

*Original Research***Evaluation of Garlic Extract in Extending Shelf Life of Paneer****Trupti P. Wanjari, Shekhar R. Badhe*, Vivek Shukla, Satish R. Yadav and Vilas M. Vaidya¹**

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Abstract

The present study was evaluated antimicrobial and antioxidant effect of different concentration of garlic extracts on shelf-life of paneer for storage period of 15 days under refrigeration condition ($4^{\circ}\text{C}\pm 1$). The antimicrobial activity of garlic extracts was evaluated against *E. coli*, *S. aureus*, *P. aeruginosa* using disc diffusion method and agar well diffusion method while its antioxidant property was evaluated based on its phenolic content (mg catechol equivalent/100 gm). The paneer cut into cubes were dipped in different concentrations of garlic extract (25%, 50% and 75% as T1, T2 and T3, respectively) and evaluated for antimicrobial and antioxidant properties against control sample. The samples were subjected to sensory evaluation when fresh and 5 days interval during 15 days storage periods. The sample were analysed for pH, TBARS, tyrosine and microbial analysis during storage. Paneer sample treated with 50% garlic extracts remained sound without changes its sensory qualities and microbiologically safe upto 10 days under refrigeration condition.

Key words: Garlic Extracts, Paneer, Shelf-life**How to cite:** Wanjari, T., Badhe, S., Yadav, S., Shukla, V., & Vaidya, V. (2019). Evaluation of Garlic Extract in Extending Shelf Life of Paneer. International Journal of Livestock Research, 9(7), 100-110. doi: 10.5455/ijlr.20190311110441**Introduction**

Paneer is heat acid coagulated milk products popular in India. Paneer is marble white in appearance, having a slightly spongy body, close-kneet texture and possessing sweetish-acidic-nutty flavour (Aneja, 2002). Paneer is rich source of protein, fat, vitamin and mineral like calcium and phosphorus. The demand of paneer is increasing throughout the world. But paneer is highly perishable milk products. The shelf life of paneer is reported to be one week at 4°C and losses its freshness after 2-4 days under refrigerated storage (Goyal and Goyal, 2016). In order to increase the shelf-life of paneer use of additives, modification in

paneer manufacturing process, surface treatments, antimicrobial substances and use of various packaging material have been proposed by the researcher. Bio-preservatives like spices having good antimicrobial and antioxidants properties with least side effects. Spices used in food preparation not only enhanced the taste and flavour but also act as antimicrobial, antioxidants, food stabilizing properties and medicinal properties (Ismile *et al.*, 2006). The role GRAS additives like cardmom, clove, cinnamon and ginger were used in enhancing shelf life of paneer (Makhal, 2000). Efficacy different spices (black paper, cardmom, cinnamon and clove) were evaluated as preservatives in paneer by Eresamet *et al.* (2015). Singh *et al.* (2014) evaluated efficacy of turmeric in extending shelf life of paneer.

Among these spices, no article was found on garlic in extending shelf life of paneer if this will be true then this work act as preliminary trial on garlic in extending shelf life of paneer and this information and data will be helpful for further research. Most of the studies revealed that garlic preservative in many food components because of its antimicrobial and antioxidant properties. It also reduces lifestyle related diseases like cardiovascular diseases, diabetes and cancer (Rahman, 2003). Garlic is therapeutically effective due to its oil and water soluble in organo-sulfur compounds. Thiosulphinate is mainly responsible for its antimicrobial activity (Hughes and Lawson, 1991). Allicin is active principle in garlic which has antibacterial, antifungal, antiviral and antiparasitic activity (Ankri, 1999). Several chemical preservative have been found but they have been found with several health hazards. Nowadays consumers are giving more emphasis on the use natural preservatives in food. By considering the above mentioned facts the present work is planned for evaluation of shelf-life of garlic treated paneer.

Materials and Methods

The present study was carried on assessment of storage stability garlic treated paneer. The materials used and methodology employed in this study are discussed below-

Fresh buffalo milk brought from nearby local market of college campus was used in all experiments. The milk was standardised to fat percentage 6 and SNF percentage 9 for optimum products characteristics. 1% Citric acid was used to coagulate the standardised milk for the preparation of paneer. Fresh garlic was procured from nearby market. The standard bacterial culture of *Staphylococcus aureus* and *E. coli* were obtained from department of Veterinary Public Health, Mumbai Veterinary College, Parel, Mumbai. Standard culture of *Pseudomonas aeruginosa* was obtained from Institute of Microbial Technology Chandigarh, India. Required chemical, glassware, chemical and reagent were procured from standard manufacturers.

Determination of Antimicrobial and Antioxidant Properties of Garlic Extracts

The fresh garlic was obtained from the local market. The garlic were peeled, cleaned, and washed in sterile distilled water. Garlic was surface sterilized using 75% (v/v) ethanol for 60 sec. In order to obtain the garlic

extract, about 100 g of washed garlic was crushed with mortar and pestle. The extract was sieved through a fine mesh cloth and sterilized using a membrane filter (0.45-micron sterile filter). This extract was considered as the 100% concentration of the extract. The concentrations, 75%, 50% and 25% was made diluting the concentrated extract with appropriate volumes of sterile distilled water (Indu *et al.*, 2006). The cultures were enriched in sterile nutrient broth for 6-8 hours at 37°C. The bacterial inoculum was uniformly spread with a sterile L-shaped spreader. Agar wells with 10 mm diameter and 20 mm apart from one another were prepared with the help of sterilized cork borer. Using a micropipette, 100 micro litres of different concentrations of garlic extract (100%, 75%, 50% and 25%) were poured into different wells in the plate. The plates were incubated at 37°C for 24 hours. The diameter of inhibition zones were measured in mm and the results were recorded.

The total phenolic content of garlic extract was determined by calorimetric spectrophotometry with Folin–Ciocalteu reagent method. This reagent contains complexes of fosfomolibdic and fosfotugstic acid. 200 micro litres of garlic extract was made up to 3 ml with distilled water, mixed thoroughly with 0.5 ml of Folin–Ciocalteu reagent for 3 min followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve and the results were expressed as mg catechol equivalent of phenol/gram sample.

Assessment of Shelf-Life of Paneer Treated with Different Level of Garlic Extract

Paneer was cut into cubes and dipped into different concentrations of garlic extract *viz.* 100%, 75%, 50% and 25%. The treated samples were analyzed for sensory evaluation. Based on the results, samples were selected for further studies. The treated paneer packed in LDPE was analyzed for physico-chemical and microbiological attributes at an interval of 0, 5, 10, and 15 days of storage (4±1°C). Sensory attributes of controlled paneer and treated paneer were judged by semi-trained panel of 6 judges on 0 day 5th, 10th and 15th days of storage by using nine point descriptive scales (Keeton, 1983). The score of 6 judges were averaged and recorded as mean value for sensory score. Each panellist evaluated 5 samples (identified by codes) in a balanced sequential order. Freshly prepared control and garlic extract treated paneer was subjected for physico-chemical, and microbial status. The samples were packaged in sterile food grade LDPE material and were kept at refrigeration temperature (4±1°C) to monitor physico-chemical and microbial changes during storage at the interval of day 0, day 5, day 10 and day 15 of storage.

Physico-Chemical Analysis of Paneer

The pH of fresh and stored paneer sample (control and garlic extract treated paneer) was determined by the method of Trout *et al.* (1992). 10 grams of sample was homogenized with 50 ml of distilled water in a

laboratory blender. The pH of suspension was recorded with the help of digital pH meter (Model-HI 99163, HANNA). TBA number of fresh and stored paneer sample (control and garlic treated paneer) was determined as per the method described by Witte *et al.* (1970) with little modification. Trichloroacetic acid (TCA) extract was prepared by blending 3 gm of sample with 7.5 ml of distilled water. After homogenization, the contents were filtered through Whatman filter paper No. 1. Two ml of aliquot of TCA extract was mixed with two ml of 0.01M 2-TBA reagent in a test tube. The test tubes were kept in a water bath at 100°C for 1 hr. After cooling the test tubes, the absorbance (A) at 530 nm was measured in a spectrophotometer (Model no. EQ 820 with wavelength range of 350-950 nm, INDIA).

Tyrosine value was determine as per procedure described by Strange *et al.* (1977) was used with slight modification. 2.5 ml TCA extract was taken and mixed with equal amount of distilled water. The mixture was blended with addition of 10 ml of 0.5 N NaOH to which 3 ml of diluted Folin–Ciocalteu reagent was added. The mixture was kept in dark at room temperature for 30 min for colour development. The optical density was measured at 730 nm using spectrophotometer. Tyrosine value was calculated as mg tyrosine per 100 gm of sample by referring to a standard graph, which was prepared as per the procedure described by Pearson, (1968).

Microbiological Analysis

Standard plate count, psychophilic count and yeast and mould count of paneer samples were estimated by following standard method of APHA (2001). Grate 10 gm of paneer sample with 90 ml of sterile normal saline solution was triturate in a sterile pestle and mortar for two min to get 10^{-1} dilution. One ml of this dilution was transferred to nine ml of sterile NSS in a test tube and mixed uniformly to get 10^{-2} dilution. Subsequent dilutions were made as per the requirement following the same procedure. For detection and enumeration of total viable count, psychophilic count, yeast and mould count, *E. coli*, *S. aureus* count. Respective standard media were prepared and processed under sterile conditions. Total viable count was enumerated by plating on nutrient agar and incubated at 37°C for 24 hrs. The procedure outlined for total plate count was followed for psychophilic count except incubation, where plates were incubated at 4°C for 5-7 days. Eosin methylene blue media was used for enumeration of *E. coli* count and incubated at 37°C for 24 hrs. *Staphylococcus aureus* was estimated with the help of mannitol salt agar and incubated at 37°C for 24 hrs. Total yeast and mould count were enumerated by plating on molten Sabouraud's dextrose agar and incubated at 25°C for 5-7 days.

Statistical Analysis

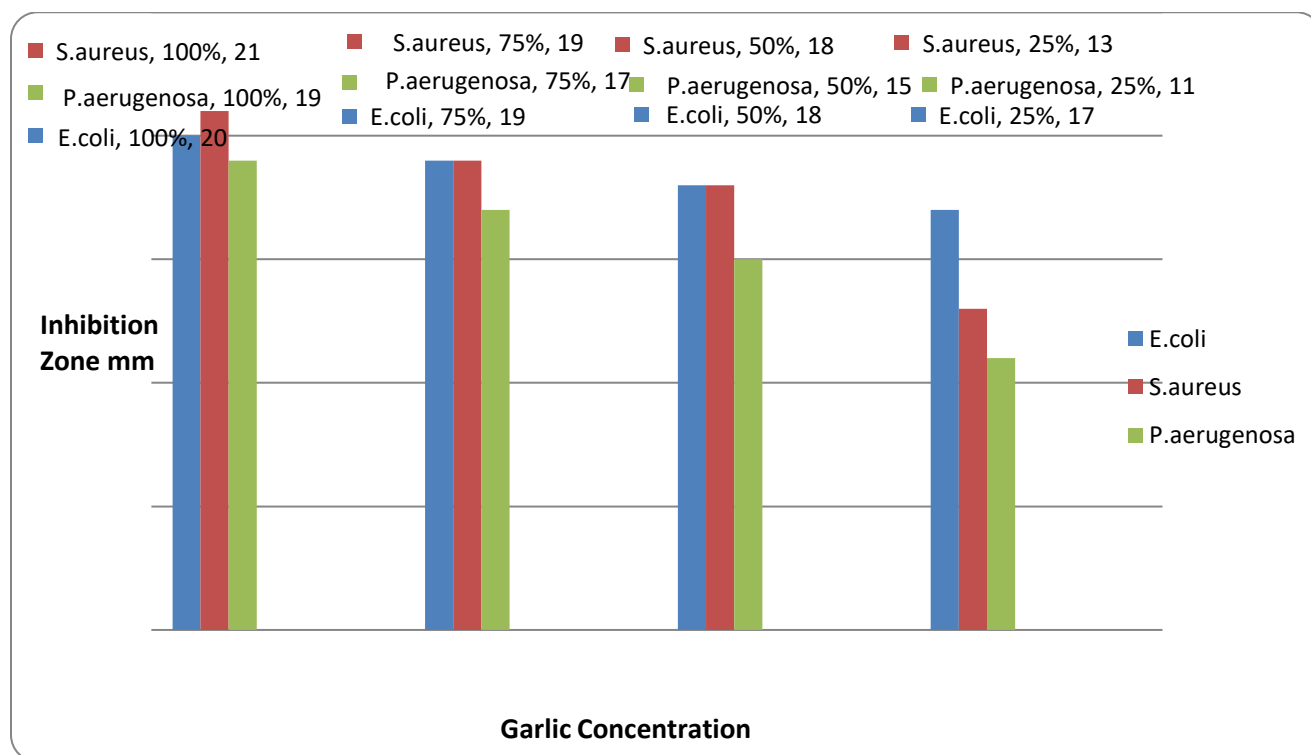
The data obtained during the experiment were analyzed usingWASP 1.0.

Results and Discussion

The present study was planned to assess the storage stability of garlic extract treated paneer at refrigeration temperature. Antimicrobial (100%, 75%, 50% and 25%) and antioxidant potentials garlic extract were tested. Garlic extract showed excellent antibacterial activity at all concentrations (100%, 75%, 50% and 25%) against *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*. Among these organisms, *Staphylococcus aureus* and *E. coli* were the most susceptible followed by *Pseudomonas aeruginosa*. Average values of zone of inhibition for the test organisms are depicted in Table 1 and Graph 1.

Table 1: Antibacterial activity of different concentration of garlic extract by disc diffusion and Agar well method

Concentrations	Mean Diameter of Inhibition Zone (mm)					
	<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>	
	Disc Diffusion	Agar Well	Disc Diffusion	Agar Well	Disc Diffusion	Agar Well
100%	20.5±0.22	21.00±0.36	21.33±0.71	21.33±0.42	19.00±0.44	19.33±0.44
75%	19.33±0.33	20.00±0.44	19.00±0.89	19.66±0.55	17.00±0.36	17.83±0.30
50%	18.33±0.21	18.33±0.33	18.00±0.68	17.33±0.66	15.33±0.33	15.83±0.30
25%	17.00±0.25	16.50±0.2	13.83±0.91	14.00±0.96	11.66±0.55	11.83±0.30



Graph 1: Antibacterial activity of different concentrations of garlic extract by disc diffusion method

The results agree with the observations of Durairaj *et al.* (2009). Arora and Kaur (1999) observed a significant bactericidal effect of garlic extract against *Staphylococcus*, *Salmonella* spp. and various yeast. The antibacterial activity of garlic is reported to be due to the action of allicin or diallyl thiosulphinic acid or diallyl disulphide. In the present study, the total phenol content of the garlic extract was determined in terms of mg catechol equivalent /g extract using the standard plot of catechol. Soobrattee *et al.* (2005) stated that the phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. The total phenolic content of garlic extract was found to be 46±0.4 mg catechol equivalent /100g extract. The value observed were in the line with Benkeblia (2005), who reported that the total phenolic content in garlic was 49 mg/100g. Bozin *et al.* (2008) extracted garlic in 80% methanol and found that total phenol content of garlic was 50 mg GAE/100g. Phenolics are the most wide spread secondary metabolite in plant kingdom.

The physico-chemical changes in the control and garlic extract treated paneer were monitored by analyzing changes in the pH, TBARS value and tyrosine value at an interval of 5 days during storage. Present study revealed that garlic extract treated paneer caused an insignificant decrease in pH value of paneer during 0 day and 5 days as well as 10 days and 15 days but there is significant decrease in pH value among 0 day and 10 days; 0 day and 15 days; 5 days and 10 days; 5 days and 15 days as shown in Table 2. The initial pH value of control and garlic extract treated paneer was 5.76 and 5.75, respectively, which decreased to 5.53 and 5.63 respectively.

Table 2: Change in physic-chemical parameters of control and treatments of paneer during storage

Treatments	Storage	C	T1	T2	T3
pH	0 day	_A 5.76±0.04 ^a	_A 5.76±0.04 ^a	_A 5.75±0.05 ^a	_A 5.75±0.05 ^a
	5 day	_A 5.72±0.06 ^a	_A 5.73±0.05 ^a	_A 5.73±0.05 ^a	_A 5.73±0.05 ^a
	10 day	_B 5.59±0.03 ^a	_B 5.66±0.04 ^a	_B 5.65±0.05 ^a	_A 5.67±0.04 ^a
	15 day	_B 5.53±0.03 ^a	_B 5.58±0.03 ^a	_B 5.60±0.02 ^a	_B 5.63±0.03 ^a
TBA	0 day	_A 0.20±0.03 ^a	_A 0.22±0.02 ^a	_A 0.22±0.04 ^a	_A 0.24±0.02 ^a
	5 day	_B 0.39±0.04 ^a	_B 0.34±0.04 ^a	_B 0.29±0.04 ^b	_B 0.32±0.04 ^b
	10 day	_C 0.72±0.1 ^a	_C 0.62±0.1 ^b	_C 0.49±0.03 ^c	_C 0.44±0.04 ^c
	15 day	_D 1.19±0.15 ^a	_D 1±0.15 ^b	_D 0.74±0.15 ^c	_D 0.63±0.12 ^d
Tyrosin	0 day	_A 12.65±0.08 ^a	_A 12.41±0.03 ^a	_A 12.45±0.02 ^a	_A 12.43±0.02 ^a
	5 day	_B 19.58±0.10 ^a	_B 18.75±0.09 ^b	_B 16.40±0.13 ^c	_B 15.61±0.06 ^d
	10 day	_C 31.90±0.23 ^a	_C 28.36±1.02 ^b	_C 25.03±0.22 ^c	_C 21.93±0.16 ^d
	15 day	_D 47.18±0.52 ^a	_D 37.92±0.42 ^b	_D 35.82±0.32 ^c	_D 31.81±0.26 ^d

*Row wise Mean bearing subscript indicates significant and non-significant difference at (p<0.05%). Column wise mean bearing superscript indicates significant and non-significant difference at (p<0.05%).

Similar result were reported by Rai *et al.* (2008); Makhali *et al.* (2014) and El-Aziz *et al.* (2012) reported that insignificant decrease in pH value of soft cheese during storage of 7 days at refrigeration temperature.

Changes in Thiobarbituric acid value of control and garlic treated paneer as shown in Table 2. The average TBA value of control and garlic treated paneer was significantly increased throughout storage period under refrigeration temperature. These values were lower in garlic treated paneer than in control one which indicates that garlic extract has antioxidant properties. TBA value of control significantly increased as compared to treatments which reached 1.19 at the end of 15 days storage under refrigeration storage. The increase in TBA value was probably due to growth of spoilage organisms. These results were in agreement with Bukhari *et al.* (2012) and Shan *et al.* (2011) studied the potential application of spice and herb extracts as natural preservatives in cheese. The TBA value of cheese was periodically tested by oxidative analyses. The initial concentrations of TBA in all treated cheeses were lower than that of the control. During the 9th day storage, the TBA values of all treated cheese samples slightly increased, whereas the TBARS values of the control sample significantly increased. The tyrosine value of freshly prepared paneer and changes occurred during storage are highlighted in Table 2. Tyrosine value for controlled was 12.65 ± 0.08 on 0 day and 47.18 on 15th day while tyrosine value for T₁ was 12.41 ± 0.03 on 0 day and 37.92 ± 0.42 on 15th day, for T₂ was 12.45 ± 0.02 on 0 day and 35.82 ± 0.32 on 15th day and for T₃ was 12.43 ± 0.02 on 0 day to 31.81 ± 0.26 on 15th day. Tyrosine value for controlled paneer was more as compared to treatments throughout its storage period. The increase in tyrosine value is an indication of proteolysis caused by microbial spoilage. These results were in accordance with Sindhu *et al.* (2000); Rai *et al.* (2008) and Singh *et al.* (2014).

During the storage study it is very important to monitor the product for various microbes. Accordingly, in present investigation the paneer samples were monitored for total viable count, psychrophilic count, yeast and mould count on day zero, and after their interval of 5 days. The paneer samples were also analyzed for contaminants like *E. coli* and *Staphylococcus aureus*. The paneer with different garlic extract treatments were subjected for total viable count (TVC log₁₀cfu/g) at day zero and subsequently at 5th, 10th and 15th day of storage. The values for TVC at day zero and during storage period are highlighted in Table 3. During the entire storage period the average TVC values were significantly increase between control and treatments. Among the treatments there were significant difference between T₁ and T₂ but there were no significant difference between T₂ and T₃ except at 15th days storage.

The average psychrophilic count of control was increased steadily during the entire storage period from 4.51 ± 0.02 to 5.15 ± 0.09 . The psychrophilic count for T₂ and T₃ were nil at the end of 5th day but average psychrophilic count for T₂ and T₃ was increase steadily up to 15th day as shown in Table 3. During the entire storage period the average yeast and mould count were significantly different between control and treatments. There was decrease in yeast and mould counts in treatments as compare to control. Among the treatments average yeast and mould count were significantly different between T₁ and T₂ but there were no significant difference observed between T₂ and T₃ as depicted in Table 3.

Table 3: Microbial count of control and treatments of paneer during storage

Parameters	Storage	C	T1	T2	T3
TVC	0 day	^A 4.8±0.02 ^a	^A 4.62±0.04 ^b	^A 4.43±0.04 ^c	^A 4.3±0.0 ^c
	5 days	^B 4.98±0.0 ^a	^B 5.00±0.0 ^a	^B 4.81±0.0 ^b	^B 4.76±0.0 ^b
	10 days	^c 5.12±0.0 ^a	^c 5.09±0.0 ^a	^c 4.97±0.0 ^b	^c 4.95±0.0 ^b
	15 days	^D 5.19±0.06 ^a	^D 5.18±0.09 ^a	^D 5.15±0.0 ^a	^D 5.1±0.01 ^{ab}
Ps	0 day	^A 4.51±0.02 ^a	^A 1.34±0.85 ^b	Nil	Nil
	5 days	^B 4.83±0.03 ^a	^B 4.63±0.01 ^a	Nil	Nil
	10 days	^{BC} 5.12±0.03 ^a	^{BC} 4.92±0.04 ^a	^A 4.73±0.02 ^{ab}	^A 4.63±0.02 ^{ab}
	15 days	^c 5.15±0.09 ^a	^c 5.13±0.01 ^a	^B 5.03±0.01 ^a	^B 4.98±0.01 ^a
Y/M	0 day	^A 2.96±0.04 ^a	^A 2.91±0.06 ^b	^A 2.77±0.02 ^c	^A 2.74±0.04 ^c
	5 days	^B 3.13±0.02 ^a	^B 3.12±0.01 ^a	^B 3.07±0.02 ^b	^B 3.06±0.02 ^b
	10 days	^c 3.41±0.01 ^a	^c 3.34±0.03 ^b	^c 3.20±0.02 ^c	^c 3.21±0.02 ^c
	15 days	^D 3.53±0.02 ^a	^D 3.46±0.01 ^b	^D 3.32±0.01 ^c	^D 3.30±0.01 ^c
<i>E. coli</i>	0 day	NIL	NIL	NIL	NIL
	5 days	NIL	NIL	NIL	NIL
	10 days	NIL	NIL	NIL	NIL
	15 days	2.98±0.33 ^a	2.96±0.22 ^a	NIL	NIL
<i>S. aureus</i>	0 day	NIL	NIL	NIL	NIL
	5 days	NIL	NIL	NIL	NIL
	10 days	NIL	NIL	NIL	NIL
	15 days	NIL	NIL	NIL	NIL

*Row wise Mean bearing subscript indicates significant and non-significant difference at ($p < 0.05\%$). Column wise mean bearing superscript indicates significant and non-significant difference at ($p < 0.05\%$).

The paneer samples were also checked for contaminants like *E. coli* and *S. aureus*. Table 3 shows the average *E. coli* and *S. aureus* counts. *E. coli* was absent in T2 and T3 throughout the storage period. In control and T1, *E. coli* was not found till 10th day of storage. However, the average (\log_{10} cfu/g) of 2.98±0.33 and 2.96±0.22 was observed in control and T1 respectively on 15th day of storage. All the samples were negative for *S. aureus* throughout the storage period. Oladipo and Jadesimi (2012) reported that garlic extract inhibits growth of bacterial strains like *E. coli*, *S. aureus* and *Salmonella* spp. The freshly prepared control and garlic extract treated paneer were evaluated organoleptically by the panel of judges on day zero and subsequent intervals of 5 days using nine point descriptive scale. Though T3 showed best results for microbial inhibition, but it had a strong garlic flavour which was not acceptable for most of the judges. A putrid smell was observed in control and T1 at day 5, indicating spoilage. Paneer treated with 50% garlic extract (T2) was best in terms of sensory evaluation (appearance, juiciness, flavour and texture) by the panel of judges. Also, the results for microbial inhibition by T2 were good. Therefore, T2 was considered the best among all samples.

The sensory scores of T2 are highlighted in Table 4 which showed a decreasing trend with advancement of storage. No significant difference was noted in the sensory scores of T2 on day 0, day 5 and day 10. Sensory

evaluation was not done on 15th day as the product was spoiled. The scores for all the samples were in line with Sanyal *et al.* (2006) and Singh *et al.* (2014) for paneer.

Table 4: Sensory scores for T2 during entire storage period

Parameters	Day 0	Day 5	Day10
Appearance	9.00±0.01 ^a	8.66±0.21 ^a	7.33±0.21 ^b
Juiciness	8.16±0.16 ^a	7.66±0.21 ^a	6.66±0.21 ^b
Flavour	8.50±0.22 ^a	7.66±0.21 ^a	7.16±0.30 ^b
Texture	9.00±0.10 ^a	8.40±0.22 ^a	7.16±0.30 ^b
Overall acceptability	8.66±0.08 ^a	8.09±0.12 ^a	7.08±0.15 ^b

Column wise mean bearing superscript indicates significant and non-significant difference at ($p < 0.05\%$).

Conclusion

The paneer samples were stored at refrigeration temperature and were monitored for changes in respect of pH, TBARS value, tyrosine content, and sensory evaluation at an interval of 5 days. They were also checked for microbial changes (TVC, psychrophilic count and yeast and mould count) at same interval. Paneer samples were also checked for contaminants like *E. coli* and *S. aureus* during the entire storage period. Though T3 showed best results for microbial inhibition, but it had a strong garlic flavour which was not acceptable for most of the judges. A putrid smell was observed in control and T1 at day 5, indicating spoilage. Paneer treated with 50% garlic extract (T2) was considered best in terms of sensory evaluation (appearance, juiciness, flavour and texture) by the panel of judges. Also, the results for microbial inhibition by T2 were good. Therefore, T2 was considered the best among all samples.

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