation of cultured low fat synbiotic buttermilk samples to assess the optimum inclusion level of them based on the sensory evaluation by the panel of sensory judges by 9 point hedonic scale. The sensory evaluation of the resultant products showed that honey and oligofructose added separately at the rate of 2 per cent level in the samples were acceptable by the sensory panel because those samples were similar to that of control. Hence the optimum inclusion level of prebiotics viz., honey and oligofructose was recommended up to 2 per cent for the preparation of prebiotic buttermilk.

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### INTRODUCTION

Historically, the value of fermented milk products had been appreciated because of their medicinal and nutritional properties. Further, the fermented milk products have been very popular in the vedic and medieval era. Buttermilk plays a major role due to its thirst quenching property and taste. Regular intake of buttermilk improves the immunity and its probiotic nature is beneficial to the gut of consumers. Buttermilk contains vitamins, potassium, calcium, and traces of phosphorus (Conway *et al*, 2014). It is a favourite traditional drink during summer (Nirgude *et al*, 2013). Furthermore, buttermilk has several therapeutic potentials such as cholesterol reduction, blood pressure reduction (Conway *et al*, 2013), antiviral (Fuller *et al*, 2013) and anticancer effects (Larsson *et al*, 2008).

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health. The important beneficial effects are antimicrobial activity, immune system modulation, antimutagenic activity, colonization resistance activity, maintenance of micro-ecology of bowel, stimulation of *Bifidobacteria*, deactivation of carcinogens *etc.* (Shiby and Mishra, 2013).

Prebiotics, the non-digestible food ingredients passing through the upper gut undigested but are fermented by colonic bacteria selectively (Ramirez-Farias *et al*, 2009). Honey contains carbohydrates: 80 per cent, ash: 0.1 per cent, and protein: 0.45 per cent (Shamala *et al*, 2000). The composition of carbohydrates (in grams) includes fructose (43), glucose (45), sucrose (2.5), maltose (4.1) and others (5.4). Itsaranuwat *et al* (2003) reported that different honeys contain specific oligosaccharides. It is likely that one or more of these compounds would prove stimulatory to *Bifidobacterium* species. The oligosaccharides are commonly added to food as prebiotics to help in the preferential growth of probiotic organism (Hattingh and Viljoen, 2001).

Combination of probiotics and prebiotics are called as synbiotics (Zeynab *et al*, 2010). *Lactobacillus acidophilus* strains are one of the most important Lactobacilli present in the intestine of human being and hence are generally used as probiotic culture in preparation of dairy products (Sandine, 1979). The study aimed to assess the optimum level of inclusion of prebiotic such as honey and Oligofructose in the preparation of low fat cultured synbiotic buttermilk.

### MATERIALS AND METHODS

Fresh cow milk obtained from AAC dairy farm after the removal of cream was utilized for the study. The freeze dried dahi cultures of mixed (Mesophilic) strain NCDC 352 and other probiotic starter cultures such as *Lactobacillus acidophilus* NCDC 014 and *Bifidobacterium bifidum* NCDC

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232 were purchased from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal. The prebiotic substance oligofructose obtained from the local essence market. Honey has been obtained directly from honey hives, were utilized as prebiotics in the study. The statistical analysis of data was carried out by applying completely randomized design (CRD) (Steel and Torrie, 1980).

**Method of preparation of curd for buttermilk**

The cultured low fat synbiotic buttermilk was prepared as per the procedure of Sukumar De (2004) with slight modification. The skim milk was preheated to 35°C and filtered to remove any foreign materials. The prebiotic like honey or oligofructose were added (in separate treatments) at 2, 3, 4 and 5 per cent level based on the treatment type (Table 1 and 2). The mix was then pasteurized at 63°C for 30 minutes. The mix was then cooled to 37°C and starter cultures were added at the rate of 2 per cent and incubated at 37°C so as to reach the pH 4.5. After attained the desired fermentation process, the product was gently stirred to break the curd, and stored at refrigeration temperature (5°C) for further analysis.

**Preparation of buttermilk from curd**

Butter milk was prepared with addition of water at the ratio of 1: 3 with curd and homogenised to achieve a uniform texture. The optimum inclusion levels of prebiotics (honey and oligofructose) in buttermilk were selected based on the sensory characteristics. The buttermilk samples packed in PET bottles and stored at 5±1°C were brought to 10°C and were evaluated for sensory qualities by nine point hedonic scale containing an expert panel of nine judges.

**Table 1** Optimization of Honey in the synbiotic buttermilk preparation

Ingredients	Control	T1	T2	T3	T4
Milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk
Starter cultures	Dahi culture	Dahi culture	Dahi culture	Dahi culture	Dahi culture
Honey (per cent)	-	2	3	4	5

**Table 2** Optimization of Oligofructose in the synbiotic buttermilk preparation.

Ingredients	Control	T5	T6	T7	T8
Milk	Skim milk	Skim milk	Skim milk	Skim milk	Skim milk
Starter cultures	Dahi culture	Dahi culture	Dahi culture	Dahi culture	Dahi culture
Oligofructose (%)	-	2	3	4	5

**RESULTS AND DISCUSSION**

The study was carried out to find out the optimum percentage of prebiotics like honey and oligofructose (as separate treatments) were included in the preparation of synbiotic buttermilk. The treatments were prepared with different combinations of prebiotics viz., Honey and Oligofructose and probiotics viz., *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, along with skim milk and dahi starter.

The sensory scores for control and honey incorporated buttermilk samples at 2, 3, 4 and 5 per cent are presented in table 3. Statistical analysis of the data revealed a significant (P<0.01) difference in the flavor and taste scores for control and treatments. As the inclusion level of honey increased, the

flavor and taste scores of the buttermilk samples decreased progressively. The mean flavor and taste scores of synbiotic buttermilk for control and 2, 3, 4 and 5 per cent inclusion levels of honey were 7.30±0.01, 7.31±0.01, 7.22±0.01, 7.17±0.01 and 7.07±0.03, respectively. The results further revealed that inclusion of honey incorporated at the rate of two per cent had the flavor and taste score similar to that of control buttermilk samples.

As the inclusion level of honey increased, the consistency score of the treatment samples decreased progressively. The mean consistency scores for control and different level of inclusion (2, 3, 4 and 5 per cent) of honey were 7.57 ± 0.01, 7.58 ± 0.02, 7.52 ± 0.01, 7.46 ± 0.02 and 7.41 ± 0.01, respectively. Honey included at the level of two per cent was at par with control buttermilk.

Similarly, the mean sensory scores for colour and appearance of the treatment samples decreased progressively as the inclusion level of honey increased. The colour and appearance scores for control and different level of inclusion of honey were 7.58±0.01, 7.63±0.01, 7.52 ±0.01, 7.46±0.01 and 7.42±0.01, respectively. However, honey included at the level of two per cent was at par with control and was acceptable by the sensory panel.

As the inclusion level of honey increased, the overall average sensory scores of the buttermilk samples decreased progressively. The overall mean sensory scores for control and 2, 3, 4 and 5 per cent inclusion levels of honey were 7.51±0.04, 7.48±0.05, 7.42±0.05, 7.37±0.03 and 7.30±0.06, respectively.

Contrary to the present findings, Macedo *et al.* (2008) prepared synbiotic milk drink with 3 per cent (w/v) of pasteurized honey along with probiotic cultures of *Lactobacillus* spp. and *Bifidobacterium* spp. cultured in 12 per cent(w/v) reconstituted non-fat dry milk. They found that the counts of *L. acidophilus* and *Bifidobacterium* species in the presence of honey were significantly higher. Sharma (2010) incorporated honey at 0, 3, 5 and 7 per cent levels in the preparation of synbiotic lassi and found that inclusion level of honey up to 5 per cent showed relatively better acceptance, better viable count of lactobacilli and optimum level of acid production. However, the results of present study revealed that the inclusion of honey above two per cent has reduced the acceptance of buttermilk. The variations in the results might be attributed to the types of drink prepared and inclusion levels of other ingredients used for the preparation of drink.

Upon analysis of the results of sensory panel, honey included at the level of two per cent was similar with control and hence, it has recommended for the preparation of honey incorporated prebiotic buttermilk.

**Table 3** Sensory scores of synbiotic buttermilk samples prepared with different inclusion levels of prebiotic honey.

Parameters	Control	Treatments			
		T1	T2	T3	T4
Flavour and Taste	7.30±0.01	7.31±0.01	7.22±0.01	7.17±0.01	7.07±0.03
Consistency	7.57±0.01	7.58±0.02	7.52±0.01	7.46 ±0.02	7.41±0.01
Colour and Appearance	7.58±0.01	7.63±0.01	7.52±0.01	7.46±0.01	7.42±0.01
Over All Sensory Scores	7.51 <sup>a</sup> ± 0.04	7.48 <sup>a</sup> ± 0.05	7.42 <sup>b</sup> ± 0.05	7.37 <sup>b</sup> ± 0.03	7.30 <sup>c</sup> ± 0.06

Means bearing (n=6) with different superscripts differ significantly (P<0.01)

More than two per cent of inclusion in the butter milk has been found a sweet taste by the sensory panel. Moreover, the presence of sweetness is not always preferred for butter milk by the consumers.

The sensory scores for different inclusion levels of prebiotic oligofructose used in the preparation of synbiotic buttermilk samples are presented in table 4. Oligofructose was included in the buttermilk at the levels of 2, 3, 4 and 5 per cent. As the inclusion level of oligofructose increased, the total sensory scores of the treatments decreased progressively as that of honey. There was a significant decrease in the sensory scores ( $P < 0.01$ ) of the oligofructose incorporated buttermilk samples as compared to the control. As the inclusion level of oligofructose increased, the flavor and taste score of the treatments decreased progressively. The average flavor and taste scores for control and 2, 3, 4 and 5 per cent inclusion levels of oligofructose were  $7.32 \pm 0.01$ ,  $7.31 \pm 0.02$ ,  $7.20 \pm 0.01$ ,  $7.07 \pm 0.03$  and  $7.07 \pm 0.02$  respectively. The results of the study revealed that buttermilk samples included with two per cent oligofructose has secured flavor and taste scores at par with control samples.

The scores for color and appearance of buttermilk also decreased as the inclusion level of oligofructose increased. The color and appearance score for control and different level of inclusion (2, 3, 4 and 5 per cent) of oligofructose were  $7.59 \pm 0.01$ ,  $7.48 \pm 0.06$ ,  $7.51 \pm 0.01$ ,  $7.45 \pm 0.01$  and  $7.39 \pm 0.01$  respectively. The color and appearance scores of buttermilk samples prepared with two per cent oligofructose were at par with control samples.

**Table 4** Overall sensory scores of synbiotic butter milk samples prepared with different inclusion levels of prebiotic oligofructose.

Parameters	Control	Treatments			
		T5	T6	T7	T8
Flavour and Taste Scores	$7.32 \pm 0.01$	$7.31 \pm 0.02$	$7.20 \pm 0.01$	$7.07 \pm 0.03$	$7.07 \pm 0.02$
Over All Sensory Scores	$7.52^a \pm 0.047$	$7.46^a \pm 0.057$	$7.39^b \pm 0.047$	$7.31^b \pm 0.037$	$7.28^b \pm 0.02$

Means bearing (n=6) with different superscripts differ significantly ( $P < 0.01$ )

As the inclusion level of oligofructose increased, the consistency score of the treatments decreased progressively. The average consistency scores for control and different level of inclusion (2, 3, 4 and 5 per cent) of oligofructose were  $7.59 \pm 0.01$ ,  $7.51 \pm 0.03$ ,  $7.48 \pm 0.01$ ,  $7.45 \pm 0.01$  and  $7.39 \pm 0.01$ , respectively. Oligofructose included at the level of two per cent was at par with control pertaining to the consistency scores.

The overall average scores for control and treatments buttermilk samples with 2, 3, 4 and 5 per cent inclusion levels of oligofructose were  $7.52 \pm 0.04$ ,  $7.46 \pm 0.05$ ,  $7.39 \pm 0.04$ ,  $7.31 \pm 0.03$  and  $7.28 \pm 0.02$ , respectively.

Buttermilk samples prepared with two per cent oligofructose had maximum sensory score of  $7.46 \pm 0.05$  and is similar that of control and hence two per cent inclusion level of oligofructose was recommended by the expert panel for the preparation of oligofructose incorporated prebiotic buttermilk. Our present findings were similar to the findings of Fornelli *et al.* (2014) and found that addition of prebiotic ingredients viz., inulin and oligofructose added at two per cent level to dairy beverages did not interfere with the overall acceptance and intention to purchase the beverages. De Castro *et al.*

(2008) also opined that fermented probiotic lactic beverages prepared with oligofructose were sensory quality preferred in relation to the control, also showing good overall acceptability.

## CONCLUSION

Traditionally, buttermilk occupies a major part of Indian's food habit. Buttermilk is known for its cooling, refreshing, thirst quenching, delicious, nutritive and digestive properties. Different levels of prebiotics such as honey and oligofructose at 2, 3, 4 and 5 per cent level were added in the preparation of cultured low fat synbiotic buttermilk to assess the optimum inclusion level of them based on the sensory evaluation. The sensory evaluation of the resultant products showed that honey and oligofructose added separately at the rate of 2 per cent level in the buttermilk samples were acceptable by the sensory panel because those samples were similar to that of normal cultured buttermilk. Hence the optimum inclusion level of prebiotics viz., honey and oligofructose were recommended at the rate of 2 per cent for consumers with health consciousness.

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