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## Comparative study of essential oil of clove and oregano treated edible film in extending shelf life of paneer

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### Abstract

The present study was conducted to compare the effect of clove and oregano essential oil treated starch and carboxymethyl cellulose (CMC) based edible packaging film in extending the shelf- life of paneer. The 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). Freshly prepared paneer sample was evaluated for physico-chemical, microbiological and sensory properties at (4±1 °C) till spoilage. The sample of paneer packed in oregano essential oil treated edible packaging film showed better and significant physico-chemical, microbiological and overall sensory properties as compared to paneer wrapped in clove essential oil treated edible packaging film. Both the paneer samples was found bland on 10<sup>th</sup> day of sensory evaluation but found microbiologically fine at 12<sup>th</sup> day of storage. The results revealed that both the samples of paneer packed in edible film treated with oregano and clove essential oil was found microbiologically safe for at least 12 days as compared to 5-6 days for normal paneer sample at 4±1 °C and oregano essential oil is more effective than clove essential oil in same concentration for preserving paneer sample.

**Keywords:** Paneer, Clove oil, Oregano oil, Edible film, Shelf-life

### 1. Introduction

Paneer refers as a dairy product obtained from the cow or buffalo milk or a combination thereof 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) <sup>[12]</sup>. Paneer shows a shelf- life of 5-6 days under refrigeration, though its freshness is lost within 3 days (Bhattacharya *et al.*, 1971) <sup>[7]</sup>. Many modern packaging methods like retort packaging, vacuum packaging etc., have been developed to increase the shelf life of paneer. In order to overcome the issue, generated by non-biodegradable or plastic packaging materials, the research has been carried out to use natural compounds for the preservation of paneer. Thus, an eco-friendly, biodegradable and different essential oil treated starch and carboxymethyl cellulose based edible packaging film was developed for this purpose. Clove essential oil (CEO) has eugenol as an active compound and have been identified for outstanding antioxidant and high antimicrobial activities against a wide range of spoilage microorganisms. Clove oil also shows plasticizing effect (Echeverría *et al.*, 2016) <sup>[10]</sup>. Below 1500 ppm FDA has granted clove essential oil the generally recognized as safe (GRAS) status. Oregano essential oil (OEO) has carvacrol and thymol as active compounds. FDA also granted generally recognized as safe (GRAS) status for oregano essential oil. The GRAS status of these essential oils gave a pave to the researchers to incorporate these essential oils in edible packaging materials. The objective of present research work was to study the efficacy of starch and carboxymethyl cellulose base edible 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

Freshly dawn buffalo milk was procured from buffalo farm of Mumbai Veterinary College and used throughout the experiments. Fat 6% and S.N.F 9% was standardized respectively for most favorable characteristics of the product. 1% citric acid was used to coagulate the milk for the preparation of paneer. In present study, paneer samples were prepared by the method suggested by Lamdande *et al.*, (2012) <sup>[17]</sup> with slight modifications.

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## 2.2 Preparation of edible film treated with essential oil

Edible film was prepared by adding starch (5 g), carboxymethyl cellulose (15% W/W starch) and glycerol (40 ml/100 g starch) in 100 mL distilled water (Ghanbarzadeh *et al.*, 2011)<sup>[13]</sup>. The suspension of edible film was agitated at 90 °C and stirred by magnetic stirrer at 500 rpm for 30 min. The suspension was cooled for 20 minutes and then 0.5% clove and 0.5% oregano essential oil was added as per the volume of the solution in two different samples. The suspension was then dried at 60 °C to obtain the film.

## 2.3 Packaging and storage of paneer

Treatment one (T1) and treatment two (T2) samples of 20 grams each were prepared. T1 paneer sample was 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 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

Physico-chemical, microbiological and sensory attributes were analyzed at an interval of 3 days till spoilage. Paneer sample was kept at 15 °C for 1 hour before analysis. All the parameters for paneer samples were evaluated in the laboratory of Department of Livestock Products Technology, Mumbai Veterinary College, Mumbai.

## 2.5 Physico-chemical analysis

T1 and T2 paneer samples were analyzed for pH in digital pH meter (Model-HI 99163, HANNA) as determined by the method of Trout *et al.*, (1992)<sup>[29]</sup>. Tyrosine value was determined for absorbance (A) at 730 nm using spectrophotometer as described by Strange *et al.*, (1977)<sup>[28]</sup> with slight modifications. Standard graph for tyrosine value was referred after calculated as milligram tyrosine per 100 gram of sample, as described by Pearson (1968)<sup>[23]</sup>. TBA value was determined by measuring absorbance (A) at 530nm in a spectrophotometer (Model no. EQ 820 with wavelength range of 350-950 nm, INDIA) as described by Witte *et al.*, (1970)<sup>[31]</sup> with little modification. Titratable acidity of the control and treated sample were determined as per AOAC (1995)<sup>[2]</sup>.

## 2.6 Microbiological counts

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

## 2.7 Sensory evaluation of paneer

The sensory evaluations of paneer samples were conducted by semi-trained panel of 6 judges and it was repeated at the interval of 5 days. The samples were judge for various sensory parameters using nine point Hedonic scales as

described by (Keeton, 1983)<sup>[14]</sup>.

## 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)<sup>[27]</sup>.

## 3. Results and Discussion

### 3.1. Physico-chemical parameters

#### 3.1.1 Change in pH

The paneer samples was analyzed for physico-chemical parameters like pH, tyrosine value, TBA value and titratable acidity at 0<sup>th</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day of storage (Table1). The initial pH values were non-significant till 6<sup>th</sup> day for T1 and T2. Significant ( $p<0.05$ ) difference among T1 and T2 was observed on day 9<sup>th</sup> to day 15<sup>th</sup> of storage. On 15<sup>th</sup> day of storage paneer packed in clove essential oil treated edible film showed more desirable value. Phenolic compound in clove and oregano essential oil helps to maintain the pH of paneer during entire storage. Results were in accordance with Shan *et al.*, (2011)<sup>[25]</sup> and Makhal *et al.*, (2014)<sup>[20]</sup> for thymol added cottage cheese.

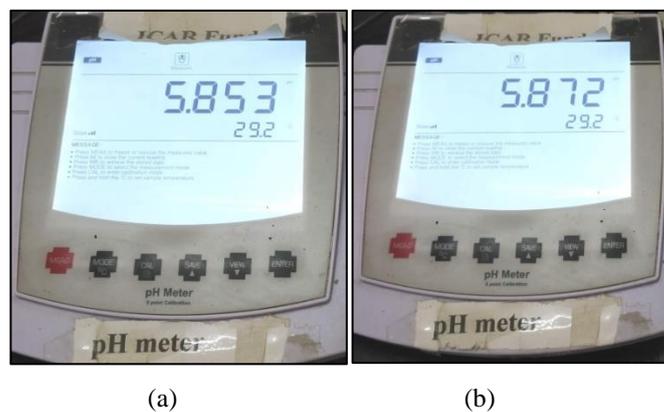


Fig 1: (a) pH of T1 on 0<sup>th</sup> day (b) pH of T2 on 0<sup>th</sup> day

#### 3.1.2 Change in tyrosine values

The average tyrosine values for T1 and T2 indicates a non-significant difference on 0 day. But a significant ( $p<0.05$ ) difference was noticed between day 3<sup>rd</sup> to day 15<sup>th</sup> of storage. Rai *et al.*, (2008)<sup>[24]</sup> showed a similar result when analyzed for chemical quality of paneer at 7±1 °C. Singh and Immanuel (2014)<sup>[26]</sup> reported similar finding of tyrosine value in paneer added with fruits peels. Yadav and Wadehra (2016)<sup>[32]</sup> also reported a similar tyrosine value at refrigeration condition for clove added spicy paneer. At the end of storage, the tyrosine value for T1 was found to be higher as compared to T2, which indicates less proteolysis in T2 paneer samples.

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

Parameters	pH		Tyrosine		TBA		Titratable Acidity	
	T1	T2	T1	T2	T1	T2	T1	T2
0 <sup>th</sup> Day	a5.85±0.03 <sup>A</sup>	a5.87±0.02 <sup>A</sup>	a12.47±0.01 <sup>A</sup>	a12.45±0.01 <sup>A</sup>	a0.22±0.02 <sup>A</sup>	a0.23±0.02 <sup>A</sup>	a0.29±0.01 <sup>A</sup>	a0.30±0.01 <sup>A</sup>
3 <sup>rd</sup> Day	a5.75±0.01 <sup>A</sup>	a5.77±0.02 <sup>A</sup>	b15.88±0.05 <sup>B</sup>	b15.84±0.04 <sup>A</sup>	b0.31±0.02 <sup>A</sup>	b0.26±0.02 <sup>A</sup>	b0.41±0.01 <sup>A</sup>	b0.40±0.02 <sup>A</sup>
6 <sup>th</sup> Day	a5.74±0.03 <sup>A</sup>	a5.75±0.03 <sup>A</sup>	c17.90±0.04 <sup>B</sup>	c17.08±0.01 <sup>A</sup>	c0.36±0.02 <sup>A</sup>	c0.32±0.06 <sup>A</sup>	c0.49±0.02 <sup>A</sup>	c0.49±0.02 <sup>A</sup>
9 <sup>th</sup> Day	b5.65±0.02 <sup>B</sup>	a5.69±0.01 <sup>A</sup>	d27.35±0.12 <sup>B</sup>	d25.16±0.02 <sup>A</sup>	d0.61±0.02 <sup>B</sup>	d0.55±0.02 <sup>A</sup>	d0.55±0.02 <sup>A</sup>	d0.53±0.01 <sup>A</sup>
12 <sup>th</sup> Day	b5.64±0.03 <sup>B</sup>	a5.71±0.04 <sup>A</sup>	e31.51±0.13 <sup>B</sup>	e30.20±0.05 <sup>A</sup>	e0.87±0.03 <sup>B</sup>	e0.74±0.03 <sup>A</sup>	e0.63±0.02 <sup>B</sup>	e0.59±0.01 <sup>A</sup>
15 <sup>th</sup> Day	c5.63±0.04 <sup>A</sup>	b5.69±0.03 <sup>B</sup>	f37.66±0.08 <sup>B</sup>	f35.65±0.22 <sup>A</sup>	f0.94±0.07 <sup>B</sup>	f0.86±0.08 <sup>A</sup>	f0.70±0.01 <sup>A</sup>	f0.71±0.01 <sup>B</sup>

\*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 TBA values

The average TBA values for T1 and T2 shows a non-significant difference from 0<sup>th</sup> day to 6<sup>th</sup> day of storage. But a significant ( $p<0.05$ ) difference was noticed in T1 and T2 from 9<sup>th</sup> day to 15<sup>th</sup> 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, indicating that the oregano essential oil protected paneer sample more against lipid oxidation. The results were in agreement with Shan *et al.*, (2011) [25] for cheese added with oregano herbs extract and Chauhan *et al.*, (2015) [8] for paneer.

### 3.1.4 Change in titratable acidity

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 T1 and T2 on 12<sup>th</sup> day and 15<sup>th</sup> 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 the reports of Verma and Khan (2009) [30] for panner and Yadav *et al.*, (2019) [33] for thyme herbs added paneer.

### 3.2 Microbiological parameters

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

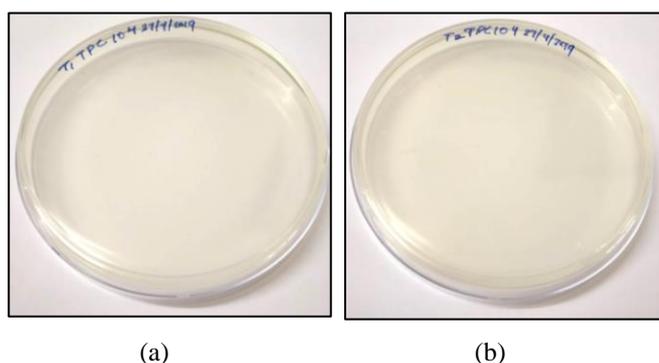
**Table 2:** Microbiological count (log cfu/g) of T1 and T2 paneer sample during storage period.

Parameters	Total Plate Count		Psychrophilic count		Yeast and Mold Count	
	T1	T2	T1	T2	T1	T2
0 <sup>th</sup> Day	NIL	NIL	NIL	NIL	NIL	NIL
3 <sup>rd</sup> Day	a1.27±0.18 <sup>A</sup>	a1.20±0.12 <sup>A</sup>	NIL	NIL	NIL	NIL
6 <sup>th</sup> Day	b2.47±0.18 <sup>A</sup>	b2.20±0.12 <sup>A</sup>	NIL	NIL	a2.73±0.07 <sup>A</sup>	a2.53±0.07 <sup>A</sup>
9 <sup>th</sup> Day	c3.60±0.12 <sup>B</sup>	c3.40±0.12 <sup>A</sup>	a3.67±0.02 <sup>B</sup>	a3.57±0.02 <sup>A</sup>	b3.14±0.01 <sup>B</sup>	b3.06±0.01 <sup>A</sup>
12 <sup>th</sup> Day	d4.77±0.09 <sup>A</sup>	d4.57±0.12 <sup>A</sup>	b4.84±0.02 <sup>A</sup>	b4.81±0.02 <sup>A</sup>	c3.31±0.02 <sup>B</sup>	b3.18±0.01 <sup>A</sup>
15 <sup>th</sup> Day	e5.47±0.09 <sup>A</sup>	e5.37±0.09 <sup>A</sup>	c5.17±0.02 <sup>B</sup>	c5.00±0.01 <sup>A</sup>	d3.43±0.02 <sup>B</sup>	c3.31±0.02 <sup>A</sup>

\*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

Total plate count (TPC log<sub>10</sub>cfu/g) for T1 and T2 was nil on zero day. A significant ( $p<0.05$ ) difference was noticed only on 9<sup>th</sup> day of storage in T1 and T2. Both the samples were under acceptable limit on 12<sup>th</sup> 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) [22] and for clove essential oil treated paneer stored at 8±1 °C was reported by Khatkar *et al.*, (2017) [15].



**Fig 2:** (a) Nil Total Plate Count of T1 on 0<sup>th</sup> day. (b) Nil Total Plate count of T2 on 0<sup>th</sup> day

### 3.2.2 Change in psychrophilic count

Psychrophilic count of T1 and T2 samples on 0<sup>th</sup> day to 6<sup>th</sup> day were nil. A significant ( $p<0.05$ ) difference was noticed by T1 and T2 on 9<sup>th</sup> day and 15<sup>th</sup> day of storage. Gradual increase in psychrophilic count was observed in T1 than T2 upon 15<sup>th</sup> day of storage. Numerous studies show that clove essentials oils (Badhe and Fairoze 2012) [5], Liu *et al.*, (2017) [18] and oregano essentials oils (Lambert *et al.*, 2001) [16] have strong bactericidal effect. Similar result was reported by Makhil *et al.*, (2014) [20] 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 0<sup>th</sup> and 3<sup>rd</sup>, the average yeast and mold count for T1 and T2 were nil. A significant ( $p<0.05$ ) difference was observed in T1 and T2 from 6<sup>th</sup> day to 15<sup>th</sup> day of storage. Owing the antifungal action, the clove essential oil (Lopez *et al.*, 2005) [19] and the oregano essential oil (Munhuweyi *et al.*, 2018) [21] 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 15<sup>th</sup> day of storage than T1. The results of yeast and mold count were in agreement with Eresam *et al.*, (2015) [11] 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<sup>th</sup>, 5<sup>th</sup> and 10<sup>th</sup> day of storage (Table 3).

#### 3.3.1 Changes in appearance score

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

#### 3.3.2 Changes in Juiciness score

There was a gradual and significant ( $p<0.05$ ) decrease in

juiciness of T1 paneer sample from 8.42 to 6.11 in 10 days. The T2 paneer sample also showed a significant ( $p<0.05$ ) decrease of 8.35 to 6.49 from initial day to 10<sup>th</sup> day of storage.

### 3.3.3 Changes in Flavour score

There was a significant ( $p<0.05$ ) decrease in flavour of T1 paneer sample from 8.43 to 7.52 in 10 days was observed. The T2 paneer sample showed a significant ( $p<0.05$ ) decrease of 8.72 to 7.68 from initial day to 10<sup>th</sup> day of storage. T2 paneer sample gave a better significant ( $p<0.05$ ) values than T1 paneer sample on 10<sup>th</sup> day of storage.

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

Day	0 <sup>th</sup> Day	5 <sup>th</sup> Day	10 <sup>th</sup> Day
<b>Sensory Evaluation</b>			
<b>Appearance</b>			
Treatment1	a8.98±0.15 <sup>A</sup>	b8.43±0.01 <sup>A</sup>	a7.35±0.07 <sup>B</sup>
Treatment2	a8.97±0.02 <sup>A</sup>	b8.60±0.12 <sup>A</sup>	a7.52±0.10 <sup>A</sup>
<b>Juiciness</b>			
Treatment1	a8.42±0.15 <sup>A</sup>	b7.61±0.12 <sup>A</sup>	a6.11±0.22 <sup>B</sup>
Treatment2	a8.35±0.10 <sup>A</sup>	b7.76±0.15 <sup>A</sup>	a6.49±0.14 <sup>A</sup>
<b>Flavour</b>			
Treatment1	a8.43±0.17 <sup>A</sup>	b7.66±0.17 <sup>A</sup>	a7.52±0.30 <sup>B</sup>
Treatment2	a8.72±0.16 <sup>A</sup>	b7.50±0.23 <sup>A</sup>	a7.68±0.24 <sup>A</sup>
<b>Texture</b>			
Treatment1	a8.97±0.02 <sup>A</sup>	b8.42±0.14 <sup>A</sup>	a7.02±0.12 <sup>B</sup>
Treatment2	a8.95±0.03 <sup>A</sup>	b8.36±0.16 <sup>A</sup>	a7.14±0.15 <sup>A</sup>
<b>Overall Acceptability</b>			
Treatment1	a8.70±0.07 <sup>A</sup>	b8.03±0.11 <sup>A</sup>	a7.00±0.08 <sup>B</sup>
Treatment2	a8.75±0.06 <sup>A</sup>	b8.05±0.08 <sup>A</sup>	a7.20±0.08 <sup>A</sup>

\*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

### 3.3.4 Changes in Texture score

There was a gradual and significant ( $p<0.05$ ) decrease in texture score of T1 paneer sample from 8.97 to 7.02 in 10 days. The T2 paneer sample showed a significant ( $p<0.05$ ) decrease of 8.95 to 7.14 from initial day to 10<sup>th</sup> day of storage.

### 3.3.5 Changes in Overall Acceptability

There was significantly ( $p<0.05$ ) decrease in overall acceptability of both treatment samples with the increase in storage period. The overall acceptability of both treatment samples was within desirable range, but slightly poorer for T1 paneer samples as compared to T2 paneer sample. The overall acceptability score of both the paneer samples decreased significantly ( $p<0.05$ ) from an initial 8.70 to 7.00 in 10 days for T1 paneer sample and 8.75 to 7.20 in 10 days for T2 paneer sample. 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) [9] and Ahmed & Bajwa (2019) [11].

## 4. Conclusion

In the present study paneer samples coated with edible film treated with 0.5% clove essential oil and 0.5% oregano essential oil was microbiologically and physico-chemically accepted till 12<sup>th</sup> day of storage. Whereas, paneer sample packed in edible film treated with essential oil of oregano was found microbiologically and physico-chemically more desirable and significant for consumption than paneer sample

packed in edible film treated with essential oil of clove. Further, conclusion can be drawn that carboxymethyl cellulose and starch based film can be used as edible packaging material. Clove and oregano essential oil can be successfully incorporated in edible packaging film to increase shelf life of perishable products.

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