Influence of Microbial Load on Bael (*Aegle marmelos* (L.) Corr.) Pulp fruit Powder Incorporated Ice Cream Premix

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Abstract

The present study was undertaken to evaluate the shelf stability of bael fruit powder incorporated ice cream pre mix. Microbial quality is an important in standardization of the formulation. In the present study, during initial days of storage total bacterial count (3.59log10cfu/mL), yeast count (2.49log10cfu/mL), mould count (3.28log10cfu/g) were detected higher levels in T_4 compared to control. Whereas, lower number of colonies of bacterial (0.80log10cfu/mL), yeast (1.15log10cfu/mL) and mould (1.53log10cfu/mL) were noticed in T_1. After three months of storage in bael powder incorporated ice cream premix sample the decrease in the number colonies were observed and found maximum number of colonies for bacterial (2.14log10cfu/mL), yeast (1.26log10cfu/mL) and mould (1.88log10cfu/mL) in treatment T_4. Whereas the lower total bacterial count (2.03log10cfu/mL), yeast count (1.15log10cfu/mL), mould count (1.53log10cfu/mL) were detected in T_1.

Index terms: Ice-cream premix, Total bacterial count, Yeast count, Mould count, Bael powder and Microbial quality

Introduction

During manufacture of ice-cream, delicate flavours are more preferred than the stronger ones. The intensity of flavour should be just enough to be easily recognized and delicately pleasing to taste (Arbuckle, 1986). Flavouring materials are available in natural, artificial and blended forms (mix of natural and artificial). Vanilla is the most popular ice-cream flavour that is extracted from vanilla planifolia. It also used as flavour enhancer with other flavours such as chocolate, cocoa, fruits and nuts. Some herbal icecreams also have been developed using mint, green tea, ginger, asparagus etc as flavouring agents intended to provide the medicinal properties to consumers through this largely consumed snack.

Bael fruits are available in many forms and these include raw and ripe whole fruits, fruit powder, fruit extract, fruit extract powder, bael seeds, dried unripe fruit slices, ripe fruit drink, bael fruit tea, bael fruit juice, fruit jelly, jam and other large number of products such as candy, toffee, jam, juice etc. with the help of various post-harvest technologies. These technologies help to reduce the post-harvest losses resulting in increase in the shelf life of bael products. However, in recent years, there has been observed increasing demand for so called dairy-based products. The increasing demand and purchasing power of consumers for dairy products with 'functional' properties is a key factor driving growth in sales value developed markets. Dairy products have long been advertised as being excellent sources of nutrition. Hence the study was under taken for microbial quality of premix prepared from bael fruit pulp powder.

Material and Methods

Shelf-life studies of bael fruit powder incorporated icecream premix (0-3mths)

The bael fruit powder incorporated into ice cream premix was packed in aluminum pouches and shelf life study was carried out under ambient conditions.

Observations recorded

Total microbial count (log10 cfu/mL)

Bael fruit incorporated ice cream premix was subjected to microbial analysis by employing the serial dilution method

Water activity (a_w)

Water activity of ice cream premix was measured by using water activity meter. Water activity of sample was measured by placing 5g of sample in the chamber. The observation was directly read in the instrument after it was stabilized.

Non enzymatic browning (OD)

The non-enzymatic browning of the ice cream premix was estimated by using spectrophotometric method suggested by Srivastava and Sanjeevkumar. (1998). 10gm of sample was centrifuged at 4000rpm for 15 minutes. To the supernatant, 15 ml of 60 percent alcohol was thoroughly mixed and filtered. The absorbance of the filtrate at 220nm was recorded in spectrophotometer using 60 percent alcohol as blank. The value of non-enzymatic browning was expressed as optical density (OD).

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.

Experimental results

Water activity (a_w) at initial days of storage

Table 1 depicts the data related to water activity of bael fruit powder incorporated ice cream premix at initial days of storage. No significant difference was observed among the treatments. The mean water activity was 0.38 (a_w). The maximum water activity was observed in T_4 (15% bael powder + 44% sugar + 34% skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.41), followed by T_3 (0.37) whereas minimum water activity was observed in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.37).

Water activity (a_w) after three months of storage

The data showed that after three months of storage, water activity in ice cream premix was found non-significant among the treatments. The maximum water activity was observed in T_4 (15% bael powder + 44% sugar + 34% skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.56) followed by T_3 (0.56). And minimum water activity was observed in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.50).

Water activity is in close association with the physical, chemical and biological properties of food. It influences on texture, colour, aroma and stability of the food products (Rockland and Nishi, 1980). No significant difference was observed among the treatments during storage period. Throughout the storage period (initial 0.38% and final 0.53%) the mean water activity was highest in T_4 (15% bael fruit powder + 44% sugar + 34% SMP + 0.2% gelatin + 0.2% GMS + 0.2% CMC) and lowest in T_1 (59% sugar + 34% SMP + 0.2% gelatin + 0.2% GMS + 0.2% CMC). This may be due to the increased moisture content along the treatments and also upon storage. This may be due to the high water binding strength of bael fruit powder. Water activity of all the formulation was lower than that of 0.6, which further correlates to the shelf stability of the powders. This study is in parallel with the findings of Kumar *et al*. (2022), where they found over the storage period, water activity of instant banana milk powder increased with increase in powder level.

Non enzymatic browning (OD)

Initial non-enzymatic browning (OD)

The data pertaining to the initial non-enzymatic browning (OD) is represented in Table 1. The mean initial non-enzymatic browning (OD) is 0.90. A significant minimum initial non-enzymatic browning (OD) was observed in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.84). Whereas, the significant maximum initial non-enzymatic browning was noted in T_2 (0.94) which is on par with T_3 (0.93) and T_4 (15% bael powder + 44% sugar + 34% skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.93).

Non enzymatic browning is a characteristic change that occurs in food products. Millard reaction is a non-enzymatic interaction between reducing sugar and amino acid, peptide or protein resulting in a variety of by-products called melanoidins, which results in the change in colour, taste and aroma of processed and stored foods (Manzocco *et al.*, 2000).

Treatment details	Water activity		Nonenzymatic browning	
	Initial	**Final**	**Initial**	**Final**
T_1: 59% sugar + 34 % SMP (Control)	0.37	0.50	0.84^{b}	0.87^{b}
T_2: 5% Bael fruit powder + 54% sugar + 34% SMP	0.38	0.52	0.93^{ab}	1.28^{a}
T_3: 10% Bael fruit powder + 49% sugar + 34% SMP	0.38	0.56	0.93^{a}	1.37^{a}
T_4: 15% Bael fruit powder + 44% sugar + 34% SMP	0.41	0.56	0.94^{ab}	1.45^{a}
Mean	**0.38**	**0.53**	**0.90**	**1.24**
S.Em±	0.02	0.02	0.02	0.09
CD at 1%	NS	NS	0.09	0.36

In the current experiment, the data pertaining to the non-enzymatic browning reactions during storage period is depicted in Table 1. During the shelf life study the mean initial 0.90 and final 1.24OD values were observed. The non-enzymatic browning reaction was highest in T_4 (15% bael fruit powder + 44% sugar + 34% SMP + 0.2% gelatin, + 0.2% GMS + 0.2% CMC) and lowest was observed in T_1 ((59% sugar + 34 % SMP+ 0.2% gelatin, + 0.2% GMS + 0.2% CMC). The increasing trend in non -enzymatic browning was observed. The possible reason for increase in browning is the increased moisture content during storage and also storage temperature. Further, the interaction between sugar and nitrogenous content which resulted in the Millard reaction. These findings are in similar to the findings of Kumar *et al*. (2022) they observed the increase in the browning reactions of the instant banana milk powder with increase in levels of unripe banana powder over the period of storage.

Table 1: Effect of bael fruit powder incorporation on water activity and nonenzymatic browning of ice cream premix storage

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

The above treatments include the following common ingredients:

Corn flour: 6.4g | Gelatin: 0.2g | Glycerol Monostearate: 0.2g

Carboxy methylcellulose: 0.2g | SMP: Skimmed milk powder 34g

Non enzymatic browning (OD) after three months of storage

The data related to non-enzymatic browning (OD) after three months of storage is represented in table 1. The mean non-enzymatic browning (OD) after storage is 1.24. A significant minimum non-enzymatic browning (OD) was observed in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.87). Whereas, the significant maximum non-enzymatic browning was noted in T_4 (15% bael powder + 44% sugar + 34% skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 1.45) which is found on par with T_3 (1.28) and T_4 (1.37).

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

Table 2 represents the change in *L**, *a** and *b** value for bael fruit incorporated ice cream premix.

Instrumental *L value**

The results pertaining to the change in the instrumental *L** value was notably different at initial and after three months of storage of bael fruit incorporated ice cream premix. However, the significantly highest *L** value was found in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 95.08), on the other hand significantly lowest *L** value was found in T_4 (15% bael powder + 44% sugar + 34% skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 88.14).

After three months of storage, the significantly highest L* value was found in T1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 93.64), on the other hand significantly lowest L* value was found in T4 (15% bael powder + 44% sugar + 34% skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 86.26). 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).

Instrumental *a value**

The results pertaining to the change in the instrumental *a** value was notably different at initial and after three months of storage of bael fruit incorporated ice cream premix. However, at initial days of storage the significantly highest *a** value was found in T_4 (3.24), on the other hand significantly lowest was found in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: -1.54).

After three months of storage significantly highest a* value was found in T4 (3.63), on the other hand significantly lowest was found in T1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 0.71). Increase in darkness value also reflected in a* values of the pomegranate seed powder (Harish *et al*, 2022).

Instrumental *b value**

The results pertaining to the change in the instrumental *b** value was notably different at initial and after three months of storage of bael fruit incorporated ice cream premix. However, the significantly highest *b** value was found in T_4 (18.46), on the other hand significantly lowest was found in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 9.35) at the initial days of storage.

After three months of storage significantly highest *b** value was found in T_4 (19.46), on the other hand significantly lowest was found in T_1 (59% sugar + 34 % skimmed milk powder + 0.2% CMC + 0.2% GMS + 0.2% gelatin: 12.91).

Colour is an important characteristic of quality as it influences on the consumer acceptability in fruit and vegetable products. The maximum values for *L** was found in T_1 (initial 95.08, final 93.64) and minimum values were found in T_4 (initial 88.14, final 86.26). Due to incorporation of bael fruit powder the lightness of the premix has decreased indicating the darkening of the surface colour and upon storage also lightness has decreased because of non-enzymatic reaction occurred in the premix.

Also, the maximum values for *a** was found in T_4 (initial 3.24, final 3.63) and minimum *a** values was found in T_1 (initial -1.54, final 0.71). Maximum *b** values were found in T_4 (initial 18.46, final 19.46) and minimum values were found in T_1 (initial 9.35, final 12.91). This increase of *a** and *b** indicates the more of red and more of yellow colour in ice cream premix. This is due to the bael fruit carotenoids. And upon storage the *a** and *b** values were also increased because of increased non-enzymatic browning reaction. These findings are similar to study of Liu *et al.* (2010) in which tomato powder instrumental colour values like *L** values decreased, *a** values increased and *b** values also increased upon storage.

Total microbial count (log10cfu/mL)

Table 2 depicts the microbial quality for bael powder incorporated ice cream premix during three months of storage under ambient condition. The initial microbial count (log10cfu/mL) was recorded high in treatment T_4 (bacteria: 3.59, yeast: 2.49 and mould count: 3.28) which is on par with T_3 and lowest microbial count was observed in T_1. After three months of storage significant maximum total microbial load [(bacteria: 2.14, yeast: 1.26 and mould count: 1.88] was found in T_4 (15%bael powder+ 44% sugar +34% skimmed milk powder + 0.2% CMC+0.2g GMS+ 0.2g gelatin).

Table 2: Effect of bael fruit powder incorporation on instrumental colour (*L, *a**and *b**) values of ice cream premix storage (0-3months) at ambient conditions**

Treatment details	Instrumental colour values					
	Initial			Final		
	*L**	*a**	*b**	*L**	*a**	*b**
T_1: 59% sugar + 34 % SMP (Control)	95.08^{a}	-1.54^{d}	9.35^{d}	93.64^{a}	0.71^{d}	12.91^{b}
T_2: 5% Bael fruit powder + 54% sugar + 34% SMP	91.92^{b}	0.65^{c}	13.17^{c}	89.92^{b}	1.36^{c}	14.21^{b}
T_3: 10% Bael fruit powder + 49% sugar + 34% SMP	90.31^{c}	2.12^{b}	15.28^{b}	88.83^{c}	2.27^{b}	16.10^{a}
T_4: 15% Bael fruit powder + 44% sugar + 34% SMP	88.14^{d}	3.24^{a}	18.46^{a}	86.26^{d}	3.63^{a}	19.46^{a}
Mean	**91.36**	**1.89**	**14.07**	**89.66**	**1.99**	**15.67**
S.Em±	0.00	0.02	0.26	0.19	0.14	0.32
CD at 1%	0.02	0.09	1.09	0.80	0.57	1.34

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

The above treatments include the following common ingredients:

Corn flour: 6.4g	Gelatin: 0.2g	Glycerol Monostearate: 0.2g
Carboxy methylcellulose: 0.2g	SMP: Skimmed milk powder 34g	

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). During the storage period, the mean of total microbial load [initial (bacteria-3.59log10cfu/mL, yeast- 2.49log10cfu/mL, mould- 3.28log10cfu/mL) and final (bacterial- 2.14log10cfu/mL, yeast- 1.26log10cfu/mL, mould-1.88log10cfu/mL)] were maximum in treatment T_4. (Table 3). The minimum number of total microbial [initial (bacteria-0.80log10cfu/mL, yeast- 0.80log10cfu/mL, mould- 0.80log10cfu/mL) and final (bacterial- 2.03log10cfu/mL, yeast- 1.15log10cfu/mL, mould-1.53log10cfu/mL)] were found in T_1.

No significant difference was observed for yeast and fungi counts among the treatments during the storage period. Along the treatments the total microbial load decreased because of the increased phenolic content which hindered the growth. Upon storage the microbial load decreased, which may be due to the composition of ice cream premix. These findings are similar to the results of Yusufu *et al.* (2014) where with increase in inclusion of firm ripe plantain fruit powder the microbial load decreased and upon storage the microbial counts increased. 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.

Table 3: Effect of bael fruit powder incorporation on total microbial count of ice cream premix storage (0-3months) at ambient conditions

Treatment details	Total microbial load (log 10cfu/ml)					
	Bacteria		Yeast		Mould	
	Initial	Final	Initial	Final	Initial	Final
T_1: Control	0.80	2.03b	0.80	1.15	0.80	1.53^{b}
T_2: 5% BFP + 54% sugar	1.60	2.06^{ab}	0.80	1.21	1.60	1.83^{ab}
T_3: 10% BFP + 49% sugar	2.61	2.09^{a}	2.40	1.24	2.49	1.87^{a}
T_4: 15% BFP + 44% sugar	3.59	2.14^{a}	2.49	1.26	3.28	1.88^{a}
Mean	**2.15**	**2.08**	**1.62**	**1.22**	**2.04**	**1.78**
S.Em±	0.94	0.02	0.91	0.11	0.91	0.07
CD at 1%	NS	0.09	NS	NS	NS	0.29

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

The above treatments include the following common ingredients:

Corn flour: 6.4g | Gelatin: 0.2g | Glycerol Monostearate: 0.2g

Carboxy methylcellulose: 0.2g | SMP: Skimmed milk powder 34g

Conclusion: It is also concluded that developed bael pulp powder incorporated ice cream premix [T_3(10% bael fruit powder + 49% Sugar + 34% SMP + 0.2g gelatine + 0.2g CMC + 0.2g GMS)] were showed excellent microbiological parameters and acceptable under the 3 months storage study.

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