

Original Research Article

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## Effect of Edible Packaging Film Treated with Essential Oil of Oregano in Extending the Shelf Life of Paneer

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### ABSTRACT

The present study was conducted to evaluate the shelf- life of paneer wrapped with oregano essential oil treated starch and carboxymethyl cellulose (CMC) based edible packaging film. The edible film was prepared by adding starch (5g), carboxymethyl cellulose (15 % W/W starch) in distilled water (100mL) and glycerol (40ml/100g starch). Physico-chemical, microbiological and sensory properties at (4±1°C) till spoilage was evaluated for paneer samples. Control sample was unacceptable after 5<sup>th</sup> day of storage for sensory evaluation and found microbiologically unfit on 9<sup>th</sup> day of storage. The paneer packed in edible film treated with 0.5% oregano essential oil 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 paneer packed in edible film treated with oregano essential oil was found microbiologically safe for at least 12 days as compared to 5-6 days for control sample at 4±1°C.

#### Keywords

Paneer, Oregano essential oil, CMC, Starch, Shelf-life

#### Article Info

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### Introduction

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). Like other dairy products, paneer also shows a shelf- life of 5-6 days under

refrigeration, though its freshness is lost within 3 days (Bhattacharya *et al.*, 1971). Retort packaging, vacuum packaging and many more modern packaging methods have been developed to increase the shelf life of paneer. The packaging materials mostly used in the food industries are non- biodegradable and the problems generated by non- degradable or plastic waste are of great concern.

In order to overcome the issue, the research has been carried out to use natural compounds for the preservation of paneer. Thus, an eco-friendly, biodegradable and oregano essential oil treated starch and carboxymethyl cellulose based edible packaging film was developed for this purpose. Oregano essential oil (OEO) is obtained from Oregano plant (*Origanum vulgare*) and has carvacrol and thymol as major active compounds. These compounds possess high antimicrobial activity and excellent antioxidant properties against a wide range of spoilage and pathogenic microorganisms. Generally recognized as safe (GRAS) status of oregano essential oil by FDA gave researcher a paved way to incorporate this oil in starch and carboxymethyl cellulose based edible film for extensive studies. The objective of present research work was to study the effectiveness of starch and carboxymethyl cellulose based edible film, incorporated with oregano essential oil to extend the shelf life of paneer stored at refrigeration temperature ( $4\pm 1^{\circ}\text{C}$ ).

## **Materials and Methods**

### **Preparation of paneer**

Freshly dawn buffalo milk was procured from cattle and buffalo farm of Mumbai Veterinary College and used throughout the experiments. The fat percentage and S.N.F was standardized to 6% and 9% respectively for most favorable product characteristics. Strength of 1% citric acid was used to coagulate the milk for preparation of paneer. In present study, paneer samples were prepared by the method suggested by Kumar *et al.*, (2014) with slight modifications.

### **Preparation of edible film treated with essential oil**

The suspension of edible film was prepared by adding starch (5 g), carboxymethyl

cellulose (15% W/W starch) in 100 mL distilled water and added glycerol (40 ml/100 g starch). The Suspension was agitated by magnetic stirrer at 500rpm for 30 min in water bath at  $90^{\circ}\text{C}$ . After that the suspension was cooled for 20 minutes and 0.5% oregano essential oil was added as per the volume of the solution. The desired concentration of oregano essential oil was determined by Minimum inhibition concentration. The suspension was then dried at  $60^{\circ}\text{C}$  to obtain the film (Ghanbarzadeh *et al.*, 2011).

### **Packaging and storage of paneer**

Control (C) and treatment (T) samples of 20 grams each was prepared. Control sample contained paneer packed in LDPE bag and kept at  $4\pm 1^{\circ}\text{C}$ . Treatment (T) sample contained paneer wrapped in edible packaging film treated with 0.5% oregano essential oil and then packed in LDPE bag and stored at  $4\pm 1^{\circ}\text{C}$ . Before analysis the control and treatment paneer samples were kept at  $15^{\circ}\text{C}$  for 1 hour.

### **Physico-chemical and microbiological analysis**

Control and treated paneer sample were analyzed for pH as determined by the method of Trout *et al.*, (1992). Digital pH meter (Model-HI 99163, HANNA) was used to record the pH of the suspension. TBA value was determined by the procedure described by Witte *et al.*, (1970) with little modification and the absorbance (A) was measured at 530nm in a spectrophotometer (Model no. EQ 820 with wavelength range of 350-950 nm, INDIA). The procedure of tyrosine value was determined as per described by Strange *et al.*, (1977) with slight modifications. The absorbance (A) was measured at 730 nm using Spectrophotometer. Tyrosine value was calculated as milligram tyrosine per 100 gram of sample by referring to a standard graph, as

per described by Pearson (1968). Titratable acidity of the control and treated sample were determined as per AOAC (1995). Total plate count, psychrophilic count, yeast and mold count and coliform count of paneer samples were estimated by the standard methods of APHA (1992).

### **Sensory evaluation of paneer**

The sensory evaluations of fresh and stored 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 attributes using nine point Hedonic scales as described by (Keeton, 1983).

### **Statistical analysis**

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

## **Results and Discussion**

### **Physico-chemical parameters**

Physico-chemical parameters of paneer samples like pH, tyrosine, TBA value and titratable acidity were analyzed 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 9<sup>th</sup> day for control and treatment. Significant (p<0.05) difference among control and treatment was observed on day 12<sup>th</sup> and day 15<sup>th</sup> of storage.

Compounds like carvacrol and thymol in oregano essential oil helps to maintain the pH of paneer during entire storage. Results were in accordance with Rai *et al.*, (2008) and Makhhal *et al.*, (2014) for thymol added

cottage cheese. The average tyrosine values for control and treatment shows non-significant difference on 0<sup>th</sup> day. A significant (p<0.05) difference was observed between 3<sup>rd</sup> day to 15<sup>th</sup> day of storage. Rai *et al.*, (2008) analyzed chemical quality of paneer at 7±1°C and showed a similar finding.

Singh and Immanuel (2014) also reported similar result of tyrosine value in paneer added with fruits peels. At the end of storage, the tyrosine value for control was found to be much higher as compared to treatment, which indicates less proteolysis in treated paneer samples.

The average TBA values for control and treatment shows a non-significant difference on 0 day. But a significant (p<0.05) difference was noticed for control and treatment from 3<sup>rd</sup> day to 15<sup>th</sup> day of storage. The value of TBA in paneer sample treated with oregano essential oil was considerably lower than that of the control, indicating that the oregano essential oil protected paneer against lipid oxidation. The results were in agreement with the reports of Shan *et al.*, (2011) for cheese added with oregano herbs extract and Chauhan *et al.*, (2015) for paneer.

Initial acidity of the control paneer samples were 0.29%. Slow and gradual increase in acidity was developed during storage. The average titratable acidity values for control and treatment showed a non-significant difference on 0<sup>th</sup> day. But a significant (p<0.05) difference was noticed in control and treatment from 3<sup>rd</sup> day to 15<sup>th</sup> day of storage. Incorporation of oregano essential oil into edible film of paneer delayed the acid development during storage, possibly because of its antimicrobial activity against the spoilage causing microorganisms. The results were in agreement with the reports of and Makhhal *et al.*, (2014) for paneer and Yadav *et al.*, (2019) for thyme herbs added paneer.

## Microbiological parameters

Control and treatment paneer samples were subjected to microbiological count subsequently 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 (Table 2). Total plate count (TPC log<sub>10</sub>cfu/g) for control and treatment was nil on 0<sup>th</sup> day. A significant difference ( $p < 0.05$ ) was observed in control and treatment from 3<sup>th</sup> day to 15<sup>th</sup> day of storage. On 9<sup>th</sup> day of storage control was on unacceptable limit whereas treatment was under acceptable limit till 12<sup>th</sup> day of storage. The reduction in total plate count was perhaps on account of the bactericidal effect of oregano essential oil. A similar result of total plate count for oregano essential oil treated cheese was reported by Olmedo *et al.*, (2013) and Rani *et al.*, (2014) for masala paneer.

Psychrophilic count of control sample was nil on 0<sup>th</sup> day and 3<sup>rd</sup> day whereas, for treatment sample psychrophilic count was nil on 0<sup>th</sup>, 3<sup>rd</sup> and 6<sup>th</sup> day of storage. On 6<sup>th</sup> day control showed the growth with count of  $4.63 \pm 0.01$  log cfu/g. A significant difference ( $p < 0.05$ ) was observed by control and treatment from 9<sup>th</sup> day to 12<sup>th</sup> day of storage. Gradual increase in psychrophilic count was observed in control than treatment upon 15<sup>th</sup> day of storage. Numerous studies show that oregano essentials oils have strong bactericidal effect Lambert *et al.*, (2001). Similar result was also reported by Makhil *et al.*, (2014) and Artiga *et al.*, (2017) that stated the lower growth of psychrotrophs in cottage cheese samples added with essential oils is due to its strong antimicrobial action.

The average yeast and mold count for control and treatment were nil on 0<sup>th</sup> day whereas on 3<sup>rd</sup> day, only control sample showed the growth (Fig.1). A significant ( $p < 0.05$ ) difference was noticed in control and treatment from 6<sup>th</sup> day to 15<sup>th</sup> day of storage. Owing to the antifungal action (Munhuweyi

*et al.*, 2018), the oregano essential oil was observed to exhibit a significant inhibitory effect against growth of yeast and molds during the entire storage period. The results of yeast and mold count were in agreement with Buch *et al.*, (2014) for paneer and Artiga *et al.*, (2017) for oregano essential oil coated cheese. The coliform count for both control and treatment samples were negative during entire period of storage. It shows that the sample was not contaminated by any coliform bacteria from any route.

## Sensory evaluation

Control and treatment paneer samples were subjected to sensory evaluation for the parameters like appearance, juiciness, flavor, texture and overall acceptability subsequently at 5 days interval (Table. 3). There was slight decrease in appearance of stored control (Fig. 2A) and treatment (Fig. 2B) paneer samples with the increase in storage period. Yellowish tint appearance was observed at the end of storage period. The appearance of control and treated samples decreased significantly ( $p < 0.05$ ) throughout the storage. Juiciness of control paneer sample showed significant ( $p < 0.05$ ) decreased from 8.46 to 7.54 in 5 days. Juiciness of treatment paneer sample showed a significant ( $p < 0.05$ ) decrease of 8.35 to 6.49 from initial day to 10<sup>th</sup> day of storage.

There was a significant ( $p < 0.05$ ) decrease in flavour of control paneer sample from 7.95 to 7.32 in 5 days. The flavor of treatment paneer sample also showed a significant ( $p < 0.05$ ) decrease of 8.72 to 7.68 from 0<sup>th</sup> day to 10<sup>th</sup> day of storage. There was also a gradual and significant ( $p < 0.05$ ) decrease in texture score of control paneer sample from 8.98 to 8.12 in 5 days. The treatment paneer sample also showed a significant ( $p < 0.05$ ) decreased texture score of 8.95 to 7.14 from initial day to 10<sup>th</sup> day of storage.

**Table.1** Physico-chemical parameters of control and treatment during storage period

Parameters	pH		Tyrosine		TBA		Titratable Acidity	
	C	T	C	T	C	T	C	T
<b>0<sup>th</sup> Day</b>	a5.85±0.03 <sup>A</sup>	a5.87±0.02 <sup>A</sup>	a12.52±0.06 <sup>A</sup>	a12.45±0.01 <sup>A</sup>	a0.22±0.01 <sup>A</sup>	a0.23±0.02 <sup>A</sup>	a0.29±0.01 <sup>A</sup>	a0.30±0.01 <sup>A</sup>
<b>3<sup>rd</sup> Day</b>	a5.83±0.04 <sup>A</sup>	a5.77±0.02 <sup>A</sup>	b15.38±0.05 <sup>B</sup>	b15.84±0.04 <sup>A</sup>	b0.36±0.01 <sup>B</sup>	b0.26±0.02 <sup>A</sup>	b0.43±0.01 <sup>B</sup>	b0.40±0.02 <sup>A</sup>
<b>6<sup>th</sup> Day</b>	a5.77±0.02 <sup>A</sup>	a5.75±0.03 <sup>A</sup>	c19.78±0.05 <sup>B</sup>	c17.08±0.01 <sup>A</sup>	c0.48±0.01 <sup>B</sup>	c0.32±0.06 <sup>A</sup>	c0.55±0.02 <sup>B</sup>	c0.49±0.02 <sup>A</sup>
<b>9<sup>th</sup> Day</b>	a5.76±0.03 <sup>A</sup>	a5.69±0.01 <sup>A</sup>	d31.14±0.02 <sup>B</sup>	d25.16±0.02 <sup>A</sup>	d0.68±0.02 <sup>B</sup>	d0.55±0.02 <sup>A</sup>	d0.62±0.02 <sup>B</sup>	d0.53±0.01 <sup>A</sup>
<b>12<sup>th</sup> Day</b>	b5.56±0.01 <sup>B</sup>	a5.71±0.04 <sup>A</sup>	e40.53±0.03 <sup>B</sup>	e30.20±0.05 <sup>A</sup>	e0.93±0.02 <sup>B</sup>	e0.74±0.03 <sup>A</sup>	e0.74±0.01 <sup>B</sup>	e0.59±0.01 <sup>A</sup>
<b>15<sup>th</sup> Day</b>	b5.55±0.03 <sup>B</sup>	b5.69±0.03 <sup>A</sup>	f47.54±0.25 <sup>B</sup>	f35.65±0.22 <sup>A</sup>	f1.16±0.06 <sup>B</sup>	f0.86±0.08 <sup>A</sup>	f0.79±0.02 <sup>B</sup>	f0.71±0.01 <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%)

**Table.2** Microbiological count (log cfu/g) of control and treatment of paneer during storage period

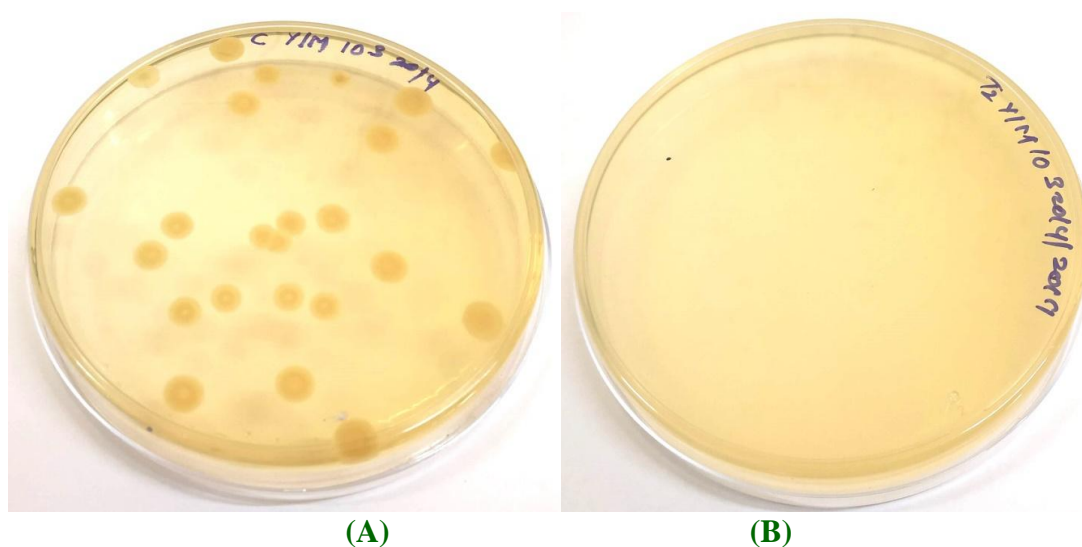
Parameters	Total Plate Count		Psychrophilic count		Yeast and Mold Count	
	C	T	C	T	C	T
<b>0<sup>th</sup> Day</b>	NIL	NIL	NIL	NIL	NIL	NIL
<b>3<sup>rd</sup> Day</b>	a1.97±0.09 <sup>B</sup>	a1.20±0.12 <sup>A</sup>	NIL	NIL	a2.53±0.18	NIL
<b>6<sup>th</sup> Day</b>	b3.30±0.15 <sup>B</sup>	b2.20±0.12 <sup>A</sup>	a4.63±0.01 <sup>A</sup>	NIL	b3.28±0.17 <sup>B</sup>	a2.53±0.07 <sup>A</sup>
<b>9<sup>th</sup> Day</b>	c5.0±0.10 <sup>B</sup>	c3.40±0.12 <sup>A</sup>	b5.03±0.01 <sup>B</sup>	a3.57±0.02 <sup>A</sup>	b3.37±0.01 <sup>B</sup>	b3.06±0.01 <sup>A</sup>
<b>12<sup>th</sup> Day</b>	d5.80±0.12 <sup>B</sup>	d4.57±0.12 <sup>A</sup>	a5.18±0.00 <sup>B</sup>	b4.81±0.02 <sup>A</sup>	b3.45±0.02 <sup>B</sup>	b3.18±0.01 <sup>A</sup>
<b>15<sup>th</sup> Day</b>	e6.77±0.03 <sup>B</sup>	e5.37±0.09 <sup>A</sup>	a5.20±0.01 <sup>B</sup>	c5.00±0.01 <sup>A</sup>	b3.52±0.01 <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%).

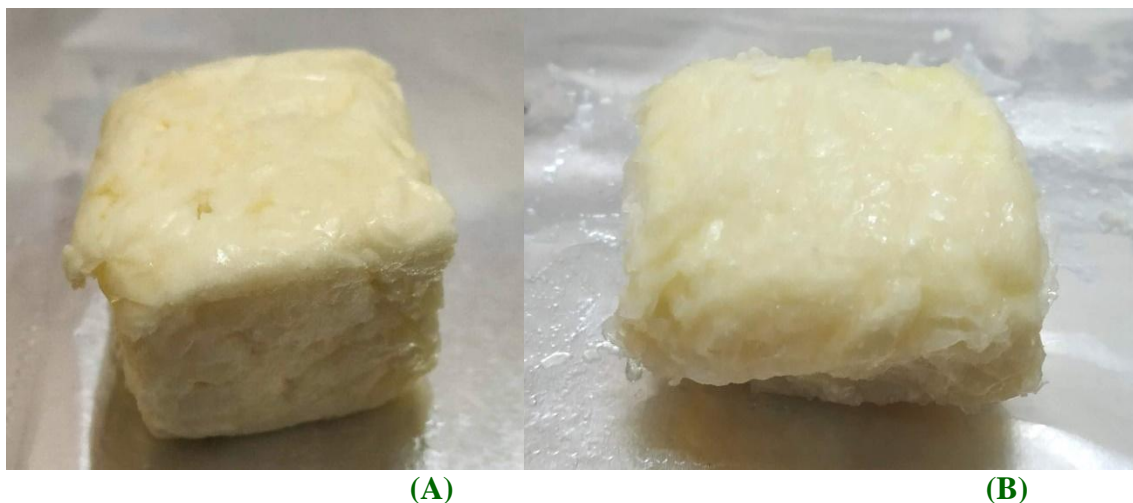
**Table.3** Sensory scores of control and treatment during storage period

Day	0 <sup>th</sup> Day	5 <sup>th</sup> Day	10 <sup>th</sup> Day
<b>Sensory Evaluation</b>			
<b>Appearance</b>			
Control	$8.98 \pm 0.02^A$	$7.97 \pm 0.12^A$	NC
Treatment	$8.97 \pm 0.02^B$	$8.60 \pm 0.12^B$	$7.52 \pm 0.10^B$
<b>Juiciness</b>			
Control	$8.46 \pm 0.14^A$	$7.54 \pm 0.18^A$	NC
Treatment	$8.35 \pm 0.10^B$	$7.76 \pm 0.15^B$	$6.49 \pm 0.14^B$
<b>Flavour</b>			
Control	$7.95 \pm 0.18^A$	$7.32 \pm 0.16^A$	NC
Treatment	$8.72 \pm 0.16^B$	$7.50 \pm 0.23^B$	$7.68 \pm 0.24^B$
<b>Texture</b>			
Control	$8.98 \pm 0.02^A$	$8.12 \pm 0.18^A$	NC
Treatment	$8.95 \pm 0.03^B$	$8.36 \pm 0.16^B$	$7.14 \pm 0.15^B$
<b>Overall Acceptability</b>			
Control	$8.59 \pm 0.04^A$	$7.74 \pm 0.08^A$	NC
Treatment	$8.75 \pm 0.06^B$	$8.05 \pm 0.08^B$	$7.20 \pm 0.08^B$

\*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) Shows yeast and mould growth on 3<sup>rd</sup> day for control sample. (B) Shows no growth of yeast and mould on 3<sup>rd</sup> day of storage for paneer sample wrapped in oregano oil treated edible film



**Fig.2** (A) Shows control paneer sample on 6<sup>th</sup> day (B) Shows treatment paneer sample wrapped in oregano essential oil treated edible film on 9<sup>th</sup> day of storage

There was significantly ( $p < 0.05$ ) decrease in overall acceptability of both control and treatment with the increase in storage period. The overall acceptability of both control and treatment samples were within desirable range, but slightly poorer for control samples as compared to treated sample. Sensory evaluation of control sample was not conducted after 5<sup>th</sup> day because it was found microbiologically unfit on 9<sup>th</sup> day of storage.

For treatment sample, overall acceptability was unfavorable on 10<sup>th</sup> day of evaluation. The scores for appearance, flavor, juiciness and texture were affected by the increase acidity and decreasing moisture content, similar finding was reported by Kumar *et al.*, (2008). Ahmed & Bajwa (2019) also stated the similar result as degradation of reducing sugar leads to pH drop and alteration of sensory qualities in paneer.

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