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Saurabh Karunamay

M.V.Sc Scholar, Department of Livestock Products Technology, Mumbai Veterinary College, MAFSU, Mumbai, Maharashtra, India

Shekhar R Badhe

Assistant Professor, Department of Livestock Products Technology, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra, India

Vivek Shulka

Assistant Professor, Department of Livestock Products Technology, Mumbai Veterinary College, MAFSU, Mumbai, Maharashtra, India

Swati Jaiswal

M.V.Sc Scholar, Department of Animal Nutrition, Mumbai Veterinary College, MAFSU, Mumbai, Maharashtra, India

Corresponding Author: Shekhar R Badhe Assistant Professor, Department of Livestock Products Technology, Nagpur Veterinary College, MAFSU, Nagpur, Maharashtra, India

Effect of essential oil of clove and oregano treated edible packaging film in extending the shelf life of paneer

Saurabh Karunamay, Shekhar R Badhe, Vivek Shulka and Swati Jaiswal

Abstract

Paneer is a very popular coagulated milk product but like other dairy products it is very perishable. Thus, a study was conducted to extend the shelf life of paneer packed with clove and oregano essential oil treated starch and carboxymethyl cellulose (CMC) based edible packaging film. Starch and CMC based edible packaging film was prepared by adding starch (5 g), carboxymethyl cellulose (15% W/W starch) and glycerol (40 ml/100 g starch) in distilled water (100 mL) and then clove and oregano essential oil was incorporated before drying. Control paneer sample and paneer samples packed in clove and oregano essential oil treated edible packaging film showed significant (p<0.05) decrease in physico-chemical, microbiological and overall sensory properties throughout the storage period but under desirable limit. Control paneer samples packed in clove and oregano essential oil treated edible packaging film was found undesirable on 10th day of sensory evaluation but found microbiologically fine at 12th day of storage. The results revealed that clove and oregano essential oil treated edible packaging film extended the shelf life of paneer at least by 4 days as compared to control that was found microbiologically unfit for consumption on 9th of storage at 4±1 °C.

Keywords: Paneer, Clove oil, Oregano oil, Starch, CMC, Shelf-life

1. Introduction

India contributes about 18.81% of global milk production. Milk production in the country is 176.35 million tonnes during 2017-18 and 187.70 million tonnes during 2018-19, (Statistics, B. A. H. (2020) ^[34]. It reflects that India has a good potential and availability of milk for the preparation of milk products. Curdled dairy products like paneer, chenna, sandesh etc., are very much liked by Indian population. But paneer is most popular curdled dairy product in India. Paneer refers as a dairy product obtained from the cow or buffalo milk or a combination there of by coagulation with sour milk, lactic acid or citric acid. It should not contain more than 70.0 per cent moisture and milk fat less than 50.0 per cent of the dry matter (FSSAI, 2011) ^[13]. Paneer shows a shelf- life of 5-6 days under refrigeration temperature but its freshness is lost within 3 days (Bhattacharya et al., 1971)^[7]. Many modern packaging methods like retort packaging, vacuum packaging etc., have been developed to increase the shelf life of paneer but plastic and other non-biodegradable packaging materials used in dairy industry are creating threat to biodiversity. In order to solve the problem this research has been carried out to use natural antimicrobial compounds like clove and oregano essential oil in edible packaging film to preserve paneer and increase its shelf life. Thus, an eco-friendly, biodegradable and essential oil treated starch and carboxylmethyl cellulose based edible packaging film was developed. Clove essential oil (CEO) has eugenol and oregano essential oil (OEO) has carvacrol and thymol as an active compound. These both essential oils have been identified for outstanding antioxidant and antimicrobial activities against a wide range of food spoilage microorganisms. Clove oil also shows plasticizing effect (Echeverría et al., 2016) [11]. FDA also granted both clove and oregano essential oils as generally recognized as safe (GRAS) status. The GRAS status of theses essential oil gave an opportunity to the researchers to incorporate clove and oregano essential oils in edible packaging materials. The objective of present research work was to study the efficacy of starch and carboxymethyl cellulose base edible packaging film, incorporated with clove and oregano essential oil in extending the shelf life of paneer stored at refrigeration temperature (4 ± 1 °C).

2 Materials and Methods

2.1 Preparation of paneer

Buffalo milk was procured from cattle and buffalo farm of Mumbai Veterinary College and used throughout the experiments. Milk was standardized to Fat 6% and S.N.F 9% for most favorable characteristics of the product. Citric acid of strength 1% was used to coagulate the milk, for the preparation of paneer. In present study, paneer samples were prepared with slight modification as by the method suggested by Lamdande *et al.*, $(2012)^{[22]}$.

2.2 Preparation of edible film treated with essential oil

Edible packaging film was prepared by adding starch (5 g), carboxylmethyl cellulose (15% W/W starch) and glycerol (40 ml/100 g starch) in 100 mL distilled water with slight modification as suggested by Ghanbarzadeh *et al.*, 2011 ^[14]. The suspension of edible film was then agitated at 90 °C and stirred by magnetic stirrer at 500 rpm for 30 min. The suspension of edible packaging film was cooled for 20 minutes and then 0.5% clove and 0.5% oregano essential oil was added as per the volume of solution in two different samples. The suspension was then dried at 60 °C until the film is obtained.

2.3 Packaging and storage of paneer

Control, treatment one (T1) and treatment two (T2) samples of 20 grams each were prepared. Control sample contained freshly prepared paneer packed in LDPE bag and stored at 4 ± 1 °C. T1 sample contained freshly prepared paneer wrapped in edible packaging film treated with 0.5% clove essential oil and then packed in LDPE bag and stored at 4 ± 1 °C. T2 sample contained freshly prepared paneer wrapped in edible packaging film treated with 0.5% oregano essential oil and then packed in LDPE bag and stored at 4 ± 1 °C.

2.4 Sampling

Control, T1 and T2 paneer samples were analyzed for physico-chemical, microbiological and sensory attributes at 3 days interval till spoilage. All the paneer samples were kept at 15 °C for 1 hour before analysis. Parameters analyzed for paneer samples were evaluated in the laboratory of Department of Livestock Products Technology and Department of Animal Nutrition, Mumbai Veterinary College, Mumbai.

2.5 Physico-chemical analysis

The moisture content of the freshly prepared paneer sample was analyzed by the method in IS: 10484-1983. For fat estimating of freshly prepared paneer sample, cheese butyrometer was used as per the method prescribed in IS: 1977:1224. The protein content of paneer prepared was determined by Kjeldahl method as described by Manefee and Overman (1940)^[26]. Control, T1 and T2 paneer samples were analyzed for pH, tyrosine value, thiobarbituric acid (TBA) value and titratable acidity. pH was analyzed in digital meter (Model-HI 99163, HANNA) as determined by the method in Trout et al., (1992) ^[36]. Absorbance (A) at 730 nm was determined for tyrosine value using spectrophotometer as described by Strange et al., (1977) [35] with slight modifications. Absorbance (A) at 530nm was measured for TBA value in a spectrophotometer (Model no. EQ 820 with wavelength range of 350-950 nm, INDIA) as described by Witte et al., (1970) [38] with little modification. Titratable acidity of the control, T1 and T2 samples were determined as per AOAC (1995) [2].

2.6 Microbiological counts

Total plate count, psychrophilic count, yeast and mold and coliform count of paneer samples were estimated by the standard methods of APHA (1992)^[4].

2.7 Sensory evaluation of paneer

The sensory evaluations of paneer samples were conducted by 6 judges of semi-trained panel and it was repeated at the interval of 5 days. The samples were judged for various sensory parameters using nine point Hedonic scales as described by (Keeton, 1983)^[18].

2.8 Statistical analysis

The data was recorded in triplicate (n=3) during the experiment and were analyzed by analysis of variance (ANOVA) using complete randomized block design (CRBD) following standard procedure as per Snedecor and Cochran, 1989^[32].

3. Results and Discussion

3.1 Physico-chemical parameters

The paneer samples was analyzed for physico-chemical parameters like pH, tyrosine value, TBA value and titratable acidity at 0^{th} , 3^{rd} , 6^{th} , 9^{th} , 12^{th} and 15^{th} day of storage (Table1).

3.1.1 Chemical composition of paneer

Freshly prepared paneer sample was analysis for proximate composition (Fig. 1). The value of Moisture content of paneer was $51.67\pm1.42\%$, fat content was 24.43 ± 0.8 and protein content was 17.70 ± 0.49 . The result for moisture content of paneer was found similar by Kumar *et al.*, (2008) ^[20] for paneer. Desale *et al.*, (2009) ^[9] stated that the fat% of paneer ranges from 16-28%. The protein % of paneer ranges from 15.06 to 20.33% (Srivastava 2004) ^[33].

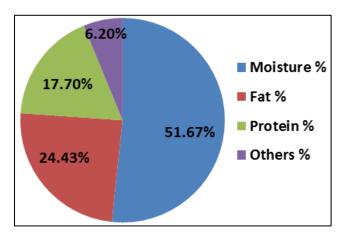


Fig.1 Proximate analysis of paneer sample

3.1.2 Change in pH

Control sample showed non-significant change till 9th day of storage but a significant (p<0.05) change was observed on 12th day. The initial pH values were non-significant till 6th day for control, T1 and T2. Significant (p<0.05) difference among control, T1 and T2 was observed on day 9th to day 15th of storage. Phenolic compound in clove and oregano essential oil helps in maintaining the pH of paneer during entire storage than that of control paneer sample. Similar results were reported by Shan *et al.*, (2011) ^[30] and Makhal *et al.*, (2014) ^[25] for thymol added cottage cheese.

Table 1: Physico-chemical parameters of control, T1 and T2 paneer samples during storage period

Parameters	рН			Tyrosine		
Day	С	T1	T2	С	T1	T2
0 th Day	a5.85±0.03 ^A	$a5.85 \pm 0.03^{A}$	$_{a}5.87{\pm}0.02^{A}$	a12.52±0.06 ^A	_a 12.47±0.01 ^A	_a 12.45±0.01 ^A
3 rd Day	$a5.83 \pm 0.04^{A}$	a5.75±0.01 ^A	a5.77±0.02 ^A	b15.38±0.05 ^A	ь15.88±0.0 ^в	ь15.84±0.04 ^С
6 th Day	_a 5.77±0.02 ^A	_a 5.74±0.03 ^A	_a 5.75±0.03 ^A	c19.78±0.05 ^C	c17.90±0.04 ^B	c17.08±0.01 ^A
9 th Day	a5.76±0.03 ^A	ь5.65±0.02 ^в	_a 5.69±0.01 ^A	d31.14±0.02 ^C	d27.35±0.10 ^B	d25.16±0.02 ^A
12 th Day	_b 5.56±0.01 ^B	_b 5.64±0.03 ^B	_a 5.71±0.04 ^A	_e 40.53±0.03 ^C	e31.51±0.13 ^B	e30.20±0.05 ^A
15 th Day	b5.55±0.03 ^A	c5.63±0.04 ^B	ь5.69±0.03 ^С	f47.54±0.25 ^C	f37.66±0.08 ^B	f35.65±0.22 ^A
Parameters		TBA			Titratable acidity	
Day	С	T1	T2	С	T1	T2
0 th Day	_a 0.22±0.01 ^A	_a 0.22±0.02 ^A	_a 0.23±0.02 ^A	_a 0.29±0.01 ^A	_a 0.29±0.01 ^A	_a 0.30±0.01 ^A
3 rd Day	_b 0.36±0.01 ^B	b0.31±0.02 ^A	b0.26±0.02 ^A	b0.43±0.01 ^A	_b 0.41±0.01 ^B	ь0.40±0.02 ^в
6 th Day	c0.48±0.01 ^B	c0.36±0.02 ^A	c0.32±0.06 ^A	c0.55±0.02 ^B	c0.49±0.02 ^A	c0.49±0.02 ^A
9 th Day	d0.68±0.02 ^C	d0.61±0.02 ^B	d0.55±0.02 ^A	d0.62±0.02 ^B	d0.55±0.02 ^A	d0.53±0.01 ^A
12 th Day	e0.93±0.02 ^C	e0.87±0.03 ^B	e0.74±0.03 ^A	e0.74±0.01 ^C	e0.63±0.02 ^B	e0.59±0.01 ^A
15 th Day	f1.16±0.06 ^C	f0.94±0.07 ^B	$_{\rm f}0.86{\pm}0.08^{\rm A}$	f0.79±0.02 ^C	f0.70±0.01 ^B	f0.71±0.01 ^A

*Note: Row wise mean bearing subscript and column wise mean bearing superscript indicates significant and nonsignificant difference at (p<0.05%).

3.1.3 Change in tyrosine values

A significant (p<0.05) change was observed in tyrosine value for control throughout the storage period. The average tyrosine values for control, T1 and T2 indicates a nonsignificant difference on 0 day. But a significant (p<0.05) difference was noticed between day 3rd to day 15th of storage. Rai *et al.*, (2008)^[29] found a similar result when analyzed for chemical quality of paneer at 7±1 °C. Singh and Immanuel (2014) ^[31] also reported similar finding of tyrosine value in paneer added with fruits peels. A similar finding was also reported by Yadav and Wadehra (2016) ^[39] for tyrosine value at refrigeration condition for clove added spicy paneer. At the end of storage, the tyrosine value for control was found to be much higher as compared to T1 and T2, which indicates less proteolysis in T1 and T2 paneer samples.

3.1.4 Change in TBA values

A significant (p<0.05) change was observed in TBA value for control sample throughout the storage period. The average TBA values for T1 and T2 shows a non-significant difference from 0th day to 6th day and for control only on 0th day as compared to treatments during storage. A significant (p<0.05) difference was noticed in T1 and T2 from 9th day to 15th day of storage. The value of TBA in paneer sample packed in edible film treated with essential oil of oregano was considerably lower than that of the clove and control paneer sample indicating that the oregano essential oil protected paneer sample more against lipid oxidation followed by clove essential oil. The results were in agreement with Shan *et al.*, (2011) ^[30] for oregano herbs extract added cheese and Chauhan *et al.*, (2015)^[8] for paneer.

3.1.5 Change in titratable acidity

A significant (p<0.05) change was observed in titratable acidity for control sample after 3rd day of storage period as compared to T1 and T2. The average titratable acidity values for T1 and T2 showed a non-significant difference from day 0 to day 9. But a significant (p<0.05) difference was noticed in control, T1 and T2 on 12th day and 15th day of storage. Incorporation of clove and oregano essential oil into edible coating of paneer delayed the acid development during storage, possibly because of antimicrobial activity against the spoilage causing microorganisms. The results were in agreement with Verma and Khan (2009) ^[37] for panner and Yadav *et al.*, (2019) ^[40] for thyme herbs added paneer.

3.2 Microbiological parameters

The paneer samples were subjected to microbiological count subsequently at 0^{th} , 3^{rd} , 6^{th} , 9^{th} , 12^{th} and 15^{th} day of storage (Table 2).

Table 2: Microbiological count (log cfu/g) of control, T1 and T2
paneer sample during storage period.

Parameters	Total Plate Count				
Day	С	T1	T2		
0 th Day	NIL	NIL	NIL		
3 rd Day	_a 1.97±0.09 ^B	a1.27±0.18 ^A	a1.20±0.12 ^A		
6 th Day	b3.30±0.15 ^B	b2.47±0.18 ^A	b2.20±0.12 ^A		
9 th Day	c5.00±0.10 ^C	c3.60±0.12 ^B	c3.40±0.12 ^A		
12 th Day	d5.80±0.12 ^B	d4.77±0.09 ^A	d4.57±0.12 ^A		
15 th Day	e6.77±0.03 ^B	e5.47±0.09 ^A	e5.37±0.09 ^A		
Parameters	P	sychrophilic cou			
Day	С	T1	T2		
0thDay	NIL	NIL	NIL		
3rdDay	NIL	NIL	NIL		
6thDay	_a 4.63±0.01 ^A	NIL	NIL		
9thDay	_b 5.03±0.01 ^C	a3.67±0.02 ^B	$_{a}3.57{\pm}0.02^{A}$		
12thDay	_a 5.18±0.00 ^B	_b 4.84±0.02 ^A	_b 4.81±0.02 ^A		
15thDay	_a 5.20±0.01 ^B	c5.17±0.02 ^B	c5.00±0.01 ^A		
Parameters	Yea	st and Mold Count			
Day	С	T1	T2		
0thDay	NIL	NIL	NIL		
3rdDay	a2.53±0.18	NIL	NIL		
6thDay	b3.28±0.17 ^B	a2.73±0.07 ^A	$a2.53\pm0.07^{A}$		
9thDay	b3.37±0.01 ^C	b3.14±0.01 ^B	b3.06±0.01 ^A		
12thDay	b3.45±0.02 ^C	c3.31±0.02 ^B	b3.18±0.01 ^A		
15thDay	b3.52±0.01 ^C	d3.43±0.02 ^B	c3.31±0.02 ^A		

^{*}Note: Row wise mean bearing subscript and column wise mean bearing superscript indicates significant and non-significant difference at (p<0.05%).

3.2.1 Change in total plate count

A significant (p<0.05) difference was observed on total plate count of control sample throughout the storage but comparing to T1 and T2, control showed significant (p<0.05) change from 3rd day of storage. Total plate count (TPC log₁₀cfu/g) for control, T1 and T2 was nil on zero day. A significant (p<0.05) difference was noticed only on 9th day of storage in T1 and T2 samples. Both the treatment samples were under acceptable limit on 12th day of storage but control sample was unacceptable on 9th day of storage. Perhaps the reduction on total plate count was due to the bactericidal effect of essential oils. A similar result of total plate count for oregano essential oil treated cheese was reported by Olmedo *et al.*, (2013)^[28]. Khatkar *et al.*, (2017)^[19] and Havanur and Adi (2020)^[15] also reported similar result for clove essential oil treated paneer stored at 8 ± 1 °C.

3.2.2 Change in psychrophilic count

Psychrophilic count was nil on 0th and 3rd day of storage for control. A significant (p<0.05) change was observed after 6th day of storage in control sample as compare to T1 and T2. Psychrophillic count of T1 and T2 samples on 0th day to 6th day were nil. A significant (p<0.05) difference was noticed by T1 and T2 on 9th day and 15th day of storage. Gradual increase in psychrophilic count was observed in control followed by T1 and then T2 upon 15th day of storage. Various studies show that clove essentials oils (Badhe and Fairoze 2012) ^[5], Liu *et al*, (2017) ^[23] and oregano essentials oils (Lambert *et al.*, 2001) ^[21] have strong bactericidal effect. Similar result was reported by Makhal *et al.*, (2014) ^[25] that stated the lower growth of psychrotrophs in cottage cheese samples added with essential oils is due to its strong antimicrobial action.

3.2.3 Change in yeast and mold count

On day 0th yeast and mold count was nil for control. A significant (p < 0.05) change was observed in control sample throughout the storage. On day 0th and 3rd, the average yeast and mold count for T1 and T2 were nil. A significant (p < 0.05) difference was observed in control from 3rd day and in T1 and T2 from 6th day to 15th day of storage. Owing the antifungal action, the clove essential oil (Lopez et al., 2005)^[24] and the oregano essential oil (Munhuweyi et al., 2018)^[27] was observed to exhibit a significant inhibitory effect against the growth of yeast and molds during the entire storage period. T2 showed less growth on 15th day of storage than T1 and very less growth as compared to control paneer sample. The results of yeast and mold count were in agreement with Eresam et al., (2015) ^[12] for clove powder incorporated paneer, Badola et al., (2018)^[6] for clove oil treated burfi and Artiga *et al.*, (2017)^[3] for oregano essential oil coated cheese.

3.2.4 Change in coliform count

The coliform count for the entire period of storage was negative. It shows that the sample was not contaminated by any coliform bacteria from any route.

3.3 Sensory evaluation

The paneer samples were subjected to sensory evaluation subsequently at 0^{th} , 5^{th} and 10^{th} day of storage (Table 3).

3.3.1 Changes in appearance score

There was slight decrease in appearance of control, T1 and T2 paneer samples with increase in storage period. Yellowish tint appearance was observed at the end of storage period, but within desirable range. Control paneer sample showed significantly (p<0.05) better appearance score on 0th day of sensory evaluation. The appearance of both T1 and T2 samples decreased significantly (p<0.05) throughout the storage but on 10th day of storage T2 paneer sample showed better significant (p<0.05) values than T1 and control.

3.3.2 Changes in Juiciness score

There was a gradual and significant (p < 0.05) decrease in juiciness of control, T1 and T2 paneer samples. On day 5th control sample showed highly decreased significant of juiciness as compared to T1 and T2 paneer sample. T1 paneer sample shows gradual and significant (p < 0.05) decrease in juiciness from 8.42 to 6.11 in 10 days whereas, T2 paneer sample showed a significant (p < 0.05) decrease of 8.35 to 6.49 from initial day to 10th day of storage.

3.3.3 Changes in Flavour score

There was a gradual and significant (p<0.05) decrease in flavour of control, T1 and T2 paneer sample. On day 5th control sample showed highly decreased significant (p<0.05) of flavour as compared to T1 and T2 paneer sample. T1 paneer sample showed significant (p<0.05) decrease in flavour from 8.43 to 7.52 in 10 days whereas, T2 paneer sample showed a significant (p<0.05) decrease of 8.72 to 7.68 from initial day to 10th day of storage. T2 paneer sample gave a better significant (p<0.05) values than T1 and control paneer sample during entire storage period.

3.3.4 Changes in Texture score

There was a gradual and significant (p<0.05) decrease in texture score of control paneer sample. On day 5th control sample showed highly decreased significant of texture score as compared to T1 and T2 paneer sample. T1 paneer sample showed gradual and significant (p<0.05) decrease in texture score from 8.97 to 7.02 in 10 days. T2 paneer sample also showed a significant (p<0.05) decrease of 8.95 to 7.14 from initial day to 10th day of storage.

Table 3: Sensory scores of control, T1 and T2 paneer samples
during storage period

Day	0 th Day	5 th Day	10 th Day			
Sensory Evaluation						
Appearance						
Control	a8.98±0.02 ^A	ь7.97±0.12 ^A	NC			
T1	$a8.98\pm0.15^{B}$	ь8.43±0.01 ^в	$a7.35\pm0.07^{B}$			
T2	$_{a}8.97{\pm}0.02^{B}$	ь8.60±0.12 ^в	a7.52±0.10 ^A			
Juiciness						
Control	$_{a}8.46\pm0.14^{A}$	ь7.54±0.18 ^A	NC			
T1	$a8.42\pm0.15^{B}$	ь7.61±0.12 ^в	a6.11±0.22 ^B			
T2	_a 8.35±0.10 ^B	_b 7.76±0.15 ^B	_a 6.49±0.14 ^A			
Flavour						
Control	_a 7.95±0.18 ^A	_b 7.32±0.16 ^A	NC			
T1	_a 8.43±0.17 ^B	ь7.66±0.17 ^в	_a 7.52±0.30 ^B			
T2	_a 8.72±0.16 ^B	_b 7.50±0.23 ^B	_a 7.68±0.24 ^A			
Texture						
Control	_a 8.98±0.02 ^A	_b 8.12±0.18 ^A	NC			
T1	$_{a}8.97{\pm}0.02^{B}$	_b 8.42±0.14 ^B	_a 7.02±0.12 ^B			
T2	a8.95±0.03 ^B	ь8.36±0.16 ^в	_a 7.14±0.15 ^A			
Overall Acceptability						
Control	$_{a}8.59\pm0.04^{A}$	_b 7.74±0.08 ^A	NC			
T1	$_{a}8.70\pm0.07^{B}$	ь8.03±0.11 ^в	a7.00±0.08 ^B			
T2	$_{a}8.75{\pm}0.06^{B}$	_b 8.05±0.08 ^B	a7.20±0.08 ^A			

*Note: Row wise mean bearing subscript and column wise mean bearing superscript indicates significant and non-significant difference at (p<0.05%).NC=Not conducted

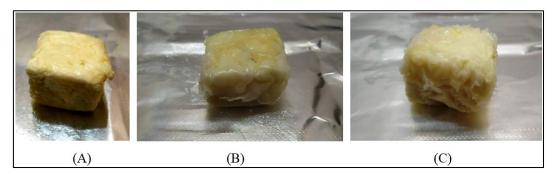


Fig.1 (A) Control paneer sample on day 5th (B) T1 paneer sample on 12th day (C) T2 paneer sample on 12th day

3.3.5 Changes in Overall Acceptability

There was a significant (p < 0.05) decrease in overall acceptability of control paneer sample as compared to T1 and T2. On day 5th control sample showed highly decreased significant (p < 0.05) overall acceptability as compared to T1 and T2 paneer sample. The overall acceptability of both T1 and T2 samples were within desirable range, but slightly poorer for T1 paneer samples as compared to T2 paneer sample. Control sample was found microbiologically unacceptable on 9th day of storage, so the sensory evaluation for control was not done on 10th day. The overall acceptability score for T1 paneer sample decreased significantly (p < 0.05)from an initial 8.70 to 7.00 in 10 days and for T2 it decreases from 8.75 to 7.20 in 10 days. The scores for appearance, flavor, juiciness and texture were affected by the increase acidity and decreasing moisture content due to degradation of reducing sugar that leads to pH drop and alteration of sensory qualities in paneer. Similar finding was reported by Deshmukh et al., (2009)^[10] and Ahmed & Bajwa (2019)^[1].

4. Conclusion

In the present study control paneer sample was found microbiologically and physico-chemically unacceptable on 9th day of storage. Paneer samples packed with edible film treated with 0.5% clove (T1) essential oil and 0.5% oregano (T2) essential oil was microbiologically and physico-chemically accepted till 12^{th} day of storage. Paneer packed in oregano essential oil treated edible film showed more significant and desirable value for consumption than paneer sample packed in edible film treated with essential oil of clove. Further, conclusion can be drawn that starch and carboxymethyl cellulose based edible film can be used as edible packaging material and essential oil of clove and oregano can be used as natural preservative in perishable dairy products.

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