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Studies on microbial parameter and shelf life of gulabjamun blended with coconut and wheat bran

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Abstract
The present investigation was to study microbial parameter of *gulabjamun* blended with coconut and wheat bran such as standard plate count, coliform count and yeast and mould. *Gulabjamun* was prepared by using different levels wheat bran and coconut, were selected as 2, 3, 15, 20 and combination of both coconut and wheat bran (20+2) per cent of wheat bran and coconut respectively for further study. The data were statistically analysed by using Completely Randomized Design (CRD). The treatment T_4 (*Khoa* + 15% of coconut) was rated best among *gulabjamun* samples and was comparable to control *gulabjamun*. The standard plate count of *gulabjamun* was 7 x 10^6 CFU/g, yeast and mould count and coliform count was nil in fresh samples of *gulabjamun*. The highest count SPC for treatment T_5 (16.78 CFU/g) on 4th days at room temperature and SPC count for treatment T_5 (19.50 CFU/g) for 28th days at refrigeration temperature. The highest YMC count for treatment T_6 (24.22 CFU/g) on 4th days stored at room temperature and YMC count for treatment T_6 (24.88 CFU/g) for 28th days stored at refrigeration temperature. The SPC count of *gulabjamun* during storage was found to increase with the increase in storage period. Shelf life of *gulabjamun* containing 15 per cent coconut was up to 28 days at refrigeration temperature (7$\pm$2 ^{0}C) and 4th days at room temperature (30 $\pm$ 2 ^{0}C).

Keywords: Gulabjamun, wheat bran, coconut and microbial parameter, shelf life

Introduction
Gulabjamun occupies unique place in the array of Indian sweets (Aneja, 1992) [1]. *Gulabjamun* is a popular *khoa* based sweet and originally it was made with *khoa* and maida. It got the name of *Gulabjamun* as it looks like monsoon fruit "Jamun" and is flavoured with "rose water". Dhap *khoa* having 40 - 45 per cent moisture is normally used for its preparation. *Gulabjamun* is largely produce by manual operation which adopts small scale batch method. However, milk is considered as nearly perfect food because it contain the wide array of nutrient and some bioactive components, however, it is deficient in certain micronutrients (iron, copper and certain vitamins), and dietary fiber. Therefore, there is obvious need to supplementing the milk with a necessary micronutrients and health promoting components from suitable sources. In recent years, cereals and its ingredients are accepted as functional food and nutraceuticals because of providing dietary fiber, proteins, energy, minerals, vitamins, and antioxidants required for human health. Fresh mature coconut is an excellent source of minerals such as copper, calcium, iron, manganese, magnesium and zinc and a very good source of vitamin B complex such as folates, riboflavin, niacin, thiamine and pyridoxine. Bhatnagar *et al*. (2009) [2] reported that coconut oil contains about 93 per cent of saturated fatty acids (SFAs) among which 65 per cent is medium chain saturated fatty acids (MUFAs) of which 50-55 per cent is lauric acid (C12:0). These MUFAs do not participate in the biosynthesis and transport of cholesterol as they are directly absorbed from the intestine and passed on to liver to be rapidly metabolized for energy production (Enig, 2004) [6]. Wheat bran is generally discarded product in the milling of the flour. The wheat bran is good source of B-complex vitamins (riboflavin, niacin and thiamine), trace minerals (Ca, K, P, Mg and Niacin) in small quantities and indigestible cellulose (Kumar *et al.*, 2011) [10]. Wheat bran is more wholesome and nourishing than flour itself. It is an excellent laxative and its laxative effect is much more superior to those of fruits or vegetables because cellulose of later is more easily broken by bacteria in intestine. Wheat bran is used as supplement source of dietary fiber for prevention of colon diseases, gastric cancer, type 2 diabetes, constipation *etc*.
Most of dietary fiber consumed by people in the form of cereal, vegetables and fruits. Incorporation of plant origin material in milk or milk products, directly or indirectly adds dietary fiber in human food. Shelf-life studies are important from commercial point of view.

According to BIS (IS: 11602- 1986) *Gulabjamun* have shelf life of 3-4 days at room temperature. The shelf life of *gulabjamun* at ambient temperature, in sugar syrup is 5-7 days which can be extended to 3 weeks by hot filling in polystyrene tubs and adding 0.1% potassium sorbate as a preservative. Product is filled hot in previously sterilized metal cans after running through a steam chest for 7-8 min. and sealed. This process is expected to give a shelf life of 6 months at room temperature (IS: 11602-1986). Singh *et al*. (2011) [12] studied the shelf life of *gulabjamun*. *Gulabjamun* samples were packed in paperboard boxes of 500 g capacity with butter paper and these were stored at ambient (26.6 to 36.25 ^{0}C) and refrigerated (about 4 to 7 ^{0}C temperature using domestic refrigerator) conditions. It was found that protein and fat content of *gulabjamun* decreased with increase in storage periods irrespective of storage condition. The shelf life of *gulabjamun* at ambient and refrigeration conditions was found to be 8 to 10 days and 10 to 14 days, respectively.

Materials and Methods

The material used and methods employed for conducting the experiments are as follows.

The *gulabjamun* was prepared from composite sample of crossbreed cow milk blended with coconut and wheat bran as different level for all the treatments. The plain treatment *gulabjamun* prepared without addition of coconut and wheat bran used as a control (T_0), *Gulabjamun* with 2% wheat bran (T_1), 3% wheat bran (T_2), 15% coconut (T_3) and 20% coconut used as a (T_4) and combination of wheat bran and coconut (2% + 20%) used as (T_5).

Preparation of *khoa*

Khoa was prepare as per the standard procedure given by Rangi *et al.* (1985) [11].

Preparation of grated coconut

First black skin of wet coconut fruit was removed, followed by grating of coconut. A small quantity of water was added in grated coconut and it was allow soaking for 20 minutes. Water soaked grated coconut was blended with the help of mixer for one minute. The grated coconut paste prepared was used in different combinations with cow milk for *gulabjamun* preparation.

Preparation of sugar syrup

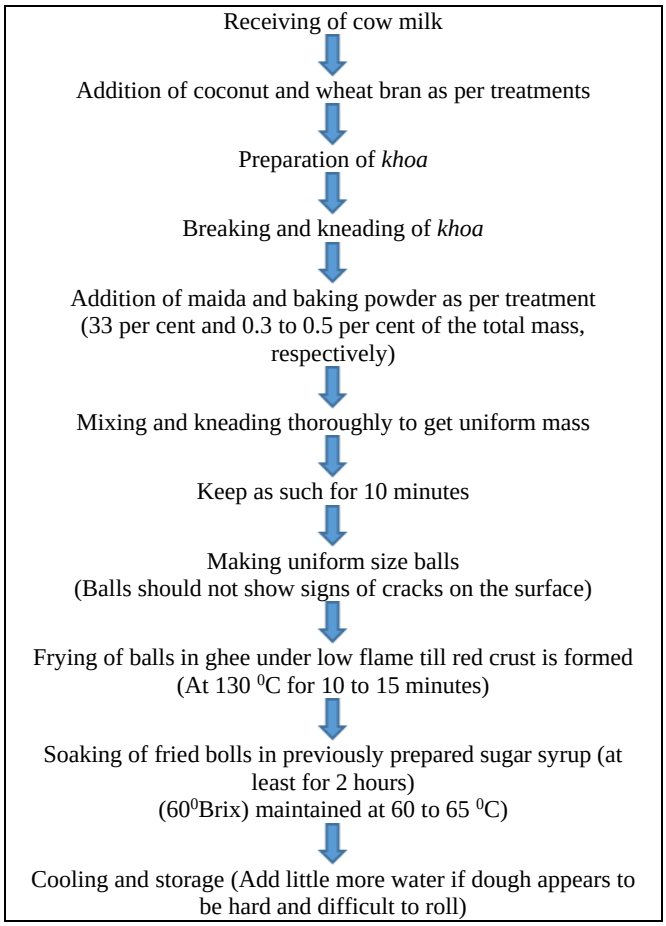
The syrup was prepare by dissolving sugar in water in the proportion of 1:1 and kept for boiling for 10 to 15 minutes. Any dirt or impurity that gathers on the surface of the syrup during boiling was removed with ladle. The syrup was ready when sugar concentration reached 60^0 Brix. *Gulabjamun* was prepared by using standardized procedure as finalized in preliminary trials.

Treatments

The most accepted 60^0 Brix sugar syrup was constant for all the treatments in final experimental trial. The *gulabjamun* prepared with different treatment combinations were studied as below;

Preparation of *Gulabjamun*

Gulabjamun was prepared as per the procedure given by Srinivasan and Anantkrishnan (1964) [14] with slight modifications as per flow diagram given below:-



Preparation of *Gulabjamun*

The samples of *gulabjamun* blended with coconut and wheat bran were analyzed for their microbial properties such as standard plate count, coliform count and yeast and mould count were analyzed.

Microbial analysis

The standard plate count of *Gulabjamun* blended with coconut and wheat bran was determined as per the procedure given in IS:5402 (1969). The coliform count of *gulabjamun* blended with coconut and wheat bran was determined as per the procedure given in IS: 5550 (1970).The yeast and mould count of *gulabjamun* blended with coconut and wheat bran was determined as per the procedure given in IS: 5403 (1969).

Shelf life study of *Gulabjamun* blended with coconut and wheat bran

Shelf life study of *gulabjamun* blended with coconut and wheat bran was done by chemical, sensory, microbial basis in plastic cups and LDPE plastic pouches at room and refrigeration temperature. Sample checked on 0, 2 and 4 days on 2 days and 7, 14, 21, 28 days on 7 days interval which was stored at room and refrigeration condition respectively.

Statistical Analysis: The data obtained was statistically analyzed by Completely Randomized Design (CRD) and for storage study F-CRD as per suggested by Snedecor, G.W. and Cochran, W.G. 1994 [13].

Results and Discussion

The results of the present investigation are presented and discussed here under following headings.

Microbiological analysis of *Gulabjamun* blended with coconut and wheat bran

All the fresh samples of *gulabjamun* blended with coconut and wheat bran were subjected to microbial analysis *viz*., standard plate count, coliform count and yeast and mould count of *gulabjamun* were studied and discussed below.

Table 1: Microbial parameter of *Gulabjamun* blended with coconut and wheat bran

Treatment/ Replication	Standard Plate Count	Coliform Count	Yeast and Mould Count
T_0	4.5	0.00	0.00
T_1	5.75	0.00	0.00
T_2	6.50	0.00	0.00
T_3	7.00	0.00	0.00
T_4	13.50	0.00	0.00
T_5	10.00	0.00	0.00
T_6	4.5	0.00	0.00
SE±	1.12	--	--
CD at 5% -	3.33	--	--

Each observation is a mean ± SD of three replicate experiments (n=3)

Standard Plate Count

From the Table 1, it inferred that the standard plate count of *gulabjmun* blended with coconut and wheat bran showed significant difference among treatments. The range of standard plate count was between 4.50 to 13.50 CFU/ml × 10^{-6}. The highest standard plate count recorded for treatment T_5 (13.50 CFU/ml) while lowest for treatment T_1 (4.50). It also showed that, standard plate count may be dependent upon environmental conditions and pre-processing factors. The standard plate count goes on increasing with increasing level of coconut and wheat bran. The results obtained in present study are in agreement with findings Vasava *et al.* (2018) [16] reveals that SPC of gluten-free *gulabjamun* was significantly ($P \leq 0.05$) influenced by storage period. The initial mean SPC of gluten-free *gulabjamun* was found zero up to 7th days of storage. Chaudhary (2016) studied the effect of storage on SPC of *gulabjamun* prepared using moraiyo as binding agent and reported that the fresh *gulabjamun* had a SPC count of 2.88 log CFU /g.

Coliform count

It was observed that coliform count was found to be completely absent in control and coconut and wheat bran added *gulabjamun*, which signifies that product was prepared in hygienic condition. The results obtained in present study are in agreement with findings Vasava *et al.* (2018) [16] reveals that coliform count of gluten-free *gulabjamun* was found zero up to 7 days of storage.

Yeast and mould

It showed that the yeast and mould in *gulabjamun* was completely absent, in freshly prepared *gulabjamun* ball soaked in sugar syrup. The results obtained in present study are in agreement with findings Chaudhary (2016) studied the *gulabjamun* prepared using moraiyo as binding agent and reported that the fresh *gulabjamun* had YMC zero up to 7th days. Vasava *et al.* (2018) [16] reveals that YMC of gluten-free *gulabjamun* was found zero up to 7th days of storage.

Effect of storage period on microbial count of *Gulabjamun* blended with coconut and wheat bran

Changes in SPC of *Gulabjamun* blended with coconut and wheat bran at room temperature

Effect of storage period SPC Count of *gulabjamun* blended with coconut and wheat bran at room temperature have been depicted in table. 2

Table 2: SPC Count of *Gulabjamun* blended with coconut and wheat bran stored at room temperature

Treatments	SPC, log cfu/g (30 ± 2 ^{0}C)						
	T_1 (control)	T_2 (2% WB)	T_3 (3%WB)	T_4 (15% C)	T_5 (20% C)	T_6 (2%WB+ 20% C)	Mean
0 day	6.00	5.67	7.33	7.67	13.67	10.00	
2nd days	12.00	11.67	11.67	16.67	19.00	15.33	8.39
4th days	12.33	12.00	10.67	16.00	17.67	16.67	14.39
Mean	10.11	9.78	9.89	13.44	16.78	14.00	14.22
				Source	Period	Treatment	P x T
				SEm (±)	0.60	0.85	1.48
				CD@5%	1.73	2.45	NS

From table 2, it observed that SPC count of *gulabjamun* blended with coconut and wheat bran goes on increasing. The highest count SPC for treatment T_5 (16.78 CFU/g) and lowest count SPC found in T_2 (9.78) on 4th days at room temperature. It was observed from tabulated values that treatment and storage period exerted significant effect on SPC count stored at room temperature whereas the interaction effect of storage period and treatment (P x T) was found to be non- significant ($P > 0.05$). The SPC count of *gulabjamun* during storage was found to increase with the increase in storage period.

Changes in coliform count of *Gulabjamun* blended with coconut and wheat bran at room temperature

Effect of storage period on coliform count of *gulabjamun* blended with coconut and wheat bran at room temperature have been depicted in table 3.

Table 3: Coliform count of *Gulabjamun* blended with coconut and wheat bran stored at room temperature

Days	Coliform Count x (10^2 CFU/g) (30 ± 2 ^{0}C)						Mean
	T_1	T_2	T_3	T_4	T_5	T_6	
0 day	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2nd days	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4th days	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			Source		Period	Treatment	P x T
			SEm (±)		--	--	--
			CD@5%		--	--	--

From table 3, it observed that coliform count of *gulabjamun* blended with coconut and wheat bran was found to be nil, which signifies that product was prepared in hygienic condition. The results obtained in present study were in close agreement with findings Vasava *et al.*, (2018) [16] revealed that during further storage of gluten-free *gulabjamun* samples stored at refrigeration temperature were found to be free from coliform at the end of 35 days of storage study.

Changes in YMC of *Gulabjamun* blended with coconut and wheat bran at room temperature

Effect of storage period YMC count of *Gulabjamun* blended with coconut and wheat bran at room temperature have been depicted in table 4.

Table 4: YMC count of *Gulabjamun* blended with coconut and wheat bran stored at room temperature

Days	YMC count x (10^2 CFU/g) (30 ± 2 ^{0}C)						Mean
	T_1	T_2	T_3	T_4	T_5	T_6	
0 day	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2nd days	18.67	13.33	21.67	19.33	32.00	35.33	23.39
4th days	21.67	19.67	24.00	27.33	36.00	37.33	27.00
Mean	13.44	11.00	15.22	15.56	22.66	24.22	16.80
				Source	Period	Treatment	P x T
				SEm (±)	1.29	1.82	3.16
				CD@5%	3.70	5.24	NS

From table 4, it observed that YMC count of *Gulabjamun* blended with coconut and wheat bran goes on increasing. The highest YMC count for treatment T_6 (24.22 CFU/g) due to increase level of coconut in *Gulabjamun*. The lowest count found in T_1 (13.44 CFU g) on 4th days. The YMC count of *Gulabjamun* during storage was found to increase with the increase in storage period. The results obtained in present study are in agreement with findings Chaudhary (2016) reported that the experimental samples of G_3 and G_6 were found free from yeast and mould on zero day but after 4 days count increased significantly at room temperature.

Changes in SPC of *Gulabjamun* blended with coconut and wheat bran at refrigeration temperature

Effect of storage period SPC Count of *gulabjamun* blended with coconut and wheat bran at refrigeration temperature have been depicted in table 5.

Table 5: SPC Count of *Gulabjamun* blended with coconut and wheat bran stored at refrigeration temperature

Treatment/Days	Standard plate count (SPC) x 10^6 CFU/g) (7±2 ^{0}C)				Mean
	7	14	21	28	
T_1	5.00	12.25	13.75	16.00	11.75^a
T_2	6.50	9.50	11.50	18.50	11.50^a
T_3	8.25	11.25	11.25	20.75	12.88^b
T_4	10.50	15.75	15.75	18.25	15.06^c
T_5	12.00	18.00	18.00	24.00	18.00^d
T_6	11.00	16.50	16.50	34.00	19.50^d
Mean	8.88^a	13.88^b	14.46^c	21.92^d	14.78
		Source	Period	Treatment	P x T
		SEm (±)	0.842	1.032	2.063
		CD @5%	2.366	2.898	5.796

From table 5, it observed that SPC count of *gulabjamun* blended with coconut and wheat bran goes on increasing. The highest count SPC for treatment T_6 (19.50 CFU/g) and lowest count SPC found in T_2 (11.50) for 28th days at refrigeration temperature. It was observed from tabulated values that treatment and storage period exerted significant effect on SPC count stored at refrigeration temperature whereas the interaction effect of storage period and treatment (P x T) was found to be significant (P>0.05). The SPC count of *gulabjamun* during storage was found to increase with the increase in storage period. The results obtained in present study are in agreement with findings Vasava *et al.*, (2018) [16] reveals that during further storage of gluten-free *gulabjamun*, increased to 3.41±0.02 log CFU/g in 35th days was observed and thereafter the product was found unacceptable due to SPC growth.

Changes in coliform count of *Gulabjamun* blended with coconut and wheat bran at refrigeration temperature

Effect of storage period on coliform count of *gulabjamun* blended with coconut and wheat bran at refrigeration temperature have been depicted in table 6

Table 6: Coliform count of *Gulabjamun* blended with coconut and wheat bran stored at refrigeration temperature

Treatment/ Days	Coliform count x (10^2 CFU/g) (7±2 ^{0}C)				Mean
	7	14	21	28	
T_1	0.00	0.00	0.00	0.00	0.00
T_2	0.00	0.00	0.00	0.00	0.00
T_3	0.00	0.00	0.00	0.00	0.00
T_4	0.00	0.00	0.00	0.00	0.00
T_5	0.00	0.00	0.00	0.00	0.00
T_6	0.00	0.00	0.00	0.00	0.00
Mean	0.00	0.00	0.00	0.00	0.00
		Source	Period	Treatment	P x T
		SEm (±)	--	--	--
		CD @5%	--	--	--

From table 6, it observed that coliform count of *gulabjamun* blended with coconut and wheat bran was found to be nil, which signifies that product was prepared in hygienic condition. The results obtained in present study are in agreement with findings Vasava *et al.*, (2018) [16] revealed that during further storage of gluten-free *gulabjamun* samples stored at refrigeration temperature were found to be free from

coliform at the end of 35 days of storage study.

Changes in YMC of *Gulabjamun* blended with coconut and wheat bran at refrigeration temperature

Effect of storage period YMC count of *gulabjamun* blended with coconut and wheat bran at refrigeration temperature have been depicted in table 7.

Table 7: YMC count of *Gulabjamun* blended with coconut and wheat bran at refrigeration temperature

Treatment/ Days	YMC count x (10^2 CFU/g) (7±2 ^{0}C)				Mean
	7	14	21	28	
T_1	0.00	13.50	18.75	20.25	13.13
T_2	0.00	18.00	17.50	23.25	14.69
T_3	0.00	18.75	20.75	21.75	15.31
T_4	0.00	20.00	21.25	28.50	17.44
T_5	0.00	25.75	34.50	32.25	23.13
T_6	0.00	28.75	35.25	35.50	24.88
Mean	0.00	20.79	24.67	26.92	18.09
		Source	Period	Treatment	P x T
		SEm (±)	1.101	1.349	2.697
		CD @ 5%	3.094	3.789	7.578

From table 7, it observed that YMC count of *gulabjamun* blended with coconut and wheat bran goes on increasing. The highest count for treatment T_6 (24.88 cfu/g) due to increase level of coconut in *gulabjamun*. The lowest count found in T_1 (13.13 cfu/g) for 28th days. It was observed from tabulated values that treatment and storage period exerted significant effect on YMC count stored at refrigeration temperature whereas the interaction effect of storage period and treatment (P x T) was found to be significant (P>0.05). The YMC count of *gulabjamun* during storage was found to increase with the increase in storage period. The results obtained in present study are in agreement with findings Chaudhary (2016) studied the *gulabjamun* prepared using moraiyo as binding agent and reported that the fresh *gulabjamun* had a YMC zero up to 7th days but were apparent after further storage and reached a count of 40 cfu/g, after 28th days of storage at refrigeration temperature (7±2 ^{0}C). Vaja (2012) [15] studied the effect of storage on YMC of *gulabjamun* prepared from sweet cream butter milk *khoa* and maida as binders. He reported that fresh *gulabjamun* had YMC of increased zero count of up to 7th days to 1.54 log cfu/g after 28th days of storage at refrigeration temperature (7±2 ^{0}C).

Conclusion

The results of this investigation would lead to conclusions as under:

It was revealed that *gulabjamun* prepared by adding 15 per cent coconut was found to the standard plate count of *gulabjamun* was 7 x 10^6 CFU/g. Coliform count was completely absent, which signifies that product was prepared in hygienic condition. The yeast and mould count in *gulabjamun* was completely absent, which was attributed as due fresh product free from yeast and mould count in *gulabjamun*. The highest count SPC for treatment T_5 (16.78 CFU/g) on 4th days at room temperature and SPC count for treatment T_5 (19.50 CFU/g) for 28th days at refrigeration temperature. The highest YMC count for treatment T_6 (24.22 CFU/g) on 4th days stored at room temperature and YMC count for treatment T_6 (24.88 CFU/g) for 28th days stored at refrigeration temperature. Shelf life of *gulabjamun* containing 15 per cent coconut was up to 28 days at refrigeration temperature (7±2 ^{0}C) and 4th days at room temperature (30 ± 2 ^{0}C). The SPC and YMC count of *gulabjamun* blended with coconut and wheat bran during storage was found to increase with the increase in storage period.

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