

Microbial studies of Bael (*Aegle marmelos* (L.) Corr.) pulp incorporated shrikhand

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Abstract

The present study was undertaken to develop and evaluate the microbial load in bael fruit incorporated shrikhand and to evaluate the storage stability of this product as influenced by treatments. Microbial quality is important in standardization of the formulation. In the present study, during initial days of storage total bacterial count ($4.68 \log_{10} \text{cfu/mL}$), yeast count ($1.60 \log_{10} \text{cfu/mL}$), mould count ($1.80 \log_{10} \text{cfu/mL}$) were detected higher levels in T₈ (35% bael pulp + 65 mL milk + 40g sugar + 1.5 mL *L. bulgaricus* curd culture). Whereas lower number of colonies of bacterial ($1.30 \log_{10} \text{cfu/mL}$), yeast ($0.60 \log_{10} \text{cfu/mL}$) and mould ($0.40 \log_{10} \text{cfu/g}$) were noticed in T₁ (100 mL milk + 40g sugar + 1.5 mL *L. bulgaricus* curd culture). After 15 days of storage in shrikhand sample the rose in the number colonies were observed.

Index terms: Shrikhand, Bael (*Aegle marmelos* (L.) Corr.), lactic acid bacteria, Microbial quality, Storage stability and Fermentation of milk

Introduction

The practice of value addition to dairy base with other fruits can serve a dual purpose of addressing malnutrition as well as contributing to control of post-harvest losses of such plant harvests. Microorganisms can thrive in food by having a favourable habitat. Yeasts, moulds and a broad spectrum of bacteria grow in milk at temperatures above 16°C (Durham, 2016). The microbial growth in milk and milk products is mostly dependent on the temperature, nutrient availability, water supply, oxygen supply and acidity of the medium. The staple desert and a fermented milk product, “Shrikhand” was obtained from chakka to which fruits, nuts, sugar, saffron and other spices can be added. Shrikhand is known for its high nutritive, characteristic flavour, taste, palatable nature and possible therapeutic value. It is very refreshing particularly during summer months and is recommended for people with obesity and cardiovascular diseases as it have low fat content.

Material and Methods

Shelf-life studies of bael fruit incorporated shrikhand

The bael fruit pulp incorporated into shrikhand was packed in polythene boxes and shelf life study was carried out under refrigerated conditions (7°C) for about fifteen days.

Observations recorded

Total microbial count (log₁₀ cfu/mL)

Ten grams of shrikhand was taken for analysis. The samples were plated on specific media following serial dilution technique. Plates were incubated for three days at 25±1°C, colonies were counted and CFU per gram was calculated. At the beginning and end of storage period, the samples were analysed for growth of bacteria, yeast and fungi contamination by using standard serial dilution plate count technique (Ben-David and Davidson, 2014). The microbial analysis was carried out at initial and after completion of storage period. The total colonies were counted and results expressed as colony forming units (log CFU/g of sample) were determined using the following formula

$$\text{Total counts} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Weight of the sample}}$$

Colour (*L**, *a** and *b**)

Colour of ice cream premix was measured with a Color Flex EZ (Model CFEZ 1919, Hunter Associates Laboratory, Inc., Reston) with a 45 mm (diameter) measuring tube using a white tile background.

Statistical analysis

The average, mean and standard deviation of basic statistical tools were adopted. The level of significance used in 'F' and 't' test was p=0.01. Critical difference values were calculated whenever 'F' test found significant.

Results and discussion

Table 1 depicts the microbial quality for bael pulp incorporated shrikhand during storage period of 15 days under refrigerated conditions (7°C). The initial microbial count (log₁₀cfu/mL) was recorded high in treatment T8 [(35% Bael pulp + 65 mL milk + 40g sugar + L. bulgaricus curd culture@ 1.5mL) (bacteria: 4.68, yeast: 1.60 and mould:1.80)] and lowest microbial load was observed in control T1 (bacteria:1.30, yeast:0.60 and mould:0.40) compared to control.

Whereas, up on storage up to 15 days, the total microbial count increased. The highest microbial load was seen in T₈ [total bacteria: 5.32 yeast: 1.40 and mould count: 1.74] and lowest microbial load was seen in T₁ (bacteria:5.10, yeast: 0.86 and mould: 0.60). No significant difference was observed among yeast and fungi during the storage period.

Table 1: Effect of bael pulp incorporation on total microbial load of shrikhanda stored (0-15days) at refrigeration (7°C) temperature

Treatment details	Total microbial load (log10cfu/mL)					
	Bacteria		Yeast		Mould	
	Initial	Final	Initial	Final	Initial	Final
T ₁ : 100 mL milk (control)	1.30 ^b	5.10 ^b	0.60	0.86	0.40	0.60
T ₆ : 25% BP + 75 mL milk	1.55 ^b	5.22 ^{ab}	1.00	0.40	0.60	0.80
T ₇ : 30% BP + 70 mL milk	2.12 ^b	5.28 ^a	1.40	0.60	1.00	0.98
T ₈ : 35% BP + 65 mL milk	4.68 ^a	5.32 ^a	1.60	1.40	1.80	1.74
Mean	2.41	5.23	1.15	0.82	0.95	1.03
S.Em±	0.41	0.04	0.38	0.34	0.37	0.38
CD at 1%	1.68	0.18	NS	NS	NS	NS

Note: 1. Similar alphabets within the column represents non significant differences at (p<0.01)

2. 100ml milk yields 32g chakka

The above treatment includes the ingredients in common such as 40g sugar and *L. bulgaricus* curd culture@1.5mL

Colour (*L**, *a** and *b**)

Table 2 represents the change in *L**, *a** and *b** value for shrikhanda at 0 and 15 days of storage under refrigerated conditions.

Instrumental *L** value

The results pertaining to the change in the instrumental *L** value was notably different at initial and at 15th day of storage of bael fruit pulp incorporated shrikhanda. Significant difference was observed among the treatments during storage period. However, the highest *L** value was found in T₁ (78.19), followed by T₂ (74.42), on the other hand lowest was found in T₈ (70.48) at the initial days of storage. At 15th day of storage highest *L** value was found in T₁ (72.57), followed by T₆ (66.92), on the other hand lowest was found in T₈ (62.76) which is on par with T₇ (63.18).

Instrumental *a** value

The results pertaining to the change in the instrumental *a** value was notably different at initial and at 15th day of storage of bael fruit pulp incorporated shrikhanda. Significant difference was observed among the treatments. However, the highest *a** value was found in T₈ (35% Bael pulp + 65 mL milk + 40g sugar + *L.*

bulgaricus curd culture@ 1.5mL: 6.24), followed by T₇ (5.20), on the other hand lowest was found in T₁ (-1.19) at the initial days of storage.

Table 2: Effect of bael pulp incorporation on instrumental colour (*L, *a** and *b**) values of shrikhand stored (0-15days) at refrigeration (7°C) temperature**

Treatment details	Instrumental colour values					
	Initial			Final		
	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>
T ₁ : 100 mL milk (control)	78.19 ^a	-1.19 ^d	9.64 ^d	72.57 ^a	-0.41 ^d	9.05 ^d
T ₆ : 25% BP + 75 mL milk	74.42 ^b	3.58 ^c	26.06 ^c	66.92 ^b	4.05 ^c	24.05 ^b
T ₇ : 30% BP + 70 mL milk	70.83 ^c	5.20 ^b	29.51 ^b	63.18 ^c	4.93 ^b	22.55 ^c
T ₈ : 35% BP + 65 mL milk	70.48 ^d	6.24 ^a	31.26 ^a	62.76 ^c	5.22 ^a	26.84 ^a
Mean	73.48	4.05	24.12	66.36	3.65	20.62
S.Em±	0.05	0.01	0.04	0.11	0.01	0.05
CD at 1%	0.22	0.04	0.16	0.46	0.04	0.22

Note: 1. Similar alphabets within the column represents non significant differences at (p<0.01)

2. 100ml milk yields 32g chakka The above treatment includes the ingredients in common such as 40g sugar and *L. bulgaricus* curd culture@1.5 mL

At 15th days of storage highest *a** value was found in T₈ (5.22), followed by T₇ (4.93), on the other hand lowest was found in T₁ (100 mL milk +40g sugar + *L. bulgaricus* curd culture@ 1.5mL: -0.41).

Instrumental *b** value

The results pertaining to the change in the instrumental *b** value was notably different at initial and at 15th day of storage of bael pulp incorporated shrikhand. However, the highest *b** value was found in T₈ (35% Bael pulp + 65 mL milk + 40g sugar + *L. bulgaricus* curd culture@ 1.5mL: 31.26), followed by T₇ (29.51), on the other hand lowest was found in T₁ (9.64) at the initial days of storage. At 15th day of storage highest *b** value was found in T₈ (26.84), followed by T₇ (22.55), on the other hand lowest was found in T₁ (100 mL milk +40g sugar + *L. bulgaricus* curd culture@ 1.5mL: 9.05).

Discussion

Microbial characteristics of sample contribute to the quality and shelf life of the product. According to World Health Organisation (WHO) standards for the analysis of the microbial load in samples, the limits for the aerobic count, yeast and mould count should be <1000 and <100 cfu/g, respectively (Anon, 2003). In the present investigation the maximum number of bacteria (4.68 log₁₀cfu/mL), yeast (1.60 log₁₀cfu/mL) and mould (1.80log₁₀cfu/mL) counts were present in T₈ (35% bael pulp + 65mL standardized milk + 40g sugar + *L. bulgaricus* curd culture@ 1.5mL). Increasing trend in the microbial load was observed among the treatments. This may be due to the increased moisture content by the addition of bael pulp into shrikhand. These findings

are in parallel with the investigations of Hole *et al.* (2017), where by the increase in level of unripe banana to the chakka increased level of microbial load was observed. Terdal *et al.*, 2017 studied that the amount of wheat, finger millet and fenugreek powder used to make composite mix blend diet was 70, 70 and 15 g each respectively. And the results showed that the microbial count was within limit.

After completion of 15 days of storage, among the different treatments the maximum microbial load (bacteria- $5.10 \log_{10} \text{cfu/mL}$, yeast- $1.40 \log_{10} \text{cfu/mL}$ and mould count- $1.74 \log_{10} \text{cfu/mL}$) counts were present in treatment T₈ (35% bael pulp + 65mL standardized milk + 40g sugar + *L. bulgaricus* curd culture@ 1.5mL) and the minimum bacterial colonies were found in T₁ (100mL standardized milk + 40g sugar + *L. bulgaricus* curd culture@ 1.5mL) while in case of both yeast and mould count no-significant difference was observed. The increasing trend in the growth of the microorganism were found upon storage. This may be due to increased moisture content and reduced levels of sugar, which acts as preservative led to the increase in microbial load upon storage. The present investigations are similar to the findings of Hole *et al.* (2017). Darshini *et al.*, 2021 studied the lightness value (L^*) was found to be maximum (63.67) in the treatment T1 i.e. control followed by T2 (48.24) and minimum (40.42) L^* value was recorded in the treatment T5 (82.5% APF +12.5% PPP + 5% DSF).

During storage, at initial day of storage the mean L^* values ranged from 70.48 to 78.19 and after 15 days of storage values ranged from 62.76 to 72.57. The decreasing trend in the lightness values were observed with increased level of bael pulp addition. During storage decreasing L^* value was found along storage, this may be due to the occurrence of Millard reaction between the sugar and nitrogen bases present in the sample.

In case of a^* and b^* values of the shrikhand had the mean values at initial ranges from -1.19 to 6.24 and 9.64 to 31.26 respectively. Also, the current findings showed the decline in redness value (-0.41 to 5.22) and yellowness (9.05 to 26.84) in the shrikhand after the 15 days of storage compared to initial. This may be due to the incorporation of bael pulp which is a good source of carotenoids have increased the a^* and b^* values along the treatments and upon storage the non-enzymatic browning reaction or the occurrence of Millard reaction might have released the melanoid pigments which led to the increased a^* and b^* values along the treatments. These findings are in parallel with the results of Barkallah *et al.* (2017) where they found that addition of spirulina has increased the L^* values and no significant difference was observed in a^* and b^* due to the chlorophyll pigment and upon storage degradation of chlorophyll pigment led to the change in the L^* , a^* and b^* values. Increase in darkness value also reflected in a^* values of the pomegranate seed powder (Harish *et al.*, 2022).

Conclusion: It is also concluded that developed bael pulp incorporated shrikhand [T₇ (30% bael pulp + 70 mL + 40g sugar + 1.5mL *L. bulgaricus* culture)] were showed excellent physical parameters and microbiologically well acceptable up to 15 days storage study.

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