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Effect of addition of clove essential oil on the storage stability of paneer

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Abstract

Paneer is a very popular soft cheese like an indigenous coagulated milk product, but has very limited shelf like other indigenous dairy products. Thus, a study was conducted to extend the shelf life of paneer with the addition of natural antimicrobial agent i.e. clove essential oil. The stored paneer samples showed highly significantly ($P < 0.01$) decrease in moisture and increase in titratable acidity, free fat content and tyrosine content, but within normal extent. The microbiological count of control samples increased highly significant ($P < 0.01$) throughout the storage period, but within the BIS specifications, while the standard plate count of paneer samples treated with clove decreased highly significantly ($P < 0.01$). There was no major perceivable defect observed in stored samples, except control, but the decreased in flavour score to less than 6.0 during storage limited their shelf life. On decreased flavor score basis, control paneer samples exhibited shelf life of only 5 days, while clove treated samples showed shelf life of 10 days when stored at $8 \pm 1^\circ\text{C}$.

Keywords: paneer, plant essential oil, shelf life, clove oil, storage study

1. Introduction

Indian dairy industry is growing with rapid pace and contributes about 17-18% of world's total milk production with an about 155.5 MT production in 2015-16 (Anonymous, 2017) [2]. For this, buffalo and cow milk contributes approx 55% and 40.5%, respectively, of total milk production which reflects good potential and availability of milk for the preparation of milk products in India. Paneer, a popular indigenous coagulated milk product, contains entire milk casein, part of denatured whey proteins, almost all fat, colloidal salts and soluble milk solids in proportion to the moisture content retained. Paneer is mainly known for its typical mild acidic flavour with slightly sweet taste. It has a firm, cohesive and spongy body and a close knit smooth texture, but like other indigenous dairy products, it is a highly perishable product. Thus, it suffers from limited shelf-life, largely because of its high moisture content (approx. 55%), of even one day when stored at higher temperatures, particularly during summer months. Its shelf-life is reported to be only 6 days under refrigeration (10°C), though its freshness is lost within three days (Bhattacharya *et al.*, 1971) [7] thus, paneer must be consumed fresh. This is the major handicap in the growth of industrial adoption of paneer production. It is, therefore, very much essential to find out suitable means of extending the shelf-life of paneer.

Academia and industry are already focusing towards storage stability of food products and have applied various processing procedures like thermal processing, freezing, drying, concentration and irradiation for controlling the microbial, chemical and physical deteriorations in the foods. However, other novel and natural procedures also need to be employed because of a variety of constraints associated with certain types of foods like use of natural food additives having antimicrobial compounds to extend the shelf-life of unprocessed or processed foods mainly by reducing the microbial growth rate or viability (Beuchat and Golden, 1989; Deans and Ritchie, 1987; Kim *et al.*, 2001) [6, 10, 19]. Plant essential (volatile) oils from medicinal and herbal plants are edible and safe to use as flavouring agents in foods since the initial ages. Essential oils along with their constituents have been extensively explored and well established for their wide spectra of antimicrobial action (Kim *et al.*, 1995a; Packiyasothy and Kyle, 2002; Alzoreky and Nakahara, 2002) [20, 26, 1], thus can be a good additive for shelf life extension of foods. The composition, structure as well as functional groups affects the antimicrobial spectra of plant essential oils (Deans *et al.*, 1995; Dorman and Deans, 2000) [11, 12]. Plant essential oils usually have several active compounds like eugenol (allspice, clove bud and leaf, bay, and cinnamon leaf), cinnamamic aldehyde (cinnamon bark, cassia oil) and citral

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which are having strong antimicrobials activity (Lis-Balchin *et al.*, 1998b; Davidson and Naidu 2000) [23, 9]. Thus, plant essential oils could also be good alternatives to heat and chemical preservatives in the field of food and dairy science, which is still unexplored in food processing practically. Further, to the best of our knowledge, there is zilch regarding such type of studies in the literature concerning the use of essential oil for direct application in paneer. Hence, keeping all these points in mind present study was planned to enhance the shelf life of paneer employing natural antimicrobial system.

2. Materials and Methods

2.1 Materials

Pasteurized full fat buffalo milk (6% fat and 9% SNF) was procured from Milk Parlour of the Vita Milk Plant, Jind and other chemicals and reagents of analytical grade were procured from S. D. Fine Chem. Ltd., Mumbai. Food grade clove plant essential oils were gifted by SABRI ITTAR 1/6, Jamal Mainsion Nowroji Hill Road No 1, Dongri. Mumbai-400009 (INDIA) and packaging materials were procured from Hitkari packaging Company, Parmanoo, Himachal Pradesh, India.

2.2 Preparation of paneer

In present study, paneer samples were prepared by following the standard method of Bhattacharya *et al.* (1971) [7] with slight modification. The vat and wooden stirrer were thoroughly cleaned and sterilized. Then filtered/ clarified and standardized (6% fat and 9% SNF) milk was taken in the vat. Occasional stirring was done during heating of milk, in order to prevent skin formation. The temperature of milk was raised to 80°C and maintained for 30 min followed by cooling to 70°C, and then coagulant (1% citric acid solution heated up to 70°C) @ 0.25% of milk was added in a thin continuous stream till complete coagulation was achieved as evidenced from the clarity of whey (greenish white tinge). The speed of stirring of milk during addition of the coagulant solution was maintained at 30-40 motions of the stirrer per min. The time taken for addition of the coagulant was approximately 60-80 sec. The pH of whey ranged from 5.6 to 5.5. Before draining the whey, the contents of the vat were left undisturbed for 15 min. The coagulated mass was then collected in muslin cloth. Pressure was applied on the top of the coagulant mass by placing weight of app. 2 kg for about 15 min. The pressed paneer was removed from the muslin cloth, and cut into 2-3" size pieces, which were then immersed in chilled water (4°C) containing 0.4% clove essential oil for 2-3 h. The pieces of chilled paneer were then removed from water and placed on wooden planks for about 10-15 min to allow loose water to drain.

2.3 Packaging and storage of paneer

LDPE pouch were used for packaging and storage of paneer samples. Immediately before packaging of paneer samples, the empty packages were sterilized under UV-light for 30 min and after that paneer samples were packed in these packaging materials under hygienic atmospheres (air) by using vertical heat sealing machine. The paneer blocks of 50 gm each were packed in different packaging material and stored at $8 \pm 1^\circ\text{C}$ and evaluated for storage studies.

2.4 Sampling

The fresh and stored ($8 \pm 1^\circ\text{C}$) paneer samples were tempered

at 15.5°C for 1 h before analysis. About 20 gm Paneer from different portions of the entire mass was taken with a trier and pooled together. It was then passed through grater and transferred to screw cap sample bottles for analysis.

2.5 Physico-chemical analysis

The moisture content of the paneer samples was determined by the method as outlined in IS: 10484-1983. For estimating the fat content of paneer samples by using cheese butyrometer, the method prescribed in IS: 1977: 1224 (Part II) [17] was adopted. The protein content of paneer was determined by semi-micro Kjeldahl method described by Manefee and Overman (1940) [24] using Kjeltac digestion and distillation equipment (2300, Kjeltac Analyzer, FOSS) followed by manual distillation and titration method. Lactose content of samples was determined as per method described by Lawrence (1968) [22]. The ash content of paneer sample was determined gravimetrically as per the method of BIS (2001a) [8]. Titratable acidity of paneer samples was determined by following the method prescribed for cheese by the AOAC (1975) [3]. The fat breakdown in paneer samples was determined by estimating free fatty acid (FFA) as % oleic acid adopting the procedure of Thomas *et al.* (1954) [39]. Proteolytic changes in terms of tyrosine content in stored paneer samples were estimated by the procedure suggested by Hull (1947) [16]. The microbiological quality of freshly prepared and stored paneer samples were analysed according to the standard methods i.e. total plate count (Rai, 2004) [31], yeast and mould counts and coliform counts (APHA, 1978) [4].

2.6 Sensory evaluation of paneer

The fresh/stored paneer samples were evaluated organoleptically by a panel of six trained judges for appearance, flavour, body & texture and overall acceptability. The test samples of paneer stored at $8 \pm 1^\circ\text{C}$ were tempered at 15.5°C for 1 h before presenting to the judges under code numbers. The samples were evaluated by judges using a 9-point Hedonic Scale. The judges were also asked to note their observations on score cards.

2.7 Statistical analysis

The data obtained were subjected to analysis of variance (i.e. one way anova and two way anova without interaction) and employing appropriate computer packages under the guidance of a statistician. Wherever required, the overall mean and standard deviation of the compositional data was also calculated.

3. Results and Discussion

3.1 Chemical composition

The proximate composition of paneer, i.e. $52.19 \pm 0.29\%$ moisture, $26.22 \pm 0.17\%$ fat, $17.09 \pm 0.11\%$ protein, $2.72 \pm 0.39\%$ lactose and $1.78 \pm 0.05\%$ ash content, in our study was similar to what has been reported earlier by Bhattacharya *et al.* (1971) [7], Pal and Garg (1989) [27], Rai (2004) [31] and Srivastava (2004) [38].

3.2 Effect of clove essential oil on physico-chemical properties

3.2.1 Changes in moisture content

There was highly significant ($P < 0.01$) decrease in moisture content of control sample with the increase in storage period from an initial 52.221 to 51.612 in 5 days, but with slight extent i.e. within 1% throughout the storage periods. While on

the other hand, clove treated paneer sample also exhibited highly significant ($P < 0.01$) decrease in moisture content, but with slower extent as compared to control paneer samples. It was observed that the moisture content of paneer samples treated with clove decreased from an initial 52.221 to 51.021 in 10 days (Table 1 and Figure 1A). Similar trend of decrease in moisture content of paneer during storage were also observed earlier by Rao *et al.*, 1984^[32], Mistry *et al.*, 1990^[25], Pal *et al.*, 1993^[28] and Pal, 1998^[29]. The gradual decrease in moisture content of paneer during storage was mostly due to expulsion of moisture from the product to surrounding, but with slower rate that might be mostly due to oily layer formed on the surface of paneer samples which act as a barrier for moisture expulsion.

3.2.2 Changes in titratable acidity (% LA)

There was highly significant ($P < 0.01$) increase in titratable acidity of all the samples with the increase in storage period. Individually, the titratable acidity of control sample and clove essential oil treated samples increased from an initial 0.16 to 0.43 in 5 days and from an initial 0.16 to 0.31 in 10 days, respectively (Table 1 and Figure 1B). The increase in titratable acidity of clove treated sample was slower than the control sample, might be due to antimicrobial action of clove plant essential oil. Similar results were obtained by Pal *et al.* (1993)^[28] during storage of wax coated fresh paneer. The increase in titratable acidity of control paneer sample is a natural process and with higher extent. The increase in titratable acidity of paneer during storage was also reported earlier by various research workers (Bhattacharya *et al.*, 1971, Shukla *et al.*, 1984, Pal and Garg, 1989 and Mistry *et al.*, 1990)^[7, 35, 27, 25].

3.2.3 Changes in free fatty acid (%)

There was highly significantly ($P < 0.01$) increase in free fatty acid of all the samples with the increase in storage period. The free fatty acid of control sample and clove treated sample increased from an initial 0.175 to 0.541 in 5 days and to 0.409 in 10 days of storage, respectively, (Table 1 and Figure 1C). The increase in free fatty acid content was mostly due to lipolytic action. The increase in free fatty acid of paneer samples was similar to earlier reported value of Sindhu *et al.* (2000)^[36], Kumar (1989)^[21] and Rai *et al.* (2008)^[30], but it was within desirable range. The free fatty acid of clove treated paneer samples increased with slower rate as compared to control samples and also lower than earlier reported values by Sindhu *et al.* (2000)^[36], Kumar (1989)^[21] and Rai (2008)^[30], that might be mostly due to antimicrobial action of clove essential oil.

3.2.4 Changes in tyrosine content

There was highly significantly ($P < 0.01$) increase in tyrosine content of all the samples with the increase in storage period. The tyrosine content of control and clove treated sample increased from an initial 12.192 to 39.336 in 5 days and to 30.549 in 10 days of storage, respectively, (Table 1 and Figure 1D). The increase in tyrosine content of clove treated sample was lesser as compared to control sample and to the earlier reported values by Pal *et al.* (1993)^[28] and Pal (1998)^[29], mostly due to antimicrobial action of clove plant essential oils which results in lesser proteolysis, while the tyrosine content of control sample was increased steeply with the increase in storage period mostly due to more proteolysis action in stored control product. Pal *et al.* (1993)^[28] and Pal (1998)^[29] also observed similar trends of increase in tyrosine content of fresh paneer sample when stored at $8 \pm 1^\circ\text{C}$.

Table 1: Effect of storage (at $8 \pm 1^\circ\text{C}$) on physico-chemical and microbiological attributes of control and clove treated paneer samples

Characteristics	Treatment	Storage Days					P-value
		0	4	5	8	10	
Moisture (%)	Control	52.221±0.002	51.708±0.001	51.613±0.001	-	-	4.67E-15**
	Clove Treated	52.221±0.002	51.931±0.001	-	51.626±0.001	51.021±0.001	1.3E-21**
Titratable acidity (%LA)	Control	0.16±0.01	0.25±0.01	0.43±0.01	-	-	5.46E-09**
	Clove Treated	0.16±0.01	0.17±0.01	-	0.23±0.01	0.31±0.01	3.6E-90**
Free fatty acid (%)	Control	0.175±0.003	0.417±0.003	0.542±0.005	-	-	8.86E-16**
	Clove Treated	0.176±0.001	0.262±0.001	-	0.307±0.001	0.409±0.001	2.43E-18**
Tyrosine content (mg/100g)	Control	12.194±0.01	29.856±0.01	39.337±0.02	-	-	2.77E-24**
	Clove Treated	12.193±0.002	18.177±0.002	-	27.609±0.001	30.552±0.003	1.25E-29**
SPC (CFU/g)	Control	3.90±0.01	4.34±0.01	4.39±0.01	-	-	1.01E-10**
	Clove Treated	3.36±0.01	3.21±0.01	-	3.33±0.01	3.32±0.01	9.76E-07**

n= 3 (mean ± S.D), ** $P < 0.01$ (Highly significant)

3.2.5 Changes in microbiological quality

There was highly significant ($P < 0.01$) variation in standard plate count of all the samples with the increase in storage period. The standard plate count (log cfu/g) of control sample increased from an initial 3.90 to 4.39 in 5 days, while clove treated samples exhibited highly significant ($P < 0.01$) decrease in standard plate count (log cfu/g) from initial 3.36 to 3.32 in 10 days (Table 1 and Figure 1E). The standard plate count of control sample was increased steeply with the increase in storage period mostly due to more availability of favorable condition for the growth of a wide variety of organisms in stored product (Gupta, 1985 and Ghodekar, 1992)^[14]. Pal *et*

al. (1993)^[28] and Pal (1998)^[29] also observed similar trends of increase in standard plate count of fresh paneer sample when stored at $8 \pm 1^\circ\text{C}$. On the other side, the decreased in standard plate count of clove treated paneer samples was mostly due to antimicrobial action of eugenol, an antimicrobial agent, of clove plant's essential oil (Helander *et al.*, 1998 and Wendakoon and Sakaguchi, 1995)^[15, 40], similar results were observed earlier by Kumar (1989)^[21], Singh *et al.* (1989)^[37] and Sachdeva and Singh (1990b)^[33] with using different antimicrobial agents during storage of paneer. The decrease in standard plate count of paneer samples during storage treated with different antimicrobial agents were also

reported earlier by various research workers (Kumar, 1989, Singh *et al.*, 1989 and Sachdeva and Singh, 1990b) [21, 37, 33].

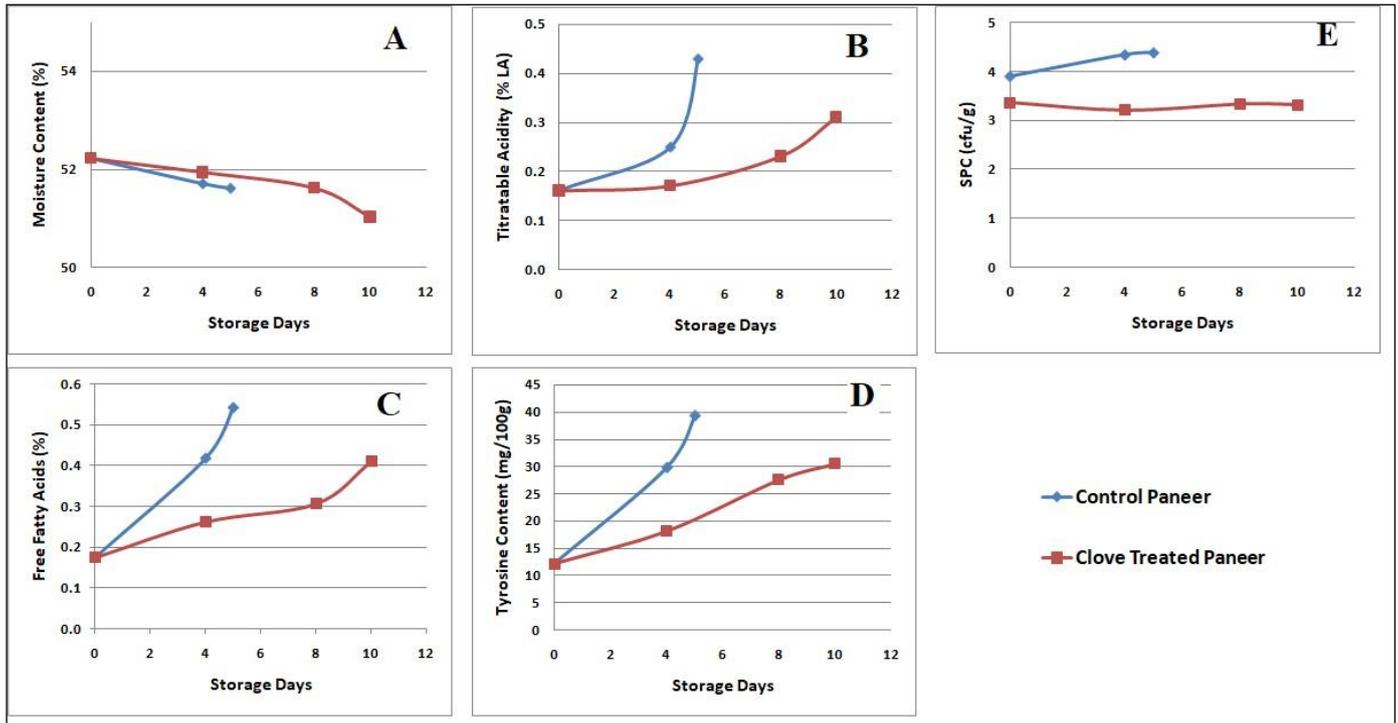


Fig 1: Effect of storage on physico-chemical (A: moisture content; B: titratable acidity; C: free fatty acid; D: tyrosine content) and microbiological quality (E: SPC) of control and clove treated paneer samples

3.2.6 Changes in appearance score

There was slight decrease in appearance of stored paneer samples with the increase in storage period. The appearance of the samples was towards yellowish tint at the end of storage period, but within desirable range. The appearance of control and clove treated samples decreased highly significantly ($P < 0.01$) from an initial 8.5 to 6.0 in 5 days and 8.0 to 7.0 in 10 days, respectively, (Table 2 and Figure 2A). Similar results were also observed earlier by various research workers (Bambha, 1998; Sachdeva, 1983 and Rai, 2004) [5, 34, 31] during storage of paneer samples. The decrease in appearance score of clove treated paneer sample was comparatively with slower extent as compared to control sample that could be mostly due to antimicrobial action of clove plant essential oil that inhibit other biochemical reaction resultant in deterioration of product.

3.2.7 Changes in flavour score

There was gradual decrease in flavour score of paneer samples during storage. The flavour score of control and clove treated samples decreased highly significantly ($P < 0.01$) from an initial 7.5 to 6.5 in 5 days and to 7 in 10 days, respectively, (Table 2 and Figure 2B).

3.2.8 Changes in body and texture

The body and texture of clove treated sample was within desirable range, but control samples showed slightly rough body and texture. There was highly significantly ($P < 0.01$) decreased in body and texture score of control samples from 8.0 to 7.0 in 5 days, while only significant ($P < 0.05$) decreased in clove treated samples from an initial 8.0 to 7.5 in 10 days with the increase in storage period (Table 2 and Figure 2C).

Table 2: Effect of storage (at $8 \pm 1^\circ\text{C}$) on sensory attributes of control and clove treated paneer samples

Characteristics	Treatment	Storage Periods (Days)					P-value
		0	4	5	8	10	
Appearance	Control	8.55±0.34	7±0.32	6±0.32	-	-	3.19E-09**
	Clove Treated	8±0.1	8±0.1	-	7±0.316	6±0.316	1.34E-12**
Flavour	Control	7.65±0.21	6.92±0.20	6.02±0.16	-	-	1.15E-09**
	Clove Treated	7.33±0.26	7.33±0.26	-	7±0.31	6±0.32	1.93E-07**
Body and Texture	Control	8±0.32	7±0.32	6.92±0.21	-	-	1.05E-05**
	Clove Treated	7.83±0.26	7.83±0.26	-	7.83±0.26	7.42±0.20	0.17*
Overall Acceptability	Control	8±0.32	6.92±0.38	6.05±0.24	-	-	8.2E-08**
	Clove Treated	7.83±0.26	7.5±0.45	-	7±0.32	7±0.45	0.002**

n = 3 (mean ± S.D), ** $P < 0.01$ (Highly significant), * $P < 0.05$ (Significant).

3.2.9 Changes in overall acceptability

There was highly significantly ($P < 0.01$) decrease in overall acceptability with the increase in storage period. The overall acceptability of both the samples was within desirable range, but slightly poorer for control samples as compared to treated

sample. Clove treated sample fetched natural look throughout storage period. The overall acceptability score of control and clove treated sample decreased highly significantly ($P < 0.01$) from an initial 8.5 to 7.0 in 5 days and 8.0 to 7.0 in 10 days, respectively, (Table 2 and Figure 2D).

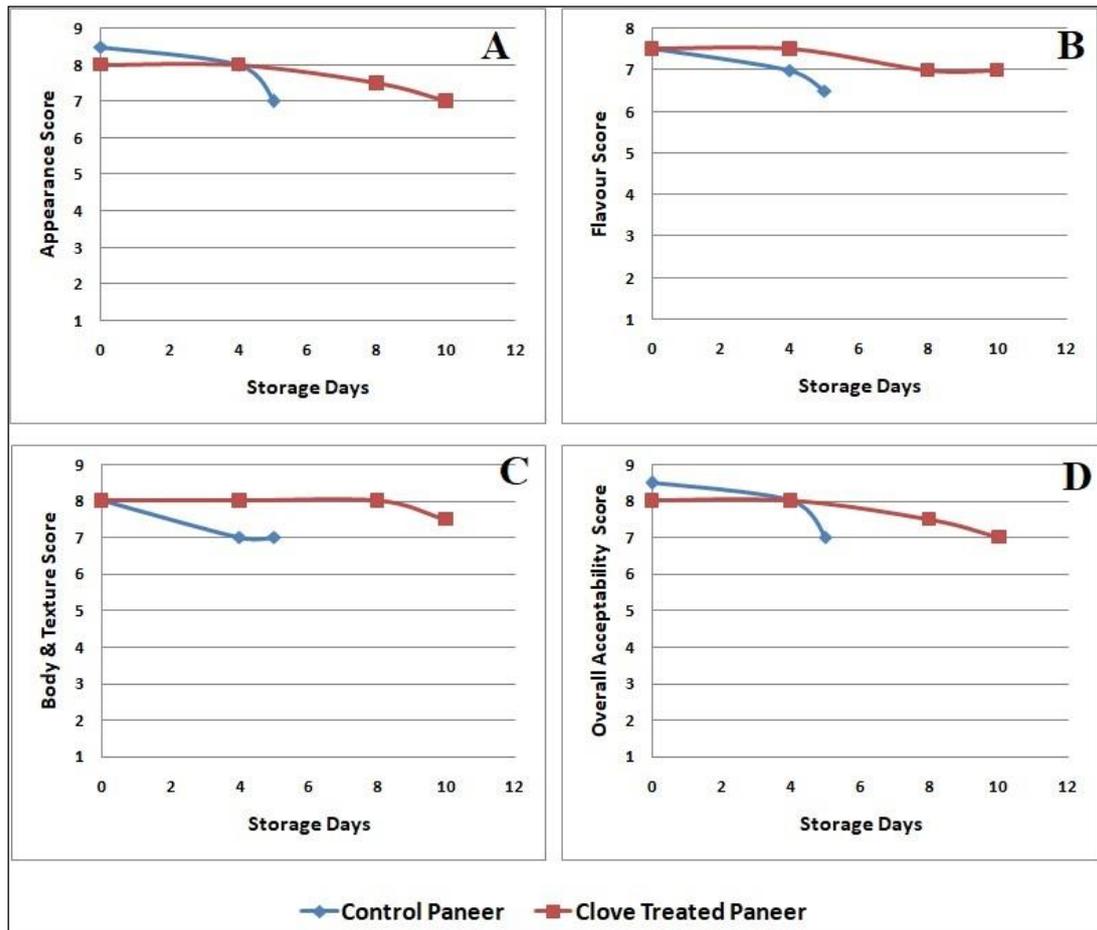


Fig 2: Effect of storage on organoleptic quality (A; appearance, B: flavour score; C: body and texture; D: overall acceptability) of control and clove treated paneer samples

4. Conclusion

There was no major perceivable defect observed in stored samples, except control, but the decrease in flavour score to less than 6.0 during storage limited their shelf life. On decreased flavour basis, control paneer sample showed shelf life of only 5 days when packed in LDPE, while clove treated sample exhibited shelf life of 10 days in LDPE at $8\pm 1^\circ\text{C}$. Hence, addition of clove essential oil increased the shelf life of paneer two times. Thus, it can be concluded that clove essential oil can be a good natural additive as an antimicrobial agent in real food environment especially for paneer and it could be helpful to overcome the problem of limited shelf life of paneer.

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