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## Physico-chemical, sensory and microbial changes during storage of gluten-free chhana cake

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**Abstract**

Physicochemical, sensory, and microbial characteristics of chhana cakes prepared from gluten-free flour blend (barnyard millet, little millet and amaranth) and control prepared using wheat flour as were investigated. From the results obtained in this study it was revealed that the scores of all the sensory attributes of gluten free chhana cake went below 7 except colour and appearance, crumb colour and crust colour score. The moisture of both chhana cakes was decreased non-significantly ( $p>0.05$ ) during storage. The specific volume of both chhana cake was decreased significantly ( $p<0.05$ ) during storage. The aerobic plate count of the both chhana cake was increased non-significantly ( $p>0.05$ ), whereas yeast and mold count was increased significantly ( $p<0.05$ ) during storage. The coliform count was found to be absent during storage period. On the 8<sup>th</sup> day of storage the overall acceptability score of gluten-free chhana cake was below 7. It was concluded that both the chhana cakes were found to be acceptable up to 6 days when packed in cling film biodegradable plastic wrap and placed in rigid PVC trays and trays were packed in LDPE pouches ( $\sim 85 \mu$ ) and stored at  $7\pm 2$  °C.

**Keywords:** Gluten-free, cake, storage period, sensory, shelf life

**Introduction**

Chhana cake/Chhena poda is a baked traditional Indian dairy product (Mukhopadhyay *et al.*, 2015) [15]. It originated in Odisha and nowadays it is popular primarily in eastern part of India. The major ingredients in chhana cake are chhana, sugar and binder's *viz.* wheat based products such as maida or suji. Maida/suji has a major role in the development of texture in cake. Especially during baking, protein interactions are important for cake structure development (Wilderjans *et al.*, 2008) [16]. Two major wheat proteins called glutenin and gliadin are responsible for development of visco-elastic property of cake dough (Lagrain *et al.*, 2005) [17]. During recent years there has been a growing need for gluten-free bakery products. Persons suffering from celiac disease and persons who are allergic to gluten are interested in wheat free foods (Rai *et al.*, 2014) [19]. Production of gluten-free bakery products causes significant technological defects in the volume and texture (Matos and Rosell, 2011) [18]. As a result, gluten-free bakery products are often less desirable in terms of their appearance, taste, aroma and texture. A gluten free chhana cake was formulated by replacing maida/suji with millets in the formulation of acceptable quality chhana cake.

To make gluten-free chhana cake a commercially viable product; it requires sufficient shelf-life. The storage stability or the shelf life of the baked products could be defined as maintaining the sensory quality and physical characteristics associated with the freshness of the product *viz.* crumb tenderness, compressibility and moistness by preventing alteration associated with staling during the storage (Baixauli *et al.*, 2008) [3]. The baking time and temperature combination affect the texture of cake and its quality like specific volume, crumb and crust colour, firmness and porosity of crumb and moisture content of the product. It also affects the evaporation of moisture during baking as well as storage. Among the different physical properties porosity of crumb is most important physical property for moisture transfer within the product during storage (Kumari *et al.*, 2015) [11]. During storage various chemical and biochemical changes occur that directly affect the sensory quality of the products.

Different methods have been used to determine the sensory characteristics of a food product using consumer data. Consumers expect cake with a light brown crust, tender crumb, moderately soft body, spongy texture, rich and caramelized flavour during the storage (Suresh *et al.*, 2019) [14]. Generally consumer rejection of the product occurs before any microbiological spoilage which makes it unsuitable for human consumption (Hough *et al.*, 2003) [5]. The microbiological quality of chhana cake becomes more important because

Microbial growth. During storage directly affect the product shelf life. The growth of the microorganism cause spoils the product and change it taste (Saranraj and Geetha, 2012) [13].

The objectives of this study were to assess the shelf life of gluten free chhana cake at refrigeration temperature i.e.  $7\pm 2$  °C and compare the control (chhana cake prepared using maida/suji as binding agents) and gluten-free chhana cake for changes in sensory, physicochemical and microbial quality during storage.

### Materials and Methods

Milk standardized to 5.5% fat was pasteurized at 75 °C for 15 seconds and stored at  $4\pm 2$  °C until use. Barnyard millet and amaranth was collected from local market of Anand city. Little millet "Gaaychap" brand, Ahmedabad was used. Sugar used in this study was of Madhur brand, Shree Renuka sugar Ltd., Mumbai. Baking powder of Blue bird brand, Mumbai was used. Extra pure glycerol monostearate (GMS) manufactured by Loba Chemie Pvt. Ltd., Mumbai used as emulsifier. Eagle brand vanilla essence manufactured by Vijay industries, Vadodara was used as flavoring agent.

### Method of manufacture of chhana

Pasteurized milk (5.5% fat) was filtered, heated to 90 °C for 5 min and cooled to 65 °C. Citric acid solution (0.5%) was heated to 65°C and gradually added to the milk, stirring gently until the milk coagulated. After 5 min., the whey was strained through muslin cloth, and the coagulated mass was hung for about 5 to 10 min. or till complete whey drainage occurred to obtain chhana.

### Method for manufacture of gluten-free chhana cake

Chhana served as the base for manufacture of gluten-free chhana cake. It was kneaded in a Hobart mixture for 1.5 to 2.0 min. GMS solution (dissolved in 15-20 times water) @ 1.66% (w/w of chhana) and vanilla flavor @ 0.4% (v/w of chhana) were added to the Hobart mixture and kneaded for 1 min. All the dry ingredients viz. 20% millet/pseudocereal blend (barnyard millet @ 6.37%, amaranth @ 6.54% little millet @ 7.09%), sugar @ 30% and baking powder @ 1.40% (all w/w of chhana) were dry blended together and added to the Hobart mixture and it was again kneaded thoroughly for 1.5 to 2.0 min and smooth dough was obtained. The dough (about 58 to 60 g each) was then transferred into silicone moulds (size of mould: 7.0 x 5.0 x 3.5) and baked in an oven at 140 °C for 80 min. Thereafter it was cooled to room temperature. Control was prepared using the method suggested by. Both the cakes were evaluated for its shelf life at refrigeration temperature i.e.,  $7\pm 2$  °C. Individual cup of chhana cake were wrapped in cling film biodegradable plastic wrap (thickness: 295 µm) and placed in rigid PVC trays. The trays were packed in LDPE pouches (~85 µm). Cardboard boxes were used as secondary package for placing the PVC trays along with laminates at  $7\pm 2$  °C. The cling film and PVC tray were pre-sterilized in UV light for 1 h. The products were analyzed for the storage related changes by the sensory evaluation till the sensory score reach below 7 or appearance of visible mold growth on the surface of the product, whichever was observed earlier. Maximum care was taken during post processing handling of chhana cake to avoid any type of contamination and to prevent growth of bacteria during storage.

The sensory attributes (colour and appearance, volume, body and texture, flavour, crumb colour, crust colour and overall acceptability scores), specific volume ( $\text{cm}^3/\text{g}$ ), moisture (%)

and microbial quality (aerobic plate count, yeast and mold count and coliform count) of stored samples of both chhana cake i.e., control chhana cake and gluten-free chhana cake were monitored at every second day at  $7\pm 2$  °C.

### Chemical analysis

Moisture in chhana cakes were analyzed by the procedure given by IS: SP 18: part XI, 1981. The fat content of the gluten-free chhana cake was analyzed by the acid hydrolysis method given by the AOAC (2005) [2]. Protein content in chhana cake was determined by semi-micro Kjeldahl method IS:1479-1961, using Kjel-plus digestion system (M/s Pelican Instruments, Chennai, Model-KES 20LVA DLS) and Kjel-plus semi-automatic distillation system (M/s Pelican Instruments, Chennai, Model- Distil M). The ash content of the gluten-free chhana cake was analyzed by the procedure given by IS: 1547 (1985) [8]. Total carbohydrate of gluten-free chhana cake was determined by subtracting the total content of moisture, fat, protein and ash from 100 as per FSSAI (2011) [4].

The specific volume of gluten-free chhana cake was determined by the rapeseed displacement method (AACC, 2000) [1]. The volume and weight were measured immediately after baking. A gluten-free chhana cake was placed in a known volume container into which rapeseeds were added until the container was full. The volume of seeds which were displaced by gluten-free chhana cake was considered the volume of gluten-free chhana cake. The unit for the measurement for specific volume was the volume ( $\text{cm}^3$ ) divided by weight (g).

Specific volume of chhana cakes were calculated by the following formula:

$$\text{Specific volume } (\text{cm}^3/\text{g}) = \frac{\text{Volume of sample in mL or cm}^3}{\text{Weight of sample in g}}$$

### Microbiological Analysis

The aerobic plate count and yeast and mold count of chhana cakes were analyzed by procedure specified in the BIS (IS: 5550, 1970). The coliform count of chhana cakes were determined by procedure specified in the IS: 1479 (Part III, 1962) [7].

### Sensory evaluation

Stored cups were withdrawn from the refrigerator at  $7\pm 2$  °C and microwaved at 2450 MHz frequency for 12 s before subjecting it for sensory evaluation. Sensory analysis was done after 24 h of manufacturing at  $23\pm 2$  °C. The sensory panelist (n=10) included staff members and post-graduate students working in the department. A 9-point hedonic scale scorecard was used for this purpose. The sensory parameters recorded were colour and appearance, volume, body and texture, flavour, crumb colour, crust colour and overall acceptability scores.

### Statistical analysis

The mean values generated from the analysis of duplicate samples of chhana cakes obtained in three replications, were subjected to statistical analysis using completely randomized design (CRD) using the software developed by Anand Agricultural University, Anand.

## Results and Discussion

### Effect of Storage on Sensory Quality of Chhana Cake

During storage various chemical and biochemical changes occur that directly affect the sensory quality of the products. The average values of colour and appearance, volume, body and texture, flavour, crumb colour, crust colour and overall acceptability score of the both chhana cake as affected by the storage at  $7\pm 2$  °C is presented in Table 1.

The changes in the colour and appearance scores of chhana cake were affected significantly ( $p<0.05$ ) by storage period and affected non-significantly ( $p>0.05$ ) by treatment. The data shown in Table 1 revealed that colour and appearance score for fresh control chhana cake (8.49) had comparatively higher than gluten-free chhana cake (8.27), which decreased significantly ( $p<0.05$ ) to 7.57 and 7.26, respectively on 8<sup>th</sup> day of storage.

The changes in the volume scores of chhana cake were affected significantly ( $p<0.05$ ) by storage period (P) as well as type of cake (T). There was a decrease in the volume scores of chhana cake during 8 days of storage. The data shown in Table 1 revealed that volume score for fresh control chhana cake (8.45) had comparatively higher than gluten-free chhana cake (8.20), which decreased significantly ( $p<0.05$ ) to 6.97 and 6.74, respectively on 8<sup>th</sup> days of storage.

The changes in the body and texture scores of chhana cake were affected by storage period. Such decrease in the body and texture score differed significantly ( $p<0.05$ ) only for the storage period (P). The treatment (T) failed to exert any significant effect on the body and texture score of chhana

cake (Table 1).

The changes in the flavour scores of chhana cake were affected significantly ( $p<0.05$ ) by storage period (P) as well as treatment (T). The data shown in Table 1 revealed that flavour score for fresh control chhana cake (8.42) had comparatively higher than gluten-free chhana cake (8.19), which decreased significantly ( $p<0.05$ ) to 6.63 and 6.22, respectively on 8<sup>th</sup> days of storage.

The data shown in Table 1 revealed that crumb colour score for fresh control chhana cake (8.40) had comparatively higher than gluten-free chhana cake (8.22), which decreased significantly ( $p<0.05$ ) to 7.20 and 7.02, respectively on 8<sup>th</sup> days of storage.

The changes in the crust colour scores of chhana cake were affected by storage period. Such decrease in the crust colour score differed significantly ( $p<0.05$ ) only for the storage period (P). The data shown in Table 1 revealed that crust colour score for fresh control chhana cake (8.39) had comparatively higher than gluten-free chhana cake (8.17), which decreased significantly ( $p<0.05$ ) to 7.22 and 7.02, respectively on 8<sup>th</sup> days of storage.

The changes in the overall acceptability scores of chhana cake were affected by storage period. Such decrease in the overall acceptability score differed significantly ( $p<0.05$ ) only for the storage period (P). The data shown in Table 1 revealed that overall acceptability score for fresh control chhana cake (8.40) had comparatively higher than gluten-free chhana cake (8.20), which was decreased significantly ( $p<0.05$ ) to 6.70 and 6.44, respectively on 8<sup>th</sup> days of storage.

**Table 1:** Change in sensory scores of chhana cake during storage at  $7\pm 2$  °C

Storage period (Days)	Sensory score (9-point hedonic scale)													
	Colour and appearance score		Volume score		Body and texture score		Flavour score		Crumb colour		Crust colour score		Overall acceptability score	
	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
0	8.49	8.27	8.45	8.20	8.31	8.17	8.42	8.19	8.40	8.22	8.39	8.17	8.40	8.20
2	8.30	8.08	8.14	7.85	8.08	7.89	8.15	7.83	8.22	7.79	8.15	7.85	8.18	7.88
4	8.10	7.86	7.78	7.49	7.83	7.48	7.70	7.32	7.86	7.55	7.79	7.59	7.85	7.51
6	7.86	7.61	7.46	7.15	7.39	7.13	7.19	6.78	7.49	7.29	7.52	7.31	7.37	7.07
8	7.57	7.26	6.97	6.74	7.03	6.83	6.63	6.22	7.20	7.02	7.22	7.02	6.70	6.44
Anova Table														
	T	P	T	P	T	P	T	P	T	P	T	P	T	P
SE m±	0.08	0.13	0.09	0.14	0.10	0.16	0.10	0.16	0.10	0.15	0.10	0.16	0.08	0.13
CD (0.05)	NS	0.39	0.26	0.41	NS	0.46	0.29	0.46	NS	0.45	NS	0.47	0.24	0.38
CV%	4.10		4.50		5.00		5.15		4.89		5.03		4.21	

Where T<sub>0</sub>= Control chhana cake, T<sub>1</sub>= Gluten-free chhana cake, T= Treatment, P= Period, NS= Non-significant

### Changes in the moisture of chhana cake during storage at $7\pm 2$ °C

There was a progressive, but gradual decrease in the moisture of both chhana cake i.e., control chhana cake and gluten-free chhana cake throughout the storage period of 8 days. The change in the moisture of chhana cake was differed significantly for treatment (T) and differed non-significantly ( $p>0.05$ ) for storage period (P). The data shown in Table 2 revealed that control chhana cake had markedly higher moisture content compared to gluten-free chhana cake. Fresh gluten-free chhana cake had average moisture of 28.84%

which decreased non-significantly ( $p>0.05$ ) to 27.54% of 8 days of storage. The fresh control chhana cake sample had average moisture 30.89% which decreased non-significantly ( $p>0.05$ ) to 30.06% of 8 days of storage.

Patel (2020) [1] also reported that moisture content of chhana based cake decreased in storage period. He observed that fresh chhana based cake had moisture of  $17.82\pm 0.92\%$  was decreased non-significantly to  $16.98\pm 0.75\%$  after 6 days and it decreased significantly ( $p<0.05$ ) on the 12<sup>th</sup> day of storage  $16.04\pm 0.54\%$  at  $20\pm 2$  °C.

**Table 2:** Change in moisture and specific volume of chhana cake during storage at 7±2 °C

Storage period (Days)	Moisture (%)		Specific volume (cm <sup>3</sup> /g)	
	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
0	30.89	28.84	1.68	1.62
2	30.76	28.57	1.66	1.59
4	30.58	28.28	1.63	1.56
6	30.36	27.94	1.60	1.51
8	30.06	27.54	1.56	1.45
Anova Table				
	T	P	T	P
SE m±	0.30	0.47	0.02	0.03
CD (0.05)	0.88	NS	0.05	0.07
CV%	3.91		3.80	

Where T<sub>0</sub>= Control chhana cake, T<sub>1</sub>= Gluten-free chhana cake, T= Treatment, P= Period, NS= Non-significant

**Changes in the specific volume of chhana cake during storage at 7±2 °C**

There was a progressive, but gradual decrease in the specific volume of both chhana cake throughout the storage period of 8 days. The change in the specific volume of chhana cake was differed significantly (*p*<0.05) for treatment (T) as well as for storage period (P). Fresh gluten-free chhana cake had average specific volume was 1.62 cm<sup>3</sup>/g of which decreased significantly (*p*<0.05) to 1.49 cm<sup>3</sup>/g of 8 days of storage. The fresh control chhana cake sample had average specific volume was 1.68 cm<sup>3</sup>/g which decreased significantly (*p*<0.05) to 1.56 cm<sup>3</sup>/g of 8<sup>th</sup> day of storage.

Patel (2020) [1] also reported that the specific volume of chhana based cake decreased significantly (*p*<0.05) in storage period. He observed that fresh chhana based cake had specific volume of 1.56±0.03 cm<sup>3</sup>/g was decreased non-significantly (*p*>0.05) to 1.52±0.01 cm<sup>3</sup>/g on the 6<sup>th</sup> day of storage and it decreased significantly (*p*<0.05) on the 12<sup>th</sup> day of storage 1.44±0.05 cm<sup>3</sup>/g at 20±2 °C.

**Changes in the aerobic plate count of chhana cake during storage at 7±2 °C**

The aerobic plate count (same as standard plate count) present in product gives an idea about quantitative presence of mesophilic bacteria. Therefore determination of aerobic plate count was considered to be an important parameter to assess the microbial quality and degree of freshness chhana cake.

The gluten-free chhana cake had higher aerobic plate count throughout the storage period as compared to control chhana cake. There was no significant (*p*>0.05) difference in aerobic plate count of the both chhana cake. The period of storage (P) had a significant (*p*<0.05) influence on the aerobic plate count. The data shown in Table 3 revealed that there was a progressive rise in the aerobic plate count during storage (i.e., 7±2 °C) of chhana cake; the increase in the aerobic plate count was found to be significant (*p*<0.05) of 8 day of storage

period. Fresh gluten-free chhana cake had average aerobic plate count 1.25 log<sub>10</sub>cfu/g of which increased significantly (*p*<0.05) to 1.87 log<sub>10</sub>cfu/g of 8 days of storage. Fresh control chhana cake had average aerobic plate count 1.23 log<sub>10</sub>cfu/g of which increased significantly (*p*<0.05) to 1.76 log<sub>10</sub>cfu/g of 8 days of storage.

Patel (2020) [1] also reported that the aerobic plate count (as log<sub>10</sub>cfu/g) of chhana based cake increased in storage period. Fresh chhana based cake had aerobic plate count 1.37±0.03 log<sub>10</sub>cfu/g was increased significantly (*p*<0.05) to 2.95±0.28 log<sub>10</sub>cfu/g of 12<sup>th</sup> days of storage at 20±2 °C.

**Changes yeast and mold count of chhana cake during storage at 7±2 °C**

Yeast and mold growth tends to be a major problem on shelf life of chhana cake. Yeast and molds are undesirable; their presence reduces the keeping quality of chhana cake.

The gluten-free chhana cake had higher yeast and mold count throughout the storage period as compared to control chhana cake. There was no significant (*p*> 0.05) difference in yeast and mold count of the both chhana cake sample. The period of storage (P) had a significant (*p*<0.05) influence on the yeast and mold count. The data shown in Table 3 revealed that there was a progressive rise in the yeast and mold count during storage (i.e., 7±2 °C) of chhana cake; the increase in the yeast and mold count was found to be significant (*p*<0.05) of 8<sup>th</sup> day of storage period. The fresh control chhana cake and gluten-free chhana cake had yeast and mold count was absent in one gram of which increased significantly (*p*<0.05) to 15.537 and 15.653 cfu/g, respectively of 8<sup>th</sup> days of storage.

Patel (2020) [1] also observed that presence of yeast and mold count was nill till 3<sup>rd</sup> day of storage of chhana based cake samples. It was increased significantly (*p*<0.05) to 19.0±2.08 cfu/g of 12 days of storage at 20±2 °C.

**Table 3:** Change in microbial count of chhana cake during storage at 7±2 °C

Storage period (Days)	Aerobic plate count (log <sub>10</sub> cfu/g)		Yeast and mold count (cfu/g)	
	T <sub>0</sub>	T <sub>1</sub>	T <sub>0</sub>	T <sub>1</sub>
0	1.23	1.25	0.0	0.0
2	1.31	1.34	2.98	3.01
4	1.42	1.45	6.40	6.61
6	1.55	1.64	10.58	10.70
8	1.76	1.87	15.54	15.65
Anova Table				
	T	P	T	P
SE m±	0.04	0.06	0.07	0.10
CD (0.05)	NS	0.18	NS	0.30
CV%	9.88		3.50	

Where T<sub>0</sub>= Control chhana cake, T<sub>1</sub>= Gluten-free chhana cake, T= Treatment, P= Period, NS= Non-significant



### Changes coliform count of chhana cake during storage at 7±2 °C

Presence of coliform in chhana cake indicates spoilage of product and severe human diseases. The entire freshly prepared and stored both chhana cake samples were free from coliform. The presence of coliform count was found absent till 8<sup>th</sup> days of storage period (i.e., 7±2 °C).

### Conclusion

From the results obtained in this study it was revealed that the scores of all the sensory attributes of gluten free chhana cake went below 7 except colour and appearance, crumb colour and crust colour score. The moisture of both chhana cakes was decreased non-significantly ( $p>0.05$ ) during storage. The specific volume of both chhana cake was decreased significantly ( $p<0.05$ ) during storage. The aerobic plate count of the both chhana cake was increased non-significantly ( $p>0.05$ ), whereas yeast and mold count was increased significantly ( $p<0.05$ ) during storage. The coliform count was found to be absent during storage period. The developed product was found to be acceptable up to 6 days when packed in cling film biodegradable plastic wrap and placed in rigid PVC trays and trays were packed in LDPE pouches (~85 µ) and stored at 7±2 °C.

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