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Effect of storage on Physico-Chemical, sensory and microbiological quality of gluten-free *gulabjamun*

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Abstract
In the present study, changes in compositional, physico-chemical, sensory and microbial properties (SPC, coliform count and yeast and mold count) of gluten-free *gulabjamun* packed in pre-sterilized polyethylene terephthalate (PET) bottles was evaluated for its shelf-life at refrigerated temperature (7±2 °C). The fat, protein, ash, total solids, total carbohydrate and acidity of sugar syrup significantly ($P<0.05$) increased during storage. The acidity, peroxide value, free fatty acids and soluble nitrogen content significantly ($P<0.05$) increased whereas; sugar syrup absorption, pH and water activity significantly ($P<0.05$) reduced during storage. Yeast and mold count was found zero up to 7th d of storage then significantly ($p<0.05$) increased during storage period. Flavour, body and texture, colour and appearance and overall acceptability scores significantly ($P<0.05$) reduced during storage at refrigeration temperature (7±2 ºC). Based on the results obtained in this study it was concluded that the shelf life of gluten-free *gulabjamun* was estimated to be 35 d when stored in pre-sterilized PET bottles at 7±2 °C.

Keywords: Shelf life, gluten-free, *gulabjamun*, *khoa*, sago, potato starch

Introduction

Gulabjamun is a milk-based sweet popular in India. Dhap variety of *khoa* is preferred for *gulabjamun* preparation. It is generally prepared from the cow or buffalo milk *khoa* by kneading with wheat flour (maida) and baking powder to form smooth dough, portioning the dough, rolling them into balls of spherical shape, deep frying the balls in oil till they turn golden brown in colour and soaking them in the sugar syrup for overnight (Nalawade *et al.*, 2015) [20]. The most liked product should have brown colour, smooth and spherical shape, soft and slightly spongy body, free from both lumps and hard central core, uniform granular texture, with cooked flavour and free from doughy feel and the sweet should be fully succulent with sugar syrup with optimum sweetness. The texture of *gulabjamun* is judged mainly on its sponginess and juiciness, with crumbliness and gumminess being the main negative attributes (Ghosh *et al*., 1986 and Patel *et al.*, 1992) [8, 23]. Traditionally in the formulation of *gulabjamun* mix, maida is used as a binding agent. Maida flour is the refined wheat flour that doesn't contain any nutrients normally present in wheat. It has 100% carbohydrates and contains no fiber. Different binding materials have been used to replace maida in preparation of dough for *gulabjamun* making. Chaudhary (2016) [5] determined the efficacy of addition of moraiyo flour in *gulabjamun* and its impact on the quality parameters. She reported that the highest overall acceptability score i.e. 8.10 was obtained in *gulabjamun* containing 30% moraiyo. Use of cassava flour prepared from fresh and soaked tubers at the rate of 15% in the *gulabjamun* preparation showed significant improvement in appearance, texture and overall acceptability of *gulabjamun* on organoleptic evaluation. It was also found that, flour prepared from soaked tubers could be used up to 20% in the *gulabjamun* preparation and found acceptable on organoleptic evaluation (Agarkar *et al*., 2004) [1]. The effect of incorporating different binder flours including shingada (water chestnut), maida (wheat flour), and sago in *khoa* on the quality of the resulting *gulabjamun* produced was studied by Londhe *et al.* (2000) [8]. A combination of sago and potato starch was found most suitable for use in gluten-free, gluten-free *gulabjamun* (Vasava, 2018) [28].

Shelf-life studies are important from commercial point of view. According to BIS (IS: 11602-1986) [4] *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) [4].

Singh *et al*. (2011) [24] studied the shelf life of *gulabjamun*. *Gulabjamun* samples were packed in paperboard boxes of 500g capacity with butter paper and these were stored at ambient (26.6 to 36.25 °C) and refrigerated (about 4 to 7 °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

Fresh, raw mixed (cow and buffalo) milk was used as the base material for manufacture of *khoa*. The average fat% of the milk was 5.3±0.2 and average MSNF content was 8.6±0.05%. Sago and potato starch powder were procured from local market at Anand, Gujarat. The approximate composition of the sago was 11% moisture, 0.4% total ash, 0.10% acid insoluble ash, 98% starch, 0.30% protein, 0.20% crude fibre; by weight, 100 ppm sulphur dioxide and 4.5-7 pH of aqueous extract, and the approximate composition of potato starch was 18% moisture, 0.1% crude protein, 0.1% crude fat, 0.1% crude fibre, 0.3% crude ash, 78.3% starch and 81.5% carbohydrate; levels/kg. Guar gum and xanthan gum were obtained from HiMedia Laboratories Pvt Ltd., Mumbai. Good quality commercial grade cane sugar of 'Madhur' brand was used for preparing sugar syrup. Refined vegetable oil / Anand cottonseed oil (Anand Regional Co-operative oilseeds growers' Union Ltd., Ahmedabad) was used as frying medium in gluten-free *gulabjamun* preparation.

Caramel flavour was prepared in the laboratory by burning 300g sugar on direct flame in a clean and dry stainless steel vessel. After caramelization was completed (as indicated by a dark brown colour) heating was stopped and 100ml water was added and the ingredients were mixed thoroughly. The mixture was cooled and stored in a clean and dry glass bottle until use. Cardamom was procured from local market at Anand, Gujarat.

Preparation of *Khoa*

Standardized milk (4.5% milk fat and 8.5% MSNF). *Khoa* was prepared by heat desiccation in a steam jacketed stainless steel open pan operated at 0.75 kg/cm^2 steam pressure with continuous manual stirring and scrapping. The process of heating stirring was continued till the product acquired desired consistency (60-65% TS). At this stage caramel flavour was added @ 1 ml/100g *khoa* and blended thoroughly in the hot mass. The finished product was subsequently transferred to enamel trays, worked to pat form. The samples were kept at room temperature (25 to 30 °C) for 18-20 h and packaged in sanitized polyethylene pouches. The pouches were then stored at refrigerated temperature (4±2 °C) till use. The approximate composition of *khoa* was 32.00±0.53% moisture, 16.92±0.24% protein, 21. 00±0.42% fat and 3.50±0.14% ash.

Process for manufacture of gluten-free *gulabjamun*

The preliminary trials were conducted employing the tentative process for processing of binders *viz*. sago and potato starch and process of manufacturing of gluten-free *gulabjamun* as depicted in Figure 1.

Preparation of Sago paste: Sago beads were subjected to dry grinding in a mixer (sieving through 80 mesh size sieve) to obtain sago powder (10g). Addition of (4 X the weight of sago) water (45 °C). The paste was then soaked for one hour and Heating to 90 °C for 2-3 min to obtain sago paste.

Potato starch / hydrocolloid paste: Potato starch powder (20g) was mixing with calculated amount of guar gum and xanthan gum and equal amount of water (45 °C) was added to form a paste. Heating to 70-75 °C for 3-4 min with continuous stirring to obtain potato starch paste.

Preparation of gluten-free *gulabjamun*

Control(C) *gulabjamun* was prepared using the method reported by Aneja *et al*. (2002) [2] using maida (refined wheat flour) as a binding agent.

Dhap *khoa* (prepared from 4.5/8.5% fat/SNF milk) + caramel flavour @ 1ml/100g *khoa*)
⇩
Mixing sago paste (1.0 g/100 g *khoa*) and potato starch/hydrocolloid paste (20 g/100g *khoa*; guar gum @ 0.05, xanthan gum @ 0.15g/100g *khoa*) and cardamom powder @ 0.1g/100g *khoa*
⇩
Kneading
⇩
Dough
⇩
Resting for 1.5 h at room temperature (28-32 °C)
⇩
Ball formation (Approx. 10g each)
⇩
Deep frying in cottonseed oil at 125 to 130 °C for 10 to 12 min
⇩
Keeping at room temperature (28-32 °C) for 5 min after frying
⇩
Soaking into sugar syrup (60 °Brix) and placing in water bath maintained at 60 °C for 1 h
⇩
Gluten-free *Gulabjamun*
⇩
Filling in pre-sterilized PET bottles
⇩
Storage at 7±2 °C

Fig 1: Procedure for manufacturing of gluten-free *gulabjamun*

Compositional Analysis

Preparation of samples: Gluten-free *gulabjamun* soaked in sugar syrup were tempered at 40 °C for 20 min. They were then kept on a sieve of about one square centimeter mesh to allow the sugar syrup to drain for 10 min. The gluten-free *gulabjamun* were then cut into small pieces and mixed thoroughly to form a paste, which was then tested separately for different chemical constituents. Total nitrogen/protein content was determined by semi-microkjeldahl method

AOAC (2002b) [3], using Kjel-plus digestion system (Model-KPS 006L, M/s.Pelican Instruments, Chennai) and Kjel-plus semi-automatic distillation system (Model- Distil M, M/s. Pelican Instruments, Chennai). Fat content of *Gulabjamun* was determined as per the procedure described in AOAC (2002b) [3]. Ash content was determined by procedure described in BIS (ISI: 1479-1961) [13]. The total solids content was determined by standard procedure using Mojonnier Milk Tester Model-D (Laboratory Manual, 1959) [16]. Total carbohydrate was derived by difference of sum total of the major constituents like moisture, protein, fat and ash from 100. The acidity of gluten-free *gulabjamun and sugar syrup* was determined by method described in BIS (IS: 1479-1962) for condensed milk. The pH of *gulabjamun* was measured using Systronic digital pH meter, Model 335. The method described by Franklin and Sharpe (1963) [7] for cheese was used. The homogenate prepared by diluting 20g sample in 20ml of glass distilled water was subjected to pH measurement. For the water activity measurement, the sample of *gulabjamun* tempered at 25 °C temperature, was measured using Rotronic Hygroskop Model: Hygrolab-3 (M/s. Rotronicag, Switzerland) connected to a sensing element (AW-DIO) with a measuring range of 0-100% relative humidity (RH). The method prescribed by Deeth *et al*. (1975) [6] was used to estimate the FFA content of burfi. Peroxide value was determined by the method as described in Indian Standard: 1479 (Part II-1961) [13]. The soluble nitrogen content of *gulabjamun* sample was determined by the procedure outlined by Kosikowski (1982) [15] using three grams of sample.

For measuring the sugar syrup absorption, fried *gulabjamun* balls (two for each treatment) with known weight was transferred to 50ml beaker containing sugar syrup, and allowed to soak for overnight at room temperature. *Gulabjamun*, after removing from syrup was allowed to drain for 10 min. on wire gauge and then weighed. Increase in weight of two *gulabjamun* over initial weight was taken as the amount of sugar syrup absorbed by *gulabjamun* and represented as percentage absorption of sugar syrup.

Sensory Evaluation: The sensory panel was composed of staff members and post graduate students working in the institution. Judges who were familiar with desirable attributes of *gulabjamun* were selected. The selection criterion was that subjects had to be regular consumer's of typical dairy sweets as well as their similar behavior between sensory evaluation sessions. The samples were subjected to sensory evaluation as described in using a 9-point hedonic scale scorecard as suggested by Stone and Sidel (2004) [26]. The judges were also requested to note down their observations/ comments for each attribute specified in the score card. The gluten-free *gulabjamun* were tempered to 35±2 ^{0}C for judging. Samples were served in odour free plastic cups covered with plastic lid. The samples were labeled with random three-digit code. The order of presentation of the samples was randomized across subjects.

Microbiological Analysis: Gluten-free *gulabjamun* sample was analyzed for the standard Plate Count (SPC), Coliform count and Yeast and Mold count (YMC) by the methods as described in IS: 5550: 2005 [12] with slight modification.

Shelf Life Studies: Gluten-free *gulabjamun*, which was prepared using a combination of binders (*viz.* sago and potato starch), was packed in composite polyethylene terephthalate PET bottles (sterilized using a solution of 150 ppm available chlorine solution for 10 min at 35 °C). The experimental samples were studied for the storage related changes. The samples packed in PET bottles were kept at refrigerated temperature (7±2 °C). The compositional, physico-chemical, rheological, sensory and microbial properties of fresh and stored samples of gluten-free *gulabjamun* were monitored at predetermined time interval after every 7th d. Stored gluten-free *gulabjamun* was rejected on basis of sensory evaluation as well as visible yeast and mold growth on the surface.

Statistical Analysis: Statistical analysis of data was carried out as per Steel and Torrie (1980) [25] using completely randomized design.

Results and Discussion

Effect of Storage on Composition of Gluten-free *Gulabjamun*

The storage changes taking place in the composition of gluten-free *gulabjamun* during storage at refrigerated temperature have been presented in Table 1. The total solid content of gluten-free *gulabjamun* significantly (P<0.05) increased and moisture decreased during the storage period up to 35th d and thereafter the product was unacceptable due to visible mold growth. There was a progressive decrease in the moisture content of the product when stored at refrigerated temperature. This effect was found to be significant (P<0.05). After 35th d of storage at refrigeration temperature the product was unacceptable due visible mold growth on the surface of storage syrup.

The values depicted in the Table 1 revealed that the samples stored at refrigerated temperature (7±2 °C) fat content of gluten-free *gulabjamun* significantly (P<0.05) increased during storage. As the storage period progresses, the sample showed an increase in the fat content from 11.17±0.02% at 0th d to 11.41±0.02% at 35th d. This may be due to the decrease in moisture content, resulting in the increased fat of gluten-free *gulabjamun*. The effect of storage on protein and ash content of *gulabjamun* was found to be non-significant (P>0.05).

Moisture loss during storage of food products has been reported resulting in progressive increase in content of other constituent's *viz.* protein, fat, carbohydrate and ash either at room temperature or refrigeration temperature. This is a natural phenomenon and has been reported by several workers which are evident in this study also. All the samples met the BIS requirement with respect to composition *viz.* fat and acidity of sugar syrup till the end of storage period *viz.* 35 d.

Table 1: Effect of storage period on composition of gluten-free *gulabjamun* (7±2 ^{0}C)

Attributes	Storage period (d)						S.Em.	C.D. (0.05)	C.V. (%)
	0	7	14	21	28	35			
Total solid (%)	67.10±0.1	67.21±0.01	67.51±0.09	67.88±0.05	68.12±0.09	68.43±0.09	0.05	0.16	0.14
Fat (%)	11.17±0.02	11.21±0.02	11.26±0.00	11.34±0.08	11.36±0.01	11.41±0.02	0.02	0.07	0.34
Protein (%)	4.89±0.02	4.90±0.02	4.88±0.01	4.90±0.01	4.91±0.01	4.90±0.02	0.01	NS	0.38
Acidity of sugar syrup (ml 0.1 N NaOH required to neutralize 100 ml syrup)	0.38±0.01	0.40±0.00	0.44±0.01	0.47±0.00	0.50±0.00	0.52±0.00	0.00	0.01	1.64
Ash (%)	1.21±0.02	1.23±0.01	1.23±0.01	1.24±0.02	1.24±0.01	1.25±0.01	0.01	NS	1.40
Total carbohydrate (%)	49.72±0.04	49.86±0.02	50.11±0.08	50.42±0.05	50.57±0.02	50.83±0.03	0.03	0.09	0.10

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

Effect of storage on Physico-Chemical Properties of Gluten-free *Gulabjamun*

Acidity: The effect of period of storage at refrigerated temperature on the acidity of gluten-free *gulabjamun* is depicted in the Table 2. The results indicated that during storage of gluten-free *gulabjamun* at refrigerated temperature, there was a significant (P<0.05) increase in acidity. The acidity increased from 0.17±0.002 in freshly prepared *gulabjamun* (i.e. 0th day) to 0.19±0.001% LA on the 35th d of storage. The progressive increase in acidity during storage could be attributed to production of acids like formic acid, acetic acid, lactic acids and other organic acids and FFA as reported by O'Brien (1997). Maillard reaction also produces many organic acids which are also responsible for increase in acidity (Goyal and Shrinivasan, 1988; 1989) [9, 10]. Increase in titratable acidity was also observed during storage of *khoa* (Kalra *et al*., 1973) [14].

Chaudhary (2016) [5] studied the effect of storage on acidity of *gulabjamun* prepared using moraiyo as a binder and reported that the acidity increased significantly (P<0.05) from 0.31 (0th d) to 0.35 (4th d)% LA at room temperature (30±2 °C) while 0.37 (28th d) at refrigeration temperature (7±2 °C). Similarly, Vaja (2012) [27] studied the effect of storage on acidity of *gulabjamun* prepared from sweet cream butter milk khoa and maida as binders. He reported that the acidity increased significantly (P<0.05) from 0.384 on 0th d to 0.406% LA on the 28th d of storage at refrigeration temperature (7±2 °C). A similar trend of increase in acidity was observed in the present study. Thus, the results obtained in the present investigations corroborates with those reported in literature.

Water activity (a_w): Water activity is a very important parameter in terms of food safety. Bacterial growth potential can be correlated with moisture content in the product concerned. Thus, water activity is helpful in deciding the shelf life of the product either by support or otherwise to the bacterial growth or change in the textural properties due to water transfer. As seen in Table 2 there was a progressive decrease in water activity of samples during storage. The a_w value of gluten-free *gulabjamun* on initial day was 0.91±0.005 which decreased to 0.87±0.005 on 35th d of storage at refrigerated condition (7±2 °C). It can be revealed from tabulated values that storage period exerts significant effect (P<0.05) on a_w of gluten-free *gulabjamun* stored at refrigeration temperature. The decrease in water activity during storage could be attributed to decrease in moisture content during storage as seen earlier in Table 4.30.

The results obtained in present study agreed with the previous researchers; Chaudhary (2016) [5] and Vaja (2012) [27] who reported that the aw decreased significantly (P<0.05) with increase in storage. Chaudhary (2016) [5] reported that the water activity of *gulabjamun* prepared using moraiyo as a binding agent decreased from 0.902 (0th d of storage) to 0.883 (28th d of storage) at refrigeration temperatures (7±2 °C). A similar trend of increase in water activity during storage was observed in the present study.

pH: The data obtained for changes in pH of *gulabjamun* during storage, along with their statistical analysis are presented in Table 2 reveals that fresh gluten-free *gulabjamun* sample had the pH values of 6.36±0.02 which decreased significantly (P<0.05) to 6.10±0.00 on 35th d of storage at refrigeration temperature (7±2 °C). It is recorded that there was progressive decrease in pH during storage at refrigeration temperatures. Chaudhary (2016) [5] studied the effect of storage on pH of gluten-free *gulabjamun* prepared using moraiyo as binding agent and reported that the pH decreased significantly (P<0.05) from 6.44 (0th d) to 6.31 (28th d) at refrigeration temperature (7±2 °C). A similar trend of decrease in pH during storage was observed in the present study.

Free fatty acids (FFA): It can be seen from Table 2 that the FFA content of gluten-free *gulabjamun* was found significantly (P<0.05) affected by the storage period. The FFA content of fresh gluten-free *gulabjamun* significantly (P<0.05) increased from 0.53±0.02 to 1.11±0.02 µ eq/g on the 35th d of storage. During storage of gluten-free *gulabjamun*, a significant (P<0.05) increase in FFA content up to 35th d was observed and thereafter the product was found unacceptable due to visible mold growth on the surface of the sugar syrup. This increase in FFA content could be attributed to hydrolysis of fat which is primarily affected by the growth of yeasts and mold. In the present investigation also the increase in FFA could be due to increase in yeast and mold count as observed in Table 2. Lad (2016) [17] reported that the free fatty acids content increased gradually in *gulabjamun* prepared from cow milk using maida as binder increased from 0.55% OA on the 0th d to 1.43% OA on the 10th d of storage at 25±2 °C. A similar trend of increase in free fatty acid content during storage was observed in the present study. Thus, the results obtained in the present investigations corroborates with those reported in literature.

Soluble Nitrogen: The tabulated values (Table 2) reveal that the soluble nitrogen of gluten-free *gulabjamun* samples stored at refrigerated temperature was significantly (P<0.05) affected by storage period. It can be seen that soluble nitrogen content of fresh gluten-free *gulabjamun* was significantly (P<0.05) increased from 0.30± 0.01 on the 0th d of storage to 0.43±0.0% N on the 35th d of storage at refrigerated temperature. Lad (2016) [17] reported that the soluble nitrogen content increased gradually in *gulabjamun* prepared from cow milk using maida as binder increased from 0.35 on the 0th d to

45% of N on the 10th d of storage at 25±2 °C. A similar trend of increase in % soluble nitrogen content during storage was observed in the present study. The increase in soluble nitrogen content on storage might be the direct consequence of degradation of protein content of gluten-free *gulabjamun*.

Peroxide value: The results indicated that during storage of gluten-free *gulabjamun* at refrigerated temperature, there was a significant (P<0.05) increase in peroxide value. The peroxide value increased from 0.15±0.01 in freshly prepared gluten-free *gulabjamun* (i.e. 0th d) to 0.29±0.01 (milli-eq. O_2/kg) on the 35th d of storage. The progressive increase in peroxide value during storage could be attributed to degradation of fat, and oxidation of fat leads into formation of peroxides. Chaudhary (2016) [5] studied the effect of storage on peroxide value of *gulabjamun* prepared using moraiyo as a binder and reported that the peroxide value increased significantly (P<0.05) from 0.35±0.16 (0th d) to 0.51±1.65 (4th d) (milli-eq. O_2/kg) at room temperature (30±2 °C) while 0.69±0.025 (28th d) (milli-eq. O_2/kg) at refrigeration temperature (7±2 °C). A similar trend of increase in acidity was observed in the present study. Thus, the results obtained in the present investigations corroborates with those reported in literature.

Sugar Syrup Absorption: Soaking of *gulabjamun* balls in sugar syrup is an important step in the manufacturing process. It leads to absorption of sugar syrup and thereby increases in weight and relative sweetness of the *gulabjamun*. The effect of period of storage at refrigerated temperature on the sugar syrup absorption of gluten-free *gulabjamun* depicted in the Table 2 indicate that during storage of gluten-free *gulabjamun* at refrigerated temperature, there was a significant (P<0.05) decrease in sugar syrup absorption. In freshly prepared gluten-free *gulabjamun* the sugar syrup absorption decreased from 116.37±0.02 (0th d) to 115.42±0.06 (35th d) g/100g during storage. However, the sensory scores for flavour as well as body and texture were reduced after 35th day of storage due to relative decrease in sweetness and increase in hardness. The reduction in sugar syrup absorption during storage might be because of increase in hardness which retards sugar syrup penetration. Chaudhary (2016) [5] studied the effect of storage on sugar syrup absorption of *gulabjamun* prepared using moraiyo as a binder and reported that the sugar syrup absorption decreased significantly (P<0.05) from 113.67 (0th d) to 109.89 (4th d) g/100 g at room temperature (30±2 °C) while 96.91 (28th d) g/100 g at refrigeration temperature (7±2 °C). Vaja (2012) [27] studied the effect of storage on sugar syrup absorption of *gulabjamun* prepared from sweet cream butter milk khoa and maida as binders. He reported that the sugar syrup absorption decreased significantly (P<0.05) from 98.08 (0th d) to 93.47 (28th d) g/100 g during storage at refrigeration temperature (7±2 °C).

Table 2: Effect of storage period on physico-chemical properties of gluten-free *gulabjamun* at refrigerated temperature (7±2 ^{0}C)

Properties	Storage period (d)						S.Em.	C.D. (0.05)	C.V. (%)
	0	7	14	21	28	35			
Acidity (% LA)	0.174± 0.002	0.176± 0.001	0.178± 0.001	0.180± 0.001	0.185± 0.001	0.190± 0.001	0.00	0.00	0.82
Water activity (aw)	0.91± 0.005	0.90± 0.00	0.89± 0.005	0.89± 0.005	0.88± 0.005	0.87± 0.005	0.00	0.01	0.59
Ph	6.36± 0.02	6.32± 0.01	6.27± 0.01	6.20± 0.01	6.15± 0.01	6.10± 0.00	0.01	0.02	0.17
Free fatty acid (µ eq/g)	0.53± 0.02	0.57± 0.02	0.72± 0.02	0.88± 0.03	0.96± 0.02	1.11± 0.02	0.01	0.05	3.24
Soluble nitrogen (% N)	0.30± 0.01	0.33± 0.01	0.38± 0.0	0.41± 0.0	0.42± 0.01	0.43± 0.0	0.01	0.02	2.47
Peroxide value (milli-eq. O_2/kg)	0.15± 0.01	0.17± 0.01	0.20± 0.01	0.23± 0.01	0.24± 0.01	0.29± 0.01	0.00	0.01	3.82
Sugar syrup absorption (g/100 g)	116.37± 0.02	116.21± 0.03	116.02± 0.04	115.95± 0.02	115.81± 0.02	115.42± 0.06	0.02	0.07	0.03

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

Effect of storage on Microbial Quality of Gluten-free *Gulabjamun*

The influence of period of storage at refrigeration temperature (7±2 °C) on the SPC of gluten-free *gulabjamun* is presented in Table 3. The mean value presented reveals that SPC of gluten-free *gulabjamun* was significantly (P≤0.05) influenced by storage period. The initial mean SPC of gluten-free *gulabjamun* was found zero up to 7th d of storage. During further storage of gluten-free *gulabjamun*, increased to 3.41±0.02 log cfu/g in 35th d was observed and thereafter the product was found unacceptable due to SPC growth. However, these values were lower than the limits specified under BIS standards (i.e. 3.47 log cfu/g), so the product was acceptable up to the 35 d of storage on the basis of microbial count. Chaudhary (2016) [5] 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, and increased significantly (P<0.05) to 3.43 log cfu/g, after 28th d of storage at refrigeration temperature (7±2 ºC). Vaja (2012) [27] studied the effect of storage on SPC of *gulabjamun* prepared from sweet cream butter milk khoa and maida as binders. He reported that fresh *gulabjamun* had a SPC count of 2.87 log cfu/g increased to 3.43 log cfu/g after 28th d of storage at refrigeration temperature (7±2 ºC).

The mean values presented reveal that YMC of gluten-free *gulabjamun* was found zero up to 7th d of storage. During further storage of gluten-free *gulabjamun*, increased to 1.34±0.03 log cfu/g in YMC up to 35th d was observed and thereafter the product was found unacceptable due to visible mold growth. However, the counts after refrigerated storage were well within the range laid down under BIS standards (i.e. 1.69 log cfu/g). Chaudhary (2016) [5] studied the *gulabjamun* prepared using moraiyo as binding agent and reported that the fresh *gulabjamun* had a YMC zero up to 7th d but were apparent after further storage and reached a count of 40 cfu/g, after 28th d of storage at refrigeration temperature

(7±2 °C). Vaja (2012) [27] 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 a YMC of increased zero count of up to 7th d to 1.54 log cfu/g after 28th d of storage at refrigeration temperature (7±2 ºC).

Gluten-free *gulabjamun* samples stored at refrigeration temperature were found to be free from coliform at the end of 35 d of storage study.

Table 3: Effect of storage period on microbial quality of gluten-free *gulabjamun* at refrigerated temperature (7±2 ^{0}C)

Attributes	Storage period (d)						S. Em.	C.D. (0.05)	C.V. (%)
	0	7	14	21	28	35			
SPC, log cfu/g	Nil	Nil	2.33±0.11	3.17±0.03	3.34±0.05	3.41±0.02	-	-	-
Coliform count, log cfu/g	Nil	Nil	Nil	Nil	Nil	Nil	-	-	-
YMC, log cfu/g	Nil	Nil	0.82±0.05	1.03±0.03	1.23±0.04	1.34±0.03	-	-	-

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

Effect of storage on Sensory Attributes of Gluten-free *Gulabjamun*

The most vital of all a product's attributes are the sensory properties, as they are most apparent to the consumers. The data on the sensory quality of gluten-free *gulabjamun* referring to flavour, body and texture, colour, appearance and overall acceptability after a storage period 35 d at refrigeration temperature (7±2 °C) are depicted in Table 4. The statistical analysis of the data indicated that the period of storage had significant (P<0.05) effect on changes in flavour of *gulabjamun* during storage. The mean value presented revealed that flavour score of gluten-free *gulabjamun* was significantly (P<0.05) reduced during the storage period. During storage of gluten-free *gulabjamun*, flavour score up to 35th d was recorded and thereafter the product became unacceptable due to visible mold growth. The initial average score of 8.23±0.06 on 0th d decreased to 6.13±0.06 on the 35th d of storage at refrigeration temperature (7±2 ºC).

The decrease in flavour score could be attributed to slight loss of freshness, which is inherent with any food product. In fresh product, the compounds formed during browning reactions are responsible for the typical flavour of the product, but as storage period progresses, the chemical reactions disturbed the delicate balance of the compounds. However, the observed decrease in body and texture score of gluten-free *gulabjamun* during storage of product at refrigeration temperature might be a result of various chemical and microbial changes. The decline in score at refrigeration temperature could be mainly attributed to the gradual increase in hardness, cohesiveness and chewiness which resulted into a harder and chewier product. Chaudhary (2016) [5] studied the effect of storage on flavour score of *gulabjamun* prepared using moraiyo as a binder and reported that the flavour score decreased significantly (P<0.05) from 8.83 (0th d) to 6.43 (28th d) at refrigeration temperature (7±2 °C). Lad (2016) [17] reported that the average flavour score of fresh *gulabjamun* prepared from cow, camel and combination of camel and buffalo milk was 8.71, 6.20 and 8.21 which decreases to 2.08, 2.79 and 4.71, respectively after 10th d of storage at 25± 2 °C.

It can be seen from Table 4 that body and texture score of gluten-free *gulabjamun* was significantly (P<0.05) influenced by the storage period at refrigeration temperature (7±2 °C). As observed from the Table 4.34, the body and texture score decreased during storage period at refrigerated temperature. During storage of gluten-free *gulabjamun* the body and texture score decreased significantly from 8.13±0.06 on 0th d to 6.57±0.12 on the 35th d of storage. At refrigerated temperature the product became dry, hard, sandy and brittle which might be ascribed to the loss of moisture and possibly due to crystallization of added sugar. This is because of dynamic structural and conformational changes, which may or may not be dependent on changes in moisture content (Navajeevan and Rao, 2005) [21]. The decline in score at refrigeration temperature could be mainly attributed to the gradual increase in hardness and gumminess which resulted into a harder and gummier product. It can be seen from Table 4.34 that there was a gradual increase in textural parameters such as hardness and gumminess up to the 21st d of storage as discussed earlier in Section 4.32. Thereafter (i.e. after 21st d of storage), the further decrease in body and texture score of gluten-free *gulabjamun* might be a result of various chemical and microbial changes. Chaudhary (2016) [5] studied the effect of storage on body and texture score of *gulabjamun* prepared using moraiyo as a binder and reported that the body and texture score decreased significantly (P<0.05) from 8.50±0.65 (0th d) to 6.10±0.36 (28th d) at refrigeration temperature (7±2 °C). Lad (2016) [17] reported that the average body and texture score of fresh *gulabjamun* prepared from cow, camel and combination of camel & buffalo milk was 8.70, 4.33 and 8.37 which decreases to 6.04, 4.04 and 6.12 respectively, after 10th d of storage at 25± 2 °C.

The mean values obtained for colour and appearance scores reveals that colour and appearance score of gluten-free *gulabjamun* significantly (P<0.05) decreased during the storage period. During storage of gluten-free *gulabjamun* at refrigerated temperature, decreased in colour and appearance score from 8.46±0.05 on 0th d to 7.50±0.10 on the 35th d of storage was observed. The decline in scores during storage of gluten-free *gulabjamun* can be attributed to microbial, chemical and textural changes in the product. Moreover in the present study, evaporation of moisture during storage might have aggravated the appearance of the gluten-free *gulabjamun* as presence of moisture enlivens the appearance of the product by reflecting incident light. Chaudhary (2016) [5] studied the effect of storage on colour and appearance score of *gulabjamun* prepared using moraiyo as a binder and reported that the colour and appearance score decreased significantly (P<0.05) from 8.67±0.17 (0th d) to 7.27±0.23 (28th d) at refrigeration temperature (7±2 °C). Lad (2016) [17] reported that the average colour and appearance score of fresh *gulabjamun* prepared from cow, camel and combination of camel and buffalo milk was 8.75, 8.44 and 8.69 which decreases to 7.23, 7.21 and 7.21 respectively, after 10th d of storage at 25± 2 °C.

The overall acceptability score of gluten-free *gulabjamun* during storage at refrigeration temperature was found to decrease with the increase in storage period. Fresh gluten-free *gulabjamun* had an overall acceptability score of 8.20±0.15; these values for overall acceptability were decreased significantly to 6.10±0.10 after 35th d of storage. Tabulated

values indicate that storage period had a significant effect (P<0.05) on overall acceptability score of gluten-free *gulabjamun* stored at refrigeration temperature. The overall acceptability scores of gluten-free *gulabjamun* was statistically different (P<0.05) from each other and the mean scores at 0, 7, 14, 21, 28 and 35 days of storage were statistically different from each other. However, the product was still acceptable by judges on sensory basis. Thus, the samples were still acceptable after 35 d of storage at refrigeration temperature. The observed decline in overall acceptability of gluten-free *gulabjamun* could partly attributed to development of change in flavour owing to development of flat, insipid taste with slight souring tinge, body and texture scores due to increase in rheological properties like hardness, chewiness and cohesiveness and also decrease in colour and appearance to some extent, in gluten-free *gulabjamun* during storage. Therefore, it can be concluded that the total score or in turn the organoleptic attributes of the product follows the trend that was evident in individual attributes of gluten-free *gulabjamun*.

Chaudhary (2016) [5] studied the effect of storage on overall acceptability score of *gulabjamun* prepared using moraiyo as a binder and reported that the overall acceptability score decreased significantly (P<0.05) from 8.67±0.01 (0th d) to 6.13±0.05 (28th d) at refrigeration temperature (7±2 °C). Lad (2016) [17] reported that the average overall acceptability score of fresh *gulabjamun* prepared from cow, camel and combination of camel and buffalo milk was 8.73, 4.04 and 8.38 which decreases to 2.12, 3.10 and 6.08 respectively, after 10th d of storage at 25± 2 °C. Veni *et al.* (2016) [29] studied the effect of storage on overall acceptability score of tikhur based *gulabjamun* prepared using tikhur flour as a binder @ 30% and reported that the overall acceptability score decreased significantly (P<0.05) from 9.0 (0th d) to 8.4 (10th d) at room temperature.

Table 4: Effect of storage period on sensory properties of gluten-free *gulabjamun* at refrigerated temperature (7±2 ^{0}C)

Attributes	Storage period (d)						S. Em.	C.D. (0.05)	C.V. (%)
	0	7	14	21	28	35			
Flavour	8.23±0.06	7.77±0.06	7.23±0.06	6.77±0.06	6.43±0.06	6.13±0.06	0.03	0.10	0.81
Body and Texture	8.13±0.06	7.87±0.06	7.47±0.06	7.23±0.06	6.87±0.06	6.57±0.12	0.04	0.13	0.96
Colour and Appearance	8.46±0.05	8.27±0.06	8.07±0.06	7.87±0.06	7.73±0.06	7.50±0.10	0.04	0.12	0.83
Overall acceptability	8.20±0.15	7.67±0.06	7.13±0.06	6.73±0.06	6.33±0.06	6.10±0.10	0.04	0.13	1.06

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

Conclusion

On the basis of this part of the study, it can be concluded that gluten-free *gulabjamun* has a shelf life of 35 d under refrigeration temperature (7± 2 °C) when packed in pre-sterilized polyethylene terephthalate (PET) bottles.

References

1. Agarkar BS, Satwadhar PN, Pawar VD, Kshirsagar RB. Effect of soaking on physico-chemical characteristics of cassava flour and its utilization in *gulabjamun*. Indian Journal of Nutrition & Dietetics. 2004; 41(11):490-493.
2. Aneja RP, Mathur BN, Chandan RC, Banerjee AK. Technology of Indian Milk Products. A Dairy India Publication, Delhi, India, 2002.
3. AOAC. Method for micro kjeldahl method. Introduction to the Chemical Analysis of foods, Association of Official Analytical Chemists, USA, 2002b, 210-211.
4. BIS. Specification for canned *Gulabjamun*. IS: 11602-1986. Indian Standards Institution, New Delhi, India, 1986.
5. Chaudhary C. Development of technology for manufacture of *gulabjamun* utilizing *moraiyo* (*panicum miliare*). Thesis submitted by SMC college of Dairy Science, AAU, Anand, 2016.
6. Deeth HC, Fitz-Gerald CH, Wood AF. A convenient method for determining the extent of lipolysis in milk. Australian Journal of Dairy Technology. 1975; 30(3):109-111.
7. Franklin JG, Sharpe ME. The incidence of bacteria in cheese milk and Cheddar cheese and their association with flavour. Journal of Dairy Research. 1963; 30:87-99.
8. Ghosh BC, Rajorhia GS, Pal D. Complete *gulabjamun* mix. Indian Dairyman, 1986; 38(6):279-282.
9. Goyal GK, Srinivasan MR. Protective role of packaging materials in refrigerated conditions on the microbiological quality of *khoa*. Asian Journal of Dairy Research. 1988; 7:66-72.
10. Goyal GK, Srinivasan MR. Effect of packaging on the chemical quality of *khoa* during storage. Indian Journal of Dairy Science. 1989; 42:165-170.
11. Indian Standard: 1479 (Part III). Bacteriological analysis of milk, Indian Standards Institution, ManakBhavan, New Delhi, India, 1962.
12. Indian Standard: 5550 Indian Standard specification for Burfi. Indian Standards Institution, New Delhi, India, 2005.
13. IS: 1479 Part III. Methods of Test for Dairy Industry. Chemical Analysis of Milk. Indian Standards Institution, New Delhi, India, 1961.
14. Kalra MS, Laxminarayan H, Dudani D. Use of nisin for extending shelf-life of processed cheese and *khoa*. Journal of Food Science & Technology. 1973; 10:92-94.
15. Kosikowaski F. Cheese and Fermented milk Products. Kosikowaski F.V. (Ed), Associates Publ. New York, 1982, 568.
16. Laboratory Manual. Methods of Analysis of Milk and its Products, 3rd Edn. Milk Industry Foundation. Washington USA, 1959, 442.
17. Lad S. Enhancing the quality of gulabjamun prepared from camel milk *khoa* Thesis submitted at Anand argicultural university, Anand, 2016.
18. Londhe GK, Joglekar NV, Patil GR, Awaz HB, Bhosale AM. Effect of different binders on the sensory attributes of *gulabjamun*. Indian Journal of Dairy & Biosciences. 2000; 11:71-74.
19. Londhe GK, Joglekar NV, Patil GR, Awaz HB, Bhosale AM. Effect of different binders on the sensory attributes of *gulabjamun*. Indian Journal of Dairy & Biosciences. 2000; 11:71-74.
20. Nalawade MR, Shinde AT, Kokare RL, Lingayat NT. Studies on preparation of *gulabjamun* blended with wheat bran. Research Journal of Animal Husbandary & Dairy

Science. 2015; 6(2):139-144.
21. Navjeevan B, Rao KJ. Physico-chemical changes in retort processed *kunda*. Indian Journal of Dairy Science. 2005; 58(6):392-398.
22. O' Brien J. Reaction chemistry of lactose: non-enzymatic degradation pathways and their significance in dairy products. In: Fox PF (Ed) Advanced Dairy Chemistry, Vol. 3. Chapman & Hall, Madras, 1997, 248.
23. Patel AA, Patil GR, Garg FC, Rajorhia GS. Textural characteristics of market samples of *gulabjamun*. Indian Journal of Dairy Science. 1992; 45(7):356-359.
24. Singh AK, Kadam DM, Saxena M, Singh RP. Effect of soy flour supplementation on the quality and shelf life of *gulabjamun*. International Journal of Food Science & Nutrition Engineering. 2011; 1(1):11-17.
25. Steel RGD, Torrie JH. Principles and procedures of statistics. Me. Graw Hill Pubi. Co. Inc., New York, 1980.
26. Stone H, Sidel JL. In: Sensory evaluation practices, 3rd Edn. Elsevier Acad. Press, NY, 2004, 88.
27. Vaja B. Suitability of sweet cream buttermilk and whey solids in form of whey and whey products in preparation of *gulabjamun*. Thesis submitted at Anand argicultural university, Anand, 2012.
28. Vasava NM. Evaluation of selected binders for manufacture of *Faraali gulabjamun.* Thesis submitted by SMC college of Dairy Science, AAU, Anand, 2018.
29. Veni BV, Khare A, Qureshi M, Nikam P. Effect of *Tikhur* (*Curcuma angustifolia* Roxb.) flour on physico-chemical and sensory qualities of *gulabjamun.* International Journal of Food & Fermentation Technolology. 2016; 6(2):281-288.