Original Research Article

Effect of chitosan-based edible films on the sensory and microbial quality of ready-to-eat *paneer* during storage

ABSTRACT

Aims: The aim of the study is to find the effect of chitosan-based edible films on the sensory and microbiological characteristics of ready-to-eat *paneer* during storage at ambient and refrigeration temperature.

Study design: This study was experimental and conducted in a lab.

Place and Duration of Study: The study was conducted at the Department of Dairy Technology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India, between October 2022 and July 2023.

Methodology: The ready to eat *paneer* prepared with 5 %fructooligosaccharides, 40 % sugar and 15 % orange juice will be aseptically wrapped with chitosan based edible film made of 1.5% chitosan and 0.75% glycerol. The product will be analysed at both ambient temperature (27±1°C) with 1days interval and refrigeration temperature (4±1°C) with 3 days interval. Sensory analysis by 9-point hedonic scale and microbial analysis of standardplate count, coliforms count, yeast andmold count were assessed. ANOVA was the method used for statistical analysis, and the significance between the control and sample was assessed using a critical difference (CD) at a 5% significance level.

Results: The sensory attributes ratings of the control *paneer* and edible chitosan film packed ready to eat *paneer* samples are non-significant ($P>0.05$) to each other and decreased consistently throughout storage at both conditions. Throughout storage, the coliform count was undetectable. The initial yeast and mold count of the chitosan film-packed ready-to-eat paneer was 0.48 $\log_{10}$cfu/g, and the standard plate count was 2.30 $\log_{10}$cfu/g. Both counts increased during storage but remained significantly ($P<0.05$) lower than those in the control *paneer*.

Conclusion: The storage studies revealed that control paneer can be stored for 1 days at ambient temperature and 6 days at refrigeration temperature without any spoilage and chitosan film packed ready to eat paneer can be stored for 2 days at ambient temperature and 9 days at refrigeration temperature without any spoilage.

Keywords: Ready to eat paneer, chitosan, edible film, sensory analysis, microbial analysis

1. INTRODUCTION

Paneer is highly perishable due to it's diverse nutrient composition, which make them an ideal environment for the growth and propagation of spoilage microorganisms and milk borne pathogens. Hence, it is imperative to adopt effective adequate preservation technologies to maintain the quality and safety. In recent years, there is substantial increase in the demand of high quality, convenient, safe milk products with extended shelf life. Packaging is an important tool for dispensing as well as for maintaining the quality of food products (Goyal and Goyal, 2016).

Paneer is primarily used in culinary dishes, but it can also be used as a ready-to-eat food product that can be consumed without further processing. The main advantage of ready-to-eat paneer is that it improves its functional properties as well as its preservation quality. (Ali *et al.,*2023).

Paneer is highly nutritious as it contains a high level of protein and fat, minerals and vitamins. However, paneer has quite low shelf life at ambient temperature mainly due to its high moisture and fat content. Several preservation techniques including packaging, heat treatment, addition of preservatives or spices, thermal processing, low temperature storage etc. have been employed to extend its shelf life (Rajinder *et al.,* 2018). Growth of microorganisms on the surface is the root cause of spoilage of paneer Because of the high moisture content (about 55 %), it has a shelf life of only one day at ambient temperature and up to a week at refrigeration (7 days) (Mishra *et al*., 2021).

Numerous advancements in packaging technology, such as vacuum packing, modified atmospheric packaging, retort packaging, etc., had proved successful in extending the shelf life of *paneer*. But almost all of the packaging components used in modern food packaging are not biodegradable. Therefore, the idea of creating eco-friendly, biodegradable and edible packaging materials that preserve paneer's distinguishing qualities while also extending its shelf life is of particular interest to the food sector.

The effects of fat content and coagulation temperature have been found significant in controlling the sensory score (Badshah *et al.,* 2023). Mishra *et al.* (2021) studied the sensory evaluation of paneer stored at refrigeration temperature for 12 days. During storage, the colour and appearance score decreased from 8.03 to 6.21, body and texture score dropped from 7.88 to 6.93, flavour score declined from 7.63 to 5.15, and overall acceptability reduced from 7.98 to 5.40 by the 12th day. Sensory evaluation of *paneer* done at room temperature for 2 days showed colour and appearance decreased in prepared *paneer* from 8.03 to 5.60 body and texture score decreased from 7.88 to 5.87 flavour score decreased from 7.63 to 5.17 and overall acceptability score reduced from 7.98 to 5.63 on 2 days of storage (Mishra *et al.,* 2021). Punnagaiarasi*et al*. (2016) evaluated the sensory quality of edible film coated *paneer* using cinnamon oil and concluded that higher sensory scores were obtained for *paneer* coated with whey protein based edible coating with cinnamon oil up to level of 1 percent.

According to Mishra *et al*. (2016), *paneer* stored at 30±1°C had an initial yeast and mold count increased to by the 1st day. The standard plate count also rose from 1st day when compared to first day. The samples became unacceptable after 1 day of storage due to visible microbial growth. *Paneer*stored at 7±1°C also showed a slower increase in microbial counts. Yeast and mold counts rose by the 8th day, while the standard plate count increased from 4.41 $\log_{10}$cfu/g to 5.47 $\log_{10}$cfu/g during the same period. The samples became unacceptable after 8 days of storage due to visible growth.

The application of chitosan-based edible films has emerged as a promising approach for enhancing the shelf life and quality of ready-to-eat paneer. Chitosan, known for its antimicrobial and biodegradable properties, forms an effective barrier against microbial growth while maintaining the sensory attributes of paneer during storage (Ghosh *et al.*, 2020). Studies have demonstrated that chitosan-coated paneer exhibits lower microbial counts and better sensory scores compared to uncoated samples, thereby improving its acceptability over an extended storage period (Kumar *et al.,* 2019). Such advancements in edible film technology not only contribute to sustainable food packaging practices but

also align with the growing consumer demand for eco-friendly and safe preservation methods.

2. MATERIAL AND METHODS

2.1 Materials

Procured fresh cow's milk from the Dairy Science College's Students ExperimentalDairy Plant (SEDP) in Hebbal, Bengaluru, citric acid from Prince Chemical Co., Bengaluru, Karnataka, Fructooligosaccharide liquid from Foslife manufactured by Revelation biotech, Hyderabad and sugar from Shree Renuka sugars ltd, Mumbai, Maharashtra. The orange juice of 35 per cent orange pulp was obtained from Mala's fruits crush, Panchgani, Maharashtra. Chitosan powder, with over 90% deacetylation, was supplied by Meron-Marine Hydrocolloids, located in Kochi, Kerala. 1% food grade acetic acid was purchased from Umang Industries in Shahdara, New Delhi. Food-grade glycerol used in the study was supplied by Bioven Ingredients, located in Greater Noida, G.B. Nagar, Uttar Pradesh.

2.2 Preparation of ready to eat *paneer*

Paneer was prepared by modified procedure of Shanaziya*et al.,* 2018. The standardised milk is heated to 90℃ with no hold and cooled to 80℃. With moderate stirring, 2% citric acid as coagulation agent is added to milk at 80℃. The added milk was left undisturbed for 2 minutes. Next, a muslin cloth is used to separate the clear whey. fructooligosaccharides 5% (to the weight of curd), powdered sugar 40% (to the weight of curd) and orange juice 15% (to the weight of curd) are combined well into the *paneer* curd. The coagulum pressed for 30 minutes (2 kg/cm^2). It is then submerged for 30 minutes in pasteurised cold water (4°C).

2.3 Preparation of chitosan based edible film

The chitosan - based edible film was prepared by modified method of Singh *et al.,* 2015. Chitosan-based edible film was made by dissolving chitosan powder of 2% in 1per cent v/v acetic acid solution and plasticizer glycerol of 0.75% and heating for 20 minutes on a

hot plate magnetic stirrer at 90°C with regular stirring. The solution was uniformly cast on fibre glass plates and dried for 48 hours at 40 °C. The dried films were peeled off and kept in a chamber with a humidity of 50 per cent and a temperature of 25°C.

2.4 Evaluation of sensory and microbial characteristics of chitosan-packaged ready-to-eat *paneer* on storage days

The ready to eat *paneer* was aseptically wrapped with chitosan based edible film and packaged in LDPE pouches and sealed. The resultant product will be subjected for storage studies to assess the shelf stability of the product. The product will be analysed at both ambient temperature (27±1°C) with 1days interval and refrigeration temperature (4±1°C) with 3 days interval. The storage study was conducted three separate times, with each analysis performed in triplicate.

2.4.1 Sensory Evaluation

The organoleptic quality of *paneer* samples was evaluated at regular intervals by semi trained judges on a 9-point hedonic scale. The samples for evaluation will be coded appropriately before serving the samples to the judges for sensory evaluation. Sensory evaluation of the samples was be carried out in the sensory evaluation lab. Five membered semi trainedpanellists are requested to grade the sample on the basis of sensory attributes: colour and appearance, flavour, body & texture and overall acceptability (Dongare*et al.,*2019).

2.4.2 Microbiological Analysis

The LDPE pouch containing the developed edible chitosan film packed ready to eat *paneer* will be opened and chitosan film was unwrapped.11 g of the product will be weighed and transfer to 99 ml of 2% sodium citrate diluent aseptically. Further dilution to desired level will be carried out by serially transferring 1 ml of diluted sample to 9 ml sterile diluent blanks (Girdharwal 2018)

2.4.2.1 Standardplate count

Standard plate count will be determined by plating 1, 2 and 3 dilutions of *paneer*. The molten media of HiMedia M091-plate count agar will be poured to diluted sample plates and incubated for 24-48 hours at 37℃ after solidification. Counts will be taken manually after the completion of incubation period.

2.4.2.2 Coliforms count

Coliform count will be determined by plating 1, 2 and 3 dilutions of *paneer*. The molten media of HiMedia M049-violet red bile agar will be poured to each diluted sample plates and incubated for 18-24 hours at 37℃ after solidification. Counts will be taken manually after the completion of incubation period.

2.4.2.3 Yeast & mold count

Yeast & mold count will be determined by plating 1, 2 and 3 dilutions of *paneer*. The pH of the molten HiMedia MH096-potato dextrose agar medium will be adjusted to 3.5 by adding 10 per cent of 1.6ml sterile lactic acid solution to 100 ml media. Media is poured to diluted plates and incubated for 3 – 5 days at 30ºC. Counts will be taken manually after the completion of incubation period.

2.5 Statistical Analysis

The data was analysed using IBM SPSS Statistics-29 software for statistical computing. Data on the response variables were collected for three replications for each of the treatments. ANOVA tables were prepared to analyse the data and where the F value was significant, the critical difference was calculated (P=0.05) and used to identify whether significant differences existed and indicated in the table using superscripts. Storage study analysis was done by repeated ANOVA method.

The formula for the critical difference (CD) is

$$\frac{\sqrt{2} \times M(E)}{r} \times t\alpha$$

Where,

MSS (E) = Mean Sum of squares of the error

r = number of replications

tα = table t value of the α level of significance at 0.95

3. RESULTS AND DISCUSSION

3.1 Effect on sensory attributes of chitosan film packed ready to eat paneer during storage at ambient temperature (27±1^{o} C)

After each day of judging there was a decrease in sensory scores. The control sample got spoiled on day 2 and the chitosan film packed ready to eat *paneer* sample got spoiled on day 3 when stored at room conditions (27±1° C). There was no significant (P<0.05) difference in thesensory score of the control sample and chitosan film packed ready to eat *paneer* sample in all days. The colour and appearance score decreased from was 8.33 to 8.25 for the control sample whereas for chitosan film packed ready to eat *paneer* it decreased from 8.0 to 7.42. Body and texture score of control *paneer* on initial day was 8.33 then later reduced to 8.17 on days 1 and for chitosan film packed ready to eat *paneer*days 0 the score was 8.17 which reduced to 7.33 on day 2.

For the control sample, the flavour score dropped from 8.17 to 7.92, whereas it dropped from 8.33 to 7.42 for ready-to-eat *paneer* packaged in chitosan film. The overall acceptability score for the control on the very initial day was 8.33 and then decreased to 7.67 on days 1 whereas the score for the chitosan film-packaged, ready-to-eat *paneer* on days 0 was 8.33 and later decreased to 7.33 on days 2. The results of this study were consistent with the findings of Mishra *et al.* (2021), who investigated the effect of black pepper extract on the sensory attributes and shelf life of paneer. Their study reported a decrease in scores during storage at ambient temperature (30±1°C). During storage days the sensory scores reduces mainly due to deterioration in its quality. The scores of the developed *paneer*, when compared to the control, were lower each day across all parameters, closely aligning with the trends observed in this research.

Table 1: Effect on sensory attributes of chitosan film packed ready to eat *paneer*during storage at ambient temperature (27±1^{o} C)

Sample	Colour & Appearance	Body & Texture	Flavour	Overall Acceptability
		DAY 0		
CP	8.33[a]	8.33[a]	8.17[a]	8.33[a]
CFRP	8.00[a]	8.17[a]	8.33[a]	8.33[a]
CD (*P*=0.05)	**NS**	**NS**	**NS**	**NS**
		DAY 1		
CP	8.25[a]	8.17[a]	7.92[a]	8.17[a]
CFRP	7.92[a]	7.50[a]	7.83[a]	7.67[a]
CD (*P*=0.05)	**NS**	**NS**	**NS**	**NS**
		DAY 2		
CP	Spoiled	Spoiled	Spoiled	Spoiled
CFRP	7.42	7.33	7.42	7.33
CD (*P*=0.05)	**-**	**-**	**-**	**-**
		DAY 3 Spoiled		

Note: CP – Control *paneer,* CFRP – Chitosan film packed ready to eat *paneer,* CD – Critical difference, NS – Non significant, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

3.2 Effect on sensory attributes of chitosan film packed ready to eat *paneer*during storage at refrigeration temperature (4±1^{o} C)

The sensory scores decreased after each day of judging. On day 3, the control sample and chitosan film packed ready to eat *paneer* sample held at room temperature (4±1°C) deteriorated. On all days, there was no significant (P>0.05) difference in sensory score between the control sample and the chitosan film packed ready to eat *paneer* sample. The colour and appearance score reduced from 8.33 to 7.92 for the control sample and from 8.0 to 7.25 for the chitosan film packed ready to eat *paneer*. The control's body and texture score on day 0 was 8.33, which was later reduced to 7.88 on day six, whereas the chitosan film packed ready to eat *paneer* score on day zero was 8.17, which was later lowered to 7.01 on day nine.

The flavour score for the control sample dropped from 8.17 to 7.58, while it dropped from 8.33 to 7.08 for ready-to-eat *paneer* packed in chitosan film. The overall acceptability

score for the control on the initial day was 8.33 and then reduced to 7.67 on day six, whereas the score for the chitosan film-packaged, ready-to-eat *paneer* on day 0 was 8.33 and then decreased to 7.00 on day nine. The findings of this study were consistent with the findings of Mishra *et al*., (2021) in their study on the effect of black pepper extract on sensory characteristics and shelf life of *paneer*, where scores dropped on storage days at refrigerated temperature (7±1°C) due to its reduction in quality and freshness on storage.

Table 2: Effect on sensory attributes of chitosan film packed ready to eat paneer during storage at refrigeration temperature (4±1° C)

Sample	Colour & Appearance	Body & Texture	Flavour	Overall Acceptability
		DAY 0		
CP	8.33 [a]	8.33 [a]	8.17 [a]	8.33 [a]
CFRP	8.00 [a]	8.17 [a]	8.33 [a]	8.33 [a]
CD (*P*=.05)	**NS**	**NS**	**NS**	**NS**
		DAY 3		
CP	8.33 [a]	8.33 [a]	8.17 [a]	8.33 [a]
CFRP	7.83 [a]	7.67 [a]	7.83 [a]	7.83 [a]
CD (*P*=.05)	**NS**	**NS**	**NS**	**NS**
		DAY 6		
CP	7.92 [a]	7.88 [a]	7.58 [a]	7.67 [a]
CFRP	7.33 [a]	7.08 [a]	7.50 [a]	7.33 [a]
CD (*P*=.05)	**NS**	**NS**	**NS**	**NS**
		DAY 9		
CP	Spoiled	Spoiled	Spoiled	Spoiled
CFRP	7.25	7.01	7.08	7.00
CD (*P*=.05)	**-**	**-**	**-**	**-**
		DAY 10 Spoiled		

Note: CP – Control *paneer,* CFRP – Chitosan film packed ready to eat *paneer,* CD – Critical difference, NS – Non significant, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

3.3 Effect on microbiological characteristics of chitosan film packed ready to eat *paneer* during storage at ambient temperature (27±1° C)

The coliform count showed nil for all the samples (control and chitosan film packed ready to eat *paneer*) throughout the storage period whereas standard plate count and yeast and molds counts increased upon increasing the period of storage. The control and chitosan film packed ready to eat *paneer* samples have standard plate counts of 2.83 and 2.30 $\log_{10}$cfu/g, respectively. On the first day of storage, the standard plate count in control climbed to 4.99 $\log_{10}$cfu/g. On the second day of storage, the standard plate count in chitosan film packed ready to eat *paneer* samples raised to 5.88 $\log_{10}$cfu/g.

The fresh control and chitosan film packed ready to eat *paneer*samples show 0.21 and 0.48 $\log_{10}$cfu/g counts of yeast and molds respectively. On first day of storage yeast and molds increased in control increased to 2.12 $\log_{10}$ cfu/g and day two of storage chitosan film packed ready to eat *paneer* increased to 2.10 $\log_{10}$cfu/g. On 2nd day of storage control *paneer* samples found unacceptable at ambient temperature. On 3rd day of storage, the chitosan film packed ready to eat *paneer* samples found unacceptable at ambient temperature. There was a significant ($P<0.05$) difference in control and chitosan film packed ready to eat *paneer* on standard plate count and yeast and mold count. The is a marked increase in bacterial count due to ambient condition for the growth bacteria in terms of temperature, moisture content and nutrient availability. Slightly acidic pH levels in many *paneer* support the growth of yeasts, and molds.The findings of this study agree with Mishra *et al.* (2016), who studied the effect of pH on the sensory, textural, microbial quality, and shelf-life of paneer which is stored at ambient temperature (30±1°C).

Table 3: Effect on microbiological characteristics of chitosan film packed ready to eat paneer during storage at ambient temperature (27±1^{o} C)

Sample	Standard plate count	Yeast and Molds	Coliform
	$\log_{10}$cfu/g		
	DAY 0		
CP	2.83 [a]	0.21 [a]	Nil
CFRP	2.30 [b]	0.48 [b]	Nil

CD (*P*=0.05)	**0.37**	**0.12**	**-**
		DAY 1	
CP	4.99 [a]	2.12 [a]	Nil
CFRP	3.80 [b]	1.64 [b]	Nil
CD (*P*=0.05)	**0.37**	**0.23**	**-**
		DAY 2	
CP	Spoiled	Spoiled	Spoiled
CFRP	5.46	2.10	Nil
CD (*P*=0.05)	**-**	**-**	**-**
		DAY 3 Spoiled	

Note: CP – Control *paneer,* CFRP – Chitosan film packed ready to eat *paneer,* CD – Critical difference, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

Fig.1: Yeast and molds plates of control paneer (left) and chitosan wrapped ready to eat paneer (right) when stored at ambient temperature on 3rd day

3.4 Effect on microbiological characteristics of chitosan film packed ready to eat *paneer* during storage at refrigeration temperature (4±1^{o} C)

The coliform count was absent for all samples (control and chitosan film packed ready to eat *paneer*) throughout the storage time, however the standard plate count, yeast and mould counts increased as the storage period increased. Standard plate counts for the

control and chitosan film packed ready to eat *paneer* samples were 2.83 and 2.30 log_{10}cfu/g, respectively. On sixth day for control and ninth day for chitosan film packed ready to eat *paneer* of storage, the standard plate count in control increased to 5.28 log_{10}cfu/g and 4.14 log10 cfu/g in chitosan film packed ready to eat *paneer*.

Fresh control and chitosan film packed ready to eat *paneer* samples have yeast and mould counts of 0.21 and 0.48 log_{10}cfu/g, respectively. On sixth day for control and ninth day for chitosan film packed ready to eat *paneer* of storage, yeast and moulds grew to 2.08 log_{10}cfu/g in the control and 2.16 log_{10}cfu/g in the chitosan film packed ready to eat *paneer*. On the seventh day of storage control paneer was unsuitable and on ninth day chitosan film packed ready to eat *paneer* was unsuitable at refrigeration temperature. There was a significant ($P<0.05$) difference in standard plate count and yeast and mould count between the control and chitosan film packed ready to eat *paneer*. There is a gradual increase in bacterial count even under refrigeration conditions due to the availability of moisture, nutrients. Additionally, the slightly acidic pH of paneer supports the growth of yeasts and molds over time. The study's findings coincide with the work of Mishra *et al.,* (2016) on Effect of pH on sensory, textural, microbiological quality and shelf-life of *paneer*, where microbial analysis of *paneer* was done under storage refrigeration temperature (7±1°C).

Table 4: Effect on microbiological characteristics of chitosan film packed ready to eat paneer during storage at refrigeration temperature (4±1° C)

Sample	Total plate count	Yeast and Molds	Coliform
	log_{10}cfu/g		
	DAY 0		
CP	2.83 [a]	0.21 [a]	Nil
CFRP	2.30 [b]	0.48 [b]	Nil
CD (*P*=0.05)	**0.37**	**0.12**	**-**
	DAY 3		
CP	4.04 [a]	1.43 [a]	Nil

CFRP	3.61 [b]	1.11 [b]	Nil
CD (*P*=0.05)	**0.16**	**0.16**	**-**
	DAY 6		
CP	5.28 [a]	2.08 [a]	Nil
CFRP	3.79 [b]	1.74 [b]	Nil
CD (*P*=0.05)	**0.18**	**0.14**	**-**
	DAY 9		
CP	Spoiled	Spoiled	Spoiled
CFRP	4.14	2.16	Nil
CD (*P*=0.05)	**-**	**-**	**-**
	DAY 10 Spoiled		

Note: CP – Control *paneer,* CFRP – Chitosan film packed ready to eat *paneer,* CD – Critical difference, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

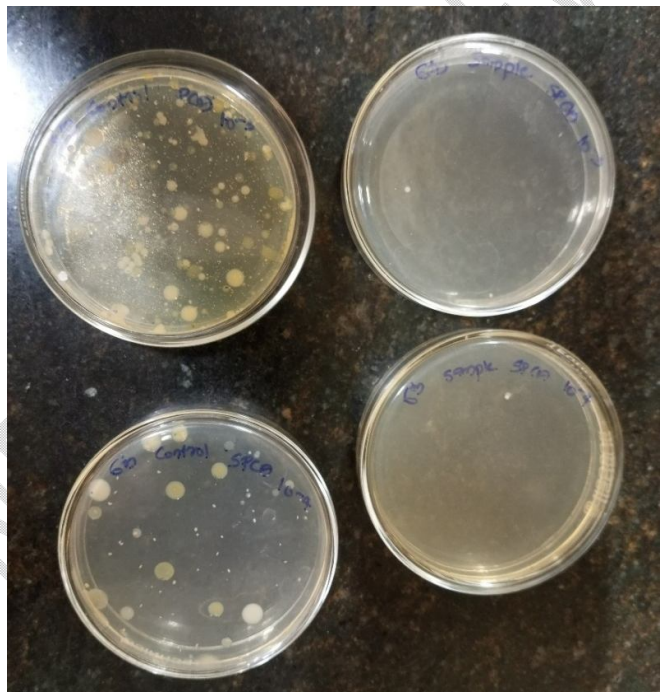

Fig:2 TPC plates of control paneer (left) and chitosan wrapped ready to eat paneer (right) when stored at refrigeration temperature on 6th day

4. CONCLUSION

The storage studies demonstrated that as the storage days progressed, the sensory attributes ratings of the control paneer and edible chitosan film packed ready to eat

paneer samples decreased consistently throughout storage at both ambient temperature (27±1°C) and refrigeration temperature (4±1°C).

The microbiological analysis was carried out for standard plate count, coliform and yeast and mould count in control and chitosan film packed ready to eat paneer during storage at both ambient temperature (27±1°C) and refrigeration temperature (4±1°C). Throughout storage, the coliform count was undetectable. During storage, the standard plate count, as well as the yeast and mould counts, increased. The standard plate count, yeast and mould count (0.48 $\log_{10}$cfu/g) and standard plate count (2.30 $\log_{10}$cfu/g) of the chitosan film packed ready to eat paneer were much lower than those of the control paneer.

The edible chitosan film prepared with 2 per cent chitosan, 0.75 per cent glycerol, 1 per cent acetic acid solution were used to wrap the ready to eat paneer prepare with addition of 5 per cent fructooligosaccharides, 40 per cent sugar, 15 per cent orange juice. The storage studies at ambient temperature (27±1°C) and refrigeration temperature (4±1°C) revealed that control paneer can be stored for 1 days at ambient temperature and 6 days at refrigeration temperature without any spoilage and chitosan film packed ready to eat paneer can be stored for 2 days at ambient temperature and 9 days at refrigeration temperature without any spoilage.

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Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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