Appendix-A

EXTENDING THE SHELF LIFE OF PANEER COATED IN EDIBLE FILM TREATED WITH ESSENTIAL OILS

T H E S I S

Submitted

in partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

LIVESTOCK PRODUCTS TECHNOLOGY

BY

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Appendix – B

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"EXTENDING THE SHELF LIFE OF PANEER COATED IN EDIBLE FILM TREATED WITH ESSENTIAL OILS"** or part thereof has not been submitted for any of the other degree or diploma of any University, nor the data have been derived from any thesis / publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Appendix – C(i)

DECLARATION OF ADVISORY COMMITTEE

Shri. **SAURABH KARUNAMAY** has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled, **"EXTENDING THE SHELF LIFE OF PANEER COATED IN EDIBLE FILM TREATED WITH ESSENTIAL OILS"** submitted by him is the result of research work is sufficient to warrant its presentation to the examination in the subject of **LIVESTOCK PRODUCTS TECHNOLOGY** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other University.

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This is to certify that the thesis entitled, **"EXTENDING THE SHELF LIFE OF PANEER COATED IN EDIBLE FILM TREATED WITH ESSENTIAL OILS"** submitted by Shri. **SAURABH KARUNAMAY** to the Maharashtra Animal and Fishery Sciences University, Nagpur, in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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Dedicated to .....

My beloved Parents

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Date:
Mumbai ***Saurabh Karunamay***

Appendix – D

TABLE OF CONTENTS

	Chapter		Page
I)	INTRODUCTION	:	1-4
II)	REVIEW OF LITERATURE	:	5-25
III)	MATERIALS & METHODS	:	26-37
IV)	RESULTS & DISCUSSION	:	38-66
V)	SUMMARY & CONCLUSIONS	:	67-68
A)	BIBLIOGRAPHY	:	i-xvi
B)	APPENDICES	:	xvii-xviii
C)	THESIS ABSTRACTS	:	xix-xxii
D)	VITA	:	xxiii

INDEX

CHAPTER NO.	SUB CHAPTER	CHAPTER	:	PAGE NO.
I)		**INTRODUCTION**	:	**1-4**
II)		**REVIEW OF LITERATURE**	:	**5-25**
	2.1	Paneer	:	**5**
	2.2	Shelf-life of paneer	:	**6**
	2.3	Physico-chemical characteristics of paneer	:	**8**
	2.3.1	pH	:	**8**
	2.3.2	Tyrosine value	:	**9**
	2.3.3	Thiobarbituric acid value	:	**10**
	2.3.4	Titrable acidity	:	**11**
	2.4	Proximate analysis	:	**12**
	2.4.1	Moisture	:	**12**
	2.4.2	Protein	:	**13**
	2.4.3	Fat	:	**14**
	2.5	Microbiological changes of paneer	:	**15**
	2.6	Sensory evaluation of paneer	:	**17**
	2.7	Edible film	:	**18**
	2.8	Glycerol	:	**21**
	2.9	Essential oils	:	**21**
	2.10	Physcio-chemical property of edible film	:	**24**
III)		**MATERIALS & METHODS**	:	**26-37**
	3.1	Materials	:	**26**
	3.1.1	Milk	:	**26**
	3.1.2	Acid coagulant	:	**26**
	3.1.3	Procurement of media chemicals, and reagent	:	**26**
	3.1.4	Procurement of glassware and plastics	:	**27**
	3.2	Experimental No.1	:	**27**
	3.2.1	Preparation and standardization of edible packaging film.	:	**27**

	3.2.2	Physicochemical characterization of edible film	:	**28**
	(a)	Thickness	:	**28**
	(b)	Moisture content	:	**28**
	(c)	Water vapour permeability	:	**28**
	(d)	Film solubility	:	**29**
	(e)	Elongation at break	:	**30**
	(f)	Colour of film	:	**30**
	(g)	pH of the film	:	**30**
	3.3	Experiment No.2	:	**31**
	3.3.1	Assessment of shelf life of paneer packed with edible film treated with clove and oregano essential oil.	:	**31**
	(a)	Preparation of paneer	:	**31**
	(b)	Sensory evaluation	:	**32**
	(c)	Physico-chemical analysis of paneer	:	**32**
	(d)	pH	:	**32**
	(e)	Thiobarbituric Acid Value	:	**33**
	(f)	Tyrosine Value	:	**33**
	(g)	Titratable acidity	:	**33**
	(h)	Proximate composition	:	**34**
	(i)	Moisture	:	**34**
	(j)	Fat	:	**34**
	(k)	Protein	:	**35**
	(l)	Microbiological analysis	:	**35**
	(m)	Preparation of serial dilution	:	**35**
	(n)	Estimation of Total Plate Count (TPC)	:	**36**
	(o)	Psychrophillic count	:	**36**
	(p)	Coliform count	:	**36**
	(q)	Yeast and mold count	:	**37**
	(r)	Statistical analysis	:	**37**
IV)		**RESULTS AND DISCUSSION**	:	**38-66**
V)		**SUMMARY AND CONCLUSIONS**	:	**67-68**
A		**BIBILIOGRAPHY**	:	**i-xv**
B		**ABSTRACT ENGLISH**	:	**xviii-xix**
		ABSTRACT MARATHI	:	**xx-xxi**
C		**VITA**	:	xx**ii**

LIST OF TABLES

Table No	Title	:	Page No.
4.1	Proximate composition of paneer	:	40
4.2	Changes in pH of control and treatment paneer during storage	:	43
4.3	Changes in tyrosine value of control and treatment paneer during storage	:	46
4.4	Changes in TBA value of control and treated paneer during storage	:	49
4.5	Changes in Titratable acidity value of control and treatment paneer during storage	:	52
4.6	Yeast and mold count of control and treatments of paneer during storage period	:	55
4.7	Psychrophilic count of control and treatments of paneer during storage period	:	57
4.8	Total plate count of control and treatments of paneer during storage period	:	60
4.9	Sensory scores of control and treatment at 0 day storage period	:	63
4.10	Sensory scores of control and treatment at 5^{th} day storage period	:	64
4.11	Sensory scores of control and treatment at 10^{th} day storage period	:	65
4.12	Cost of production for edible packaging film treated with essential oil for packaging paneer (per 100 ml)	:	66

LIST OF FIGURES

Figure No.	Title	Page No.
3.1	Preparation of paneer	31
4.1	Proximate composition of paneer	40
4.2	Changes in pH of control and treatment paneer during storage	43
4.3	Changes in tyrosine value of control and treatment paneer during storage	46
4.4	Changes in TBA value of control and treated paneer during storage	49
4.5	Changes in Titratable acidity value of control and treatment paneer during storage	52
4.6	Yeast and mold count of control and treatments of paneer during storage period	55
4.7	Psychrophilic count of control and treatments of paneer during storage period	57
4.8	Total plate count of control and treatments of paneer during storage period	60
4.9	Sensory scores of control and treatment at 0 day storage period	63
4.10	Sensory scores of control and treatment at 5 day storage period	64
4.11	Sensory scores of control and treatment at 10 day storage period	65

LIST OF PLATES

Plate No:	Title	Between pages
Plate 1	Control Paneer Sample	66-67
Plate 2	T1 Paneer Sample	66-67
Plate 3	T2 Paneer Sample	66-67
Plate 4	Edible packaging film	66-67
Plate 5	Experiment performing EAB	66-67

LIST OF ABBREVIATIONS

A	Absorbance
APHA	American Public Health Association
ASTM	American society For Testing and Materials
AOAC	Association of Analytical Chemists
Atm	Atmosphere
BP	Boiling point
Co2	Carbon dioxide
CMC	Carboxymethyl cellulose
CSA	Cassova Starch acetate
CA	Citric Acid
cfu	Colony forming unit
C	Control.
°	Degree Celsius
EAB	Elongation at break
EO	Essential Oil
FSSAI	Food safety and Standard Authority of India
GRAS	Generally Recognized as Safe
Gly	Glycerol
GOVT	Government
g	Gram
HACS	High Amylose Corn Starch
Hr	Hours
ICMR	Indian Council Of Medical Research
KGM	Konjac Glucomannan
<	Less than
Log	Logarithm

LDPE	Low Density Polyethylene
mg	Milligram
ml	Milliliter
mm	Millimeter
min	Minute
MAP	Modified atmosphere packaging
MPA	Moisture permeability
MST	Moisture-proof sealable transparent cellophane
MMT	Montmorillonite
MPN	Most Probable Number
N2	Nitrogen
OD	Optical Density
OREG	Oregano
OEO	Oregano essential Oil
T1	Paneer treated with 0.5% clove essential oil
T2	Paneer treated with 0.5% oregano essential oil
%	Per cent
±	Plus minus
pH	Potential of hydrogen
PFA	Prevention of Food Adulteration Act
Pvt ltd	Private limited
SA	Sodium Alginate
SNF	Solid-not-fat
SPI	Soya Protein Isolate
SSPS	Soyabean Polysaccharide
SPC	Standard Plate Count.
SB	Strain to break
TPA	Texture Profile Analysis
TBA	Thiobarbituric Acid
TPC	Total plate Count
TCA	Trichloroacetic acid.

UTS	Ultimate Tensile strength
UK	United Kingdom
WVP	Water vapour Permeability
Kw	Water vapour permeability constant
w/w	Weight by weight
Wt	Weight.

Introduction

1. INTRODUCTION

India is the highest milk producing country in the world. India's milk production is estimated to have increased by 6.6 per cent during the last financial year. Milk production in the country is 165.4 million tonnes during 2016-17 and 176.35 million tonnes during 2017-18, (DAHD).

India contributes about 18.81% of global milk production. It reflects that India has a good potential and availability of milk for the preparation of milk products in India. 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. An estimated 5% of total milk produced in India is converted into paneer.

Paneer means the product obtained from the cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 70.0 per cent moisture and the milk fat content shall not be less than 50.0 per cent of the dry matter (FSSAI, 2011). Paneer is mainly known for its typical mild acidic flavor with slightly sweet taste. It has a firm, cohesive and spongy body and a close knit smooth texture.

Paneer provides a good amount of protein for the vegetarian in the society. Its protein percentage ranges between 18.5 % - 20.8 %. It is one of the most common dairy food included in our day-to-day life. But like other indigenous dairy products, it is also very perishable. Thus, it suffers from limited shelf-life. Its shelf-life is reported to be only 6 days under refrigeration, though its freshness is lost within a day.

Many modern packaging methods have been developed to increase the shelf life of the paneer. The main function of the packaging methods is to maintain the

quality and safety of the product throughout its storage and transport and also helps to extend the shelf-life of the product by preventing the growth of spoilage microorganism. Some of the packaging methods for paneer are retort packaging, vacuum packaging etc., and they are very much helpful to increase the shelf-life of paneer. But, almost all the packaging material used in the food industry are non-biodegradable.

Therefore, in order to solve the problems generated by plastic waste, many efforts have been made to obtain an environmental friendly material. Most of the researches are focused on substitution of the petro-based plastics by biodegradable materials with similar properties and low in cost. So, there is a special interest in the food industry to develop eco-friendly, biodegradable and edible packaging material that not only increases the shelf-life of paneer but also maintain its flavor, texture, freshness and overall acceptability.

Several studies reported the use of cellulose and starch to prepare edible films and coatings with different properties (Bertuzzi *et al.,* 2007); (Larotonda *et al.,*2005); (Mali *et al*.,2005) .Carboxymethyl cellulose (CMC) is a cellulose derivative with carboxymethyl groups bound to some of the hydroxyl groups of the glucopyranosemonomers that make up the cellulose backbone. It is used in food under the E number E466 as a viscosity modifier or thickener, and is also used as a emulsion stabilizer in various products including ice cream.

Whereas, starch consist of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as energy storage. It is the most common carbohydrate in human diets and is contained in large amounts in staple foods like potatoes, wheat, maize, rice, and cassava. It is also used as food additives as thickeners and stabilizers in foods such as puddings, custards, soups, sauces, gravies, pie fillings etc.

To increase the efficacy of the edible packaging film, different essential oils are also incorporated by some researchers. Essential oils (EOs) are one of the most important natural products derived from plants for their various biological properties and medicinal uses. Essential oils (EOs), extracted from aromatic plants, are interesting natural products and represent an important part of the traditional pharmacopeia. An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils etc. The use of some EOs as alternative antimicrobial and pharmaceutical agents has attracted considerable interest recently. Most of the EOs and their single constituents have been reported to inhibit several phyto-pathogens, human-pathogens, and insects as well as their effective uses in food and pharmaceutical industries. Some of the essential oils used in food industry are clove oil, oregano oil etc.

Clove oils an essential oil extracted from the clove plant. It contains eugenol as a primary active compound. It has various medicinal value, it is used as anodyne (painkiller) for dental emergency, as a carminative and also as a natural anthelmintic.

Oregano oil is obtained from a perennial herb. It is considered as one of the most common culinary herbs, where its leaves can enhance the flavor of food. This species is used in traditional and modern medicine and in the pharmaceutical industry. It has some primary active ingredients like carvacrol and thymol. It has antimicrobial properties and used as folk medicine in ancient times.

Limited work has been done on developing biodegradable edible packaging film, based on CMC and starch that has treated with clove and oregano essential oils. So, this provides an opportunity to carry out further research in this area. With this view, the following research work was carried out with the following objectives:

Objectives:

1. Preparation and standardization of edible packaging film.
2. Standardization of edible packaging film with clove and oregano essential oil.
3. To study the shelf-life of paneer packaged with clove and oregano oil treated edible film.
4. To study the cost of production.

Review of Literature

2. REVIEW OF LITERATURE

According to FSSAI (2006), paneer has been defined as a product obtained from the cow or buffalo milk or combinations thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 70% moisture and milk fat content shall not be less than 50 % of the dry matter. The milk fat content of skim milk paneer shall not exceed 13.0 % of the dry matter.

2.1. Paneer

Khan and Pal (2011) represented paneer as a South Asian variety of soft cheese prepared by acid and heat coagulation of milk. It is a rich source of high quality animal protein, fat, minerals and vitamins.

Kumar *et al.,* (2011) stated that paneer is marble white in appearance, having firm, cohesive and spongy body with a close-knit texture and a sweetish-acidic-nutty flavour. Paneer is prepared using different types of milk and varied techniques results in wide variation in physico-chemical, microbiological and sensory quality of the product. Paneer keeps well for about a day at ambient temperature and for about a week under refrigeration (7 °C).

Raja *et al.,* (2014) attempted to develop processed paneer by partial addition of different levels of skimmed milk and soymilk. Soy paneer sample A (control) was prepared with 100% soymilk using citric acid at a concentration of 1.5% as coagulant. Sample B and Sample C were prepared with soymilk and skimmed milk in the ratio of 50:50 and 75:25 respectively with the addition of citric acid (1.5%) as coagulant. The result reveals sample containing 75:25 levels of soymilk and skimmed milk was liked most by the sensory panelists in comparison to other samples.

Viji *et al.,* (2017) utilizes goat milk for the preparation of paneer in combination with buffalo milk. Goat milk was incorporated at 25 percent (T1), 50 percent (T2) and 75 per cent (T3) levels with buffalo milk for the preparation of paneer. The result shows admixture of goat milk at 25 percent level with buffalo milk showed improvement in the yield of paneer.

Naik *et al.,* (2016) stated that paneer has great value in diet, as it is a rich source of high quality protein, fat, minerals and vitamins. Paneer has a short life span of about 5-7 days at refrigeration storage without much deterioration in the quality but freshness of the product is lost in three days.

Gadekar *et al.,* (2019) prepard paneer from milk of malpura sheep and found the fat per cent of paneer was for 46.17 %.

2.2. Shelf-life of paneer

Makhal (2000) investigated the role of GRAS additives like cardamom, clove, cinnamon and ginger in preservation of paneer. Paneer containing cardamom, clove, cinnamon and ginger each for low, medium and high doses showed shelf-life of 28,32 and 36 days; 24, 28 and 32 days; 24, 28 and 32 days and 32, 36 and 40 days respectively at 5±1˚C.

Rao and Patil (2001) investigated the application of Hurdle Technology for the preservation of Ready-to-Eat paneer curry. They employed more than one preservation parameter such as water activity (aw, pH and Redox-Potential) and heat treatment and recorded that paneer could be stored well for 30 days at 30˚C.

Smith *et al.,* (2001) investigated the efficiency of four plant essential oils; bay, clove, cinnamon and thyme as natural food preservatives in soft cheese against *Listeria monocytogene*and *Salmonella enteritidis* at 4°C and 10°C respectively, over a 14-day period. It was concluded that selected plant essential oils can act as potent inhibitors of *L. monocytogenes* and *S. enteritidis* in soft cheese.

Shan *et al.,* (2011) investigated the antibacterial efficiency of five spices and herb extracts (cinnamon stick, oregano, clove, pomegranate peel, and grape seed) against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica* in cheese at room temperature. Treatments with these extracts increased the stability of cheese against lipid oxidation. Clove showed the highest antibacterial and antioxidant activity. The reduction of food borne pathogens and the inhibition of lipid oxidation in cheese indicated that the extracts of these plants (especially clove) have potential as natural food preservatives.

Belewu *et al.,* (2012) studied the effect of eucalyptus oil and lemongrass oil on the shelf life of soft cheese. The treatments improved the nutrient composition and extended the shelf life of the cheese to four weeks as against three days for the control.

Mahgoub *et al.,* (2013) observed that the storage life of black cumin seed oil supplemented cheese was extended under refrigerated conditions with low microbial loads.

Oladipo and Jadesimi (2012) stated that dipping or boiling of West African soft cheese with ginger and/or garlic extract is a quite promising preservation technique.

Makhal *et al.,* (2014) revealed the effectiveness of thymol in extending keeping quality of cottage cheese. It was observed that addition of 40 ppm thymol enhanced the keeping quality by 8 days as compared to the control sample.

Singh *et al.,* (2014) reported that addition of turmeric at the rate of 0.6% by weight of expected yield of paneer extended the shelf life of paneer up to 15 days on storage at refrigeration temperature.

Eresam *et al.,* (2015) evaluated different spices (black pepper, cardamom, cinnamon and clove) for extending the shelf life of paneer. Among the studied spices, cardamom was found to be the best spice to improve shelf life of paneer up to 28 days of storage at 7 ± 1ºC. Control sample remained acceptable up to 7 days, black pepper and clove treated paneer up to 14 days and 21 days respectively.

2.3.Physico-chemical characteristics of paneer

2.3.1. pH

Belewu *et al.,* (2005) found an increasing trend in pH of soft cheese treated with garlic extract. The intial pH of control and garlic extract treated soft cheese was 6.50 and 6.20 respectively, which increased significantly.

Rai *et al.,* (2008) found that on storage of paneer for 15 days at 7 ± 1˚C, its pH decreased from 5.92 to 5.66, 5.71, and 5.63 respectively in samples packaged under vacuum packaging , MAP (100% CO2), and MAP (100% N2).

Bukhari *et al.,* (2012) observed a significantly decreasing pH value of Kaladhi (hard and dry cheese) prepared by using various organic acid coagulants.

El-Aziz *et al.,* (2012) stated that ginger extract fortified cheese caused an insignificant decrease in pH value of soft cheese during storage of 7 days at refrigeration temperature. The initial pH value of control and ginger extract fortified cheese was 5.86 and 5.80 respectively, which decreased to 4.94 and 4.78 respectively in 6 weeks.

Makhal *et al.,* (2014) reported a gradual decrease in pH of cottage cheese treated with thymol during storage. pH of fresh samples of cottage cheese ranged from 4.98 to 5.0, which steadily decreased up to a certain period of storage. The steady decline in pH was due to the slow rate of development of acidity.

2.3.2. Tyrosine value

Pal *et al.,* (1993) observed that the tyrosine content (mg/100 g) of fresh paneer increased from 13.84 to 41.53 on 10^{th}, and to 64.97 on 15^{th}day when stored at 8± 1˚C.

Sindhu *et al.,* (2000) reported that the tyrosine content (mg/g) increased from 0.14 to 0.42, and from 0.12 to 0.47 for buffalo and cow milk paneer respectively stored for 30 days at <10˚C. The increase in tyrosine value is an indication of proteolysis caused by microbial spoilage.

Rai *et al.,* (2008) studied the effect of Modified Atmosphere Packaging and Vacuum packaging on 45 storage ability of paneer. He revealed that tyrosine content value increased from 12.61 (mg/100 g) to 34.80, 29.18 and 33.21 respectively, for the samples packaged under vacuum package, MAP (100% CO2) and MAP (100% N2).

Singh *et al.,* (2014) reported that the tyrosine value of fresh paneer sample was 8.83±0.19 mg/100g paneer which increased significantly upon storage.

Khatkar *et al.,* (2017) (b) conducted a study on shelf life of paneer treated with clove essential oil .The paneer samples showed increase in tyrosine content, but within normal extent. According to 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±1ºC.

2.3.3.Thiobarbituric acid value

Okpala *et al.,* (2010) observed that the mean TBA values obtained for high pressure treated fresh cheese samples for Days 1 and 8 ranged from 1.3 to 6.4 and from 1.6 to 7.5 mg malonaldehyde/ gsample, respectively. However, TBA values of high pressure treated fresh cheese and control differed significantly ($p<0.001$). For both Days 1 and 8, TBA values of high pressure treated fresh cheese remained lower than control.

Abd-Rabou *et al.,* (2010) showed the changes in the thiobarbituric acid values (TBA) as affected by zinc fortification of Edam cheese. The average of TBA values as optical density (O.D.) was gradually increased along the ripening period. These values were higher in treated cheeses than in control. The TBA value of cheese fortified with zinc chloride was higher than other treatments, which reached 0.085 as O.D at the end of ripening period (12 weeks), but they were 0.047 and 0.035 for treatments with zinc acetate and zinc sulphate respectively, while it was 0.023 in control cheese.

Shan *et al.,* (2011) studied the potential application of spice and herb extracts (cinnamon stick, oregano, clove, pomegranate peel, and grape seed) as natural preservatives in cheese. The lipid oxidation (thiobarbituric acid-reactive substances) 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 9-day

storage, the TBA values of all treated cheese samples slightly increased, whereas the TBA values of the control sample significantly increased.

Bukhari *et al.,* (2012) reported that the TBA value (mg mal/kg) of kaladhi (hard and dry cheese) was 0.323 which significantly increased to 0.900 during storage. The increase in TBA value was probably due to growth of spoilage organisms.

Wagh *et al.,* (2014) evaluated the milk protein films for packaging of cheddar cheese. The initial TBA value of cheddar cheese was determined as 0.01 which increased to 0.05 for control and 0.03 to 0.04 for samples wrapped in protein films at the end of 30 days. There was no significant difference in the TBA values of control and film-wrapped samples at the end of 10 days. However, as storage progressed, control cheddar cheese samples exhibited considerably higher TBA value and consequently faster oxidation rate ($p < 0.05$) as compared with film-wrapped samples.

2.3.4. Titratable acidity

Khatkar *et al.,* (2017) (a) conducted a study to extend the shelf life of paneer treated with clove essential oil. The paneer samples showed increase in titratable acidity but within normal extent. According to 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±1ºC.

2.4 Proximate analysis

2.4.1. Moisture

Pal and Kapoor (2000) showed that the moisture content of paneer prepared from buffalo milk was 53.51%.

Belewu *et al.,* (2005) studied the effect of garlic extract on moisture content of West African soft cheese. They found that the moisture content in control group and garlic extract treated cheese were 62.30% and 62.15% respectively which decreased gradually with storage.

Masud *et al.,* (2007) found that the moisture content of paneer prepared from buffalo milk was 47.05%.

Kumar *et al.,* (2008) reported that moisture content was 50.98% in paneer prepared from buffalo milk (6% fat).

Rai *et al.,* (2008) studied the effect of MAP on the moisture content of paneer packed under four different atmospheres, namely air (atm 1), vacuum (atm 2), 100% CO_2 (atm 3), and 100% N_2 (atm 4) at 7±1˚C storage. The maximum loss in moisture content in the samples after 15 days of storage was observed in the case of atm 1, followed by atm 2, atm 4, and atm 3. On further storage for 45 days, the initial moisture content of 51.60% decreased to 51.16% (atm 2), 51.21% (atm 4), and 51.25% (atm 3), indicating that the minimum moisture loss had been in the samples packaged under 100% CO_2.

Desale *et al.,* (2009) observed that the moisture content of paneer ranged from 42.62 to 60.39%.

Karadbhajne and Bhoyarkar (2010) reported that moisture content in paneer prepared from buffalo milk was 51.50%.

Metwalli (2011) observed that the moisture content of Kareish cheese manufactured with 0.5% garlic extract decreased from 77.12% to 73.30% till the end of the storage.

Mahgoub *et al.,* (2013) observed that the moisture contents of control cheese & black cumin seed oil treated cheese gradually decreased during the refrigeration storage.

Makhal *et al.,* (2014) reported that moisture content of fresh cottage cheese varied from 72.90 to 73.10%; while at the end of storage period, it declined to 71.50 to 71.88%.

2.4.2.Protein

Pal and Kapoor (2000) reported that the protein content of buffalo milk paneer was 16.44%.

Belewu *et al.,* (2005) reported that the crude protein content in control and garlic extract treated West African soft cheese were 8.30% and 20.14% respectively. There was no significant reduction in protein content during storage.

Masud *et al.,* (2007) observed that protein content of buffalo milk paneer was 19.77%.

Kumar *et al.,* (2008) reported that protein content of paneer prepared from buffalo milk (6% fat) was 14.89%.

Desale *et al.,* (2009) stated that protein% of paneer ranged from 15.06 to 20.33%.

Karadbhajne and Bhoyarkar (2010) reported that protein content in paneer prepared from buffalo milk was 20.75%.

Metwalli (2011) reported that the protein content in control & garlic extract treated kareish cheese sample was 16.50 & 16.70% which decreased to 15.50 & 16.68% respectively during 30 days storage.

El-Aziz *et al.,* (2012) observed no significant change in the protein content of control sample & ginger extract fortified cheese during storage.

Mahgoub *et al.,* (2013) studied the physico-chemical properties of cheese supplemented with black cumin seed oil. They observed that the total protein content in fresh control & treatment sample were 17.50 & 17.60 respectively, which reduced to 13.90 & 13.59 % respectively during 30 days of storage..

Hamid (2014) reported that the protein content in Sudanese white cheese was 17.80%.

2.4.3. Fat

Pal and Kapoor (2000) reported that fat% of paneer prepared from buffalo milk (6% fat) was 24.12%.

Masud *et al.,* (2007) observed that fat% of buffalo milk paneer was 23%.

Kumar *et al.,* (2008) reported that fat% of paneer prepared from buffalo milk (6% fat) was 27.97%.

Karadbhajne and Bhoyarkar (2010) reported that the fat% in buffalo milk paneer was 25.88%.

Desale *et al*., (2009) stated that the fat% of paneer ranged from 16 to 28%.

Mahgoub *et al*., (2013) observed that fat% in control & black cumin seed oil treated cheese was17.50 and 17.60% respectively, which significantly increased to 21.50 & 21.90% respectively during 30 days storage.

Hamid (2014) reported that the fat content in Sudanese white cheese was 29.04%.

Gadekar *et al*., (2019) prepard paneer from milk of malpura sheep and found the fat per cent of paneer was for 46.17 %.

2.5. Microbiological changes of paneer

Wahi *et al.,* (2006) investigated the growth and survival of *Escherichia coli* O157:H7 during manufacture and storage of paneer. Milk has been inoculated with 104 CFU/mL of E. coli O157:H7 cultures for manufacturing paneer samples and vacuum-packaged and stored at 4°C, 8°C, and 28°C. Survival and growth of *E. coli* O157:H7 in paneer samples was determined after every 4 h for up to 48 h. *Escherichia coli* O157:H7 could survive the manufacturing process of paneer and were present at the end of the storage period (at 28°C). Though, refrigeration (4°C or 8°C) effectively inhibited the growth of *E. coli* O157:H7, this pathogen could survive over a period of 48 h.

Shrivastava *et al.,* (2011) conducted an experiment to see effect of MAP and storage on the microbial quality of laboratory-made paneer by packing under four different atmospheres: atmospheric air (atm 1), vacuum (atm 2), 100% CO2 (atm 3), and 100% N2 (atm 4), in pre-sterilized high barrier bags and stored at 7 ± 1°C. However, the study revealed that the initial standard plate count (SPC) log10 3.462 increased to log10 4.962, log10 3.817, and log10 4.012 respectively in samples packaged under atm 2, atm 3 and atm 4 after 45 days of storage, indicating that the minimum increase in the count had been in the samples packaged under 100% CO2 followed by atm 4 and atm 2 in ascending order. The analysis showed that the samples packaged under air had maximum anaerobic count. The initial mean value of yeast and mold count of paneer samples increased from log10 1.338 to 3.927 (atm 2), 3.109 (atm 3), and 3.468 (atm 4) after 45 days of storage, indicating that the minimum increase in yeast and mold count was in the samples packed under 100% CO2, thereby establishing the bactericidal and fungicidal effect of CO2.

Eresam *et al.,* (2015) used clove to improve shelf life of paneer. Clove at the rate of 0.6 % by weight of paneer was added and stored at 7 ±1°C for 28 days. After evaluation of standard plate counts (SPC), yeast and mold count and coliform count. The results indicated that control remained acceptable up to 7 days and clove upto 21days.

Rani *et al.,* (2014) prepared brine dipped and dry salted masala paneer products were prepared using fresh skim milk (fat 0.5% and solid not fat (SNF) 8.7%), vegetable oil (soybean oil), coriander leaves (1%), mint leaves (1%) and green chilies (0.3%), roasted and grounded cumin seeds (0.3%) and black pepper (0.3%). The paneer samples were examined for standard plate counts (SPC), yeast and mold counts and coliform counts at 2 days interval up to 8 days. SPC, coliform count and yeast and mold counts in the products were very low. The result concluded that the products were microbiologically safe for at least 6 days on refrigeration storage.

2.6. Sensory evaluation of paneer

Sanyal *et al.,* (2006) studied that the sensory scores (appearance, flavour, texture) of paneer decreased significantly with the storage

Foda *et al.,* (2008) studied and observed a decreasing trend in the sensory scores with storage in white herby cheese.

Kumar *et al.,* (2008) conducted experiment to determine the influence of different amount of lactic acid viz. 0.2, 0.4 and 0.6 per cent (w/v) on sensory attributes of buffalo milk paneer. Study revealed that the incorporation of lactic acid at the rate of 0.2 per cent brought about a significant ($P<0.05$) high sensory score.

Rai *et al.,* (2008) conducted experiment on the effect of MAP and storage at 7 ± 1°C on the sensory quality of laboratory-made paneer by packing under four different atmospheres: air (atm 1), vacuum (atm 2), 100% CO2 (atm 3), and 100% N2 (atm 4), in pre-sterilized high barrier bags. The result reveals that considering the sensory attributes, namely appearance, flavor, body, and texture and overall acceptability, the stored paneer samples packed under atm 3 were rated as best followed by atm 4, atm 2, and atm 1 in descending order.

Nandede *et al.,* (2012) prepared soy paneer from Soybean varieties JS-335, MAUS-82 and MAUS-2 and compared quality characteristics such as color, taste, appearance, body, texture and overall acceptability. The result shows significant better sensory quality of soy paneer.

Hamid (2014) studied significant variations in the colour, flavour, taste and texture of control and cumin oil treated cheese during the storage period.

Singh *et al.,* (2014) observed that the storage period had a significant effect on flavor, colour, appearance, body and texture scores of the paneer.

Chitranayak *et al.,* (2017) prepared paneer samples by applying varying pressure and time combinations and their characteristics were measured. The result reveals that as time pressing of the coagulum varied from 20-30 minutes and pressure increased from 7.6k Pa to 10.8 kPa enhances the sensory acceptance of the paneer.

Ahmed and Bajwa (2019) studied the effect of sour fruit juices as coagulants on sensory scores and texture profile analysis (TPA) using scanning electron microscopy (SEM) of paneer. The study conclude that sensory attributes of appearance/color, body and texture, mouth feel, flavor and overall acceptability was on high note but exhibited disparate values for TPA (hardness, adhesiveness, gumminess and resilience) and discrete structures under SEM.

2.7.Edible film

Larotonda *et al.,* (2005) conducted an experiment describing the use of cassava starch acetate (CSA) to impregnate Kraft paper and its influence on the water vapor permeability (Kw). The result demonstrated that CSA impregnation of Kraft paper is an interesting alternative for the improvement of the hygroscopic properties and water vapor permeability of the Kraft paper.

Bertuzzi *et al.,* (2007) studied the effect of film prepared by high amylose corn starch (HACS), and investigated film solubility in water, water sorption isotherms, opacity and crystallinity of films.

Su *et al.,* (2010) conducted an experiment on edible films based on carboxymethyl cellulose (CMC), compatibilized by glycerol, were prepared by

solution casting. The effects of CMC content on blend structure, thermal stability, water solubility and water absorption, and mechanical properties were systematically investigated. The results indicate that the structure and properties of SPI edible films were modified and improved by blending with CMC.

Falguera *et al.,* (2011) noted that edible films and coatings are thin layers of edible materials applied on food products that play an important role on their conservation, distribution and marketing.

Ghanbarzadeh *et al.,* (2010) prepared carboxymethyl cellulose (CMC) composite films by casting method. He investigated the effects of CMC addition on some physical properties of the resulted blend films . The result revealed the moisture absorption and solubility in water properties of the blend films exhibit similar trends but addition of CMC at the level of 20% W/W starch caused an increase in the ultimate tensile strength (UTS) by more than 59% in comparison to the pure starch film without any significant decrease in the strain to break (SB).

Ghanbarzadeh *et al.,* (2011) conducted an experiment by adding varying concentrations (0–20%, W/W) of citric acid (CA) and carboxymethyl cellulose (CMC) in corn starch film. And studied the effect CMC on the water vapor permeability (WVP), moisture absorption, solubility and tensile properties. The result shows at the level of 15% (W/W) CMC, the starch films showed the lowest WVP values (2.34×10−7 gPa−1 h−1m−1) and UTS increased from 6.57MPa for the film without CMC to 16.11MPa for that containing 20% CMC.

Tongdeesoontorn *et al.,* (2011) conducted an experiment based on cassava starch based edible film and concluded that plasticizer can added to improve the property of edible film.

Dashipour *et al.,* (2014) studied the effect of clove oil incorporated in Carboxymethyl cellulose (CMC) edible film for the beneficial in food packaging to retard of deterioration.

Singh *et al.,* (2014) developed polysaccharide based edible films i.e. carboxyl methyl cellulose (CMC) and sodium alginate (SA) along with glycerol (1%) as a plasticizer. The film was prepared by varying the concentration i.e., CMC (0.5,1.0,1.5%); SA(1.0,2.0,3.0%) and were evaluated for physical properties and mechanical properties. The result shows that the thickness , tensile strength , water vapour transmission rate, colour attributes increases whereas the moisture content and percentage elongation at break decreases with the increase in coating concentration irrespective of the type of film.

Šuput *et al.,* (2015) studied to extend shelf life of product base on eco-friendly edible films and coatings. The result shows edible films and coatings have potential to replace some traditional polymer packaging and it can be concluded that edible films and coatings can be chosen for food packaging purpose according to specific applications, the types of food products, and the major mechanisms of quality deterioration.

Tesfay *et al.,* (2017) investigated the potential of edible carboxyl methylcellulose (CMC) containing moringa leaf and seed extracts as a novel postharvest treatment for maintaining storage quality and controlling diseases in ‘Hass’ and ‘Gem’ avocado fruit. The result shows CMC containing moringa extract suppresses diseases, prolongs the shelf-life and maintain the overall quality avocados during postharvest supply chain.

Zillo *et al.,* (2018) studied the effect of *Eucalyptus staigeriana, Lippiasidoides and Pimentapseudocaryophyllus* essential oils (EOs)on carboxymethyl cellulose coating in the post-harvest treatment of papaya to avoid

post-harvest losses. The result shows reduction in disease severity and maintained post-harvest parameters and increases shelf-life of papayas.

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2.8.Glycerol

Perez *et al.,* (2016) studied the different contents of glycerol (Gly).
As a plasticizer in order to evaluate new edible film formulations for their potential use in food packaging applications. Additionally, potential changes in the film mechanical properties during storage at ambient and freezing conditions were considered. The result shows a better design applications of edible films containing plasticizers that may crystallize over time in order to optimize film formulation in a rational manner towards their eventual application as food packaging.

Echeverría *et al.,* (2016) conducted an experiment in potential use of biopolymer layered silicate systems as active food packaging. Active nanocomposite films were prepared by casting from aqueous dispersions containing soy protein isolates (SPI), glycerol as a plasticizer, different concentrations of MMT and clove essential oil. The result revealed that the addition of clove essential oil exerted a plasticizing effect, which was verified in a decrease in the tensile strength and elastic modulus (up to 50 and 75 %, respectively) and an increase of the water content of films (up to 20 %).

2.9. Essential oils

Havanur and Adi (2014) conducted an experiment the *in vitro* antibacterial activity of clove oil against the microorganism contaminating and spoiling panner by Agar-well diffusion assay. The result shows clove oil treated panner could extend the shelf life to 40 days.

Echeverría *et al.,* (2016) conducted the effect of clove essential oil addition to soy protein–montmorillonite (MMT) films on the material's structure, functionality, and active release. The presence of Clove essential oil enhances favored of montmorillonite into the soy protein matrix, while the nanoclay seemed to promote the release of active compounds.

Jalali *et al.,* (2016) studied the effects of carboxyl methylcellulose composite coating incorporated with clove essential oil on quality of silver carp fillet chilled storage (4 + 1 °C). were examined over a period of 16 days. The results indicate carboxyl methylcellulose composite coating with clove essential oil may be recommended as a preservative in the meat products.

Artigas *et al.,* (2017) conducted an experiment to prepare nanoemulsion-based edible coatings containing oregano essential oil (OEO) and studied antimicrobial effect onto low-fat cut cheese. The result shows effective antimicrobial property against *Staphylococcus aureus* and helps to increase Shelf-life of the low-fat cut cheese.

Khatkar *et al.,* (2017) (a) analysed to extend the shelf life of paneer with the addition of natural antimicrobial agent i.e. clove essential oil. The result revealed that 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±1ºC

Khatkar *et al.,* (2017) (b) investigated the shelf life of paneer treated with cinnamon oil and the result revealed that samples showed shelf life of only 10 days with metalized polyester, 8 days with nylon and 5 days with LDPE, while cinnamon treated samples showed shelf life of 18 days with metalized polyester, 14 days with nylon and 9 days with LDPE at 8±1ºC.

Munhuweyi *et al.,* (2018) investigated the physical and antimicrobial properties of fabricated nano-fibre for active packaging systems. Active microcapsules and nano-fibres derived from precipitating β-cyclodextrin (β-CD) with oregano (OREG) essential oils (EOs) were developed and their in vitro antifungal activity against *Botrytis sp*., an important fruit pathogen was determined. The result shows the nano-fibres ability to release oregano (OREG) EO volatiles and high antifungal activity.

Pathania *et al*., (2018) noted the review emphases on clove essential oil based nanoemulsions which are prepared with different ingredients which hence, enhance the antimicrobial action in food items.

Liu *et al.,* (2019) conducted an experiment and used oregano essential oil to developed fabricated soluble soybean polysaccharide (SSPS)-based films. The result shows immobilization effect held more essential oils within the film matrix during film preparation and showed prolonged antimicrobial property against pathogenic bacteria.

2.10. Physcio-chemical properties of edible film

Rodrı´guez *et al.,* (2006) made film of potato starch were and glycerol as plasticizer and Tween 20, Span 80, and soy lecithin as surfactants. Water vapour

permeability (WVP) and plasticizer effect were analysed . The result shows films with glycerol and high level of any of the surfactants behaved as films with larger amount of plasticizer (with lower tensile strength, higher elongation, and higher WVP).

Bifani *et al.,* (2007) studied the water vapor permeability (WVP) of carboxymethylcellulose (CMC)-based films .The possible effect shows SC acts only as barrier to the oxygen.

Cheng *et al.,* (2008) analyzed the Water vapour permeability prepared from konjacglucomannan (KGM), carboxymethylcellulose (CMC), with and without alkali (KOH) and studied water vapour permeability. The result shows smaller lipid globules, with a homogeneous distribution, increased the apparent hydrophobicity and ‘‘tortuosity” of an emulsion film for water molecule transmission, thereby resulting in reduced WVP.

Su *et al.,* (2010) prepared edible films based on carboxymethyl cellulose (CMC) and soy protein isolate (SPI), compatibilized by glycerol, were prepared by solution casting. The effects of CMC content on blend structure, thermal stability, water solubility and water sorption, and mechanical properties were systematically investigated. The results indicate that the structure and properties of SPI edible films were modified and improved by blending with CMC.

Ramírez *et al.,* (2012) studied wettability and water-barrier properties of film solution based on carboxymethylcellulose (CMC) with murta leave (Ugnimolinae Turcz) extract. The result shows the WVP was not affected by murta leave extract incorporation.

Kibar and Ferhunde (2013) investigated the effect of the addition of methylcellulose and carboxymethyl cellulose on the thermal, mechanical and water adsorption properties of starch-based films plasticized with glycerol or polyethylene glycol (PEG). Eventually, it can be concluded that film forming properties of starch can be improved by incorporation of methylcellulose and carboxymethyl cellulose to the polymer matrix.

Ebrahimi *et al.,* (2018) studied, elongation at break and water vapor permeability ofedible films based on carboxymethyl cellulose (CMC).

Materials and Methods

3. MATERIALS AND METHODS

The present study on extending the shelf life of paneer packed with edible film treated with essential oils was performed in various phase. The material used and the methodology employed during the study are discussed here under.

3.1. Materials

3.1.1. Milk

Freshly drawn buffalo milk was brought from cattle and buffalo farm of Mumbai Veterinary College and used in all the experiment. The fat percentage and S.N.F was standardized to 6% and 9 % respectively for optimum product characteristics.

3.1.2. Acid coagulant

GDL was used to coagulate the standardized milk for the preparation of paneer. The strength of the coagulant was 1%.

3.1.3. Procurement of media, Chemicals and reagents

Chemicals and reagents required for experiment (Starch, Carboxyl methyl cellulose, Glycerol, Citric acid, Trichloroacetic acid, TBA reagent, Folin and Ciacalteu's reagent, etc.) were procure from standard manufacturers. All the chemicals of analytical grade, required for various analyses were procured from standard firm's viz., Himedia chemicals Pvt Ltd, and S.D Fine Chemicals Pvt Ltd, etc. Whereas clove and oregano essential oil was procured from Synthite industries Pvt Ltd.

3.1.4. Procurement of glassware and plastics

Glassware required for experiment (pH, TBA, Tyrosine, Titratable acidity, Moisture, Protein, Fat, Water-vapor permeability, Film solubility and Microbiological examination) were procured from standard manufacturers Himedia chemicals Pvt Ltd, and S.D Fine Chemicals Pvt Ltd, etc.

3.2 .Experimental No.1

3.2.1. Preparation and standardization of edible packaging film.

Preparation and standardization of edible film was be done by adding Starch (5 g) at (25°C for 5 min) with distilled water(100 mL) and glycerol (40 ml/100 g starch) and 0.5 CA (10% W/W starch).These suspensions will then agitated by magnetic stirrer (500rpm) for 30 min in water bath (90 °C). Carboxymethyl cellulose (15 % W/W starch) was selected as film produced from (0%,5%,10% CMC added to starch(W/W) was very brittle and film with 20% CMC added W/W starch was producing a very thick film. So, 0.75 gm CMC was solubilized in 75 ml of distilled water at 75 °C for 10 min. The CMC and starch solutions was mixed together (75 ml CMC-solution+100 ml starch-CA-solution) and stirred at 75 °C for 10 min (PH=5.5). Dispersions were then cooled at 40 °C and mixed gently for 20 min to release all air bubbles. (Ghanbarzadeh *et al*.2011). Then, about 40 mL of the sample will be poured onto petri dish and allow to settle down to form film. Then, the following attributes will be analysed.

3.2.2. Physicochemical characterization of edible film

(a) Thickness

The thickness of each film sample was measured using a 0-10-mm manual micrometer (Swastik Scientific Company, Mumbai) with an accuracy of 0.01 mm. Measurements were taken at eight different points on each film sample, and the average values were represented as the film thickness.

(b) Moisture content

The moisture content of each film sample was measured according to the method of Soradech *et al.*(2013). For this, each film sample was cut into small pieces (3×2 cm) and dried in a hot air oven at 100°C for 12 h. After drying, the weight loss was measured as moisture content and expressed as a percentage based on the initial weight of film. All experiments were carried out in triplicate:

$$\text{Moisture \%} = \frac{W1 - W2}{W1} \times 100$$

Where,

W1 = initial weight of the film; and

W2 = weight of the film after drying

(c) Water vapor permeability

Water vapor permeability (WVP) was measured using a modified ASTM (American Society for Testing and Materials) method as described by Gontard *et al.* (1992) with slight modification. The film samples were sealed on a conical flask containing dried silica gel (0 per cent relative humidity) with the help of parafilm. The flasks were placed at 20°C in a dessicator containing distilled water, followed by

weighing after every 1 h interval for up to 8 h until the weight became constant. WVP of the film was calculated as follows:

$$WVP\ (g\ m\ s^{-1}\ Pa^{-1}) = w \times 1 \times A^{-1} \times t^{-1} \times (P2 - P1)^{-1}$$

Where,

W = weight gain of the flask (g);

l = film thickness (mm).

A = exposed area of film (m2);

t = time of gain (s); and

(P2 - P1) = vapour pressure difference across the film (2237 Pa at 20°C).

(d) <u>Film solubility</u>

The solubility of film samples in water was measured by the method of Ghanbarzadeh *et al.*(2010) with slight modifications. Film samples were cut into 3 × 2 cm pieces and dried at 100°C for 12 h. The dried film samples were dissolved in distilled water (20 ml) under gentle agitation using a magnetic stirrer at room temperature. After 2 minutes dissolution, the film was taken out and dried in the oven at 105°C until the weight became stable. Before and after dissolution, the accurate weights of the dried film sample pieces were measured, and the weight difference was considered as soluble solids. The water solubility of film was calculated as a percentage based on the initial weight of film. All the experiments were performed in triplicate:

$$\text{Film solubility (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where,

W1 = dried weight of the film before dissolution; and

W2 = dried weight of the film after dissolution.

(e) Elongation at break

For calculation of EAB, edible films were cut into 8 × 2 cm strips, then fixed on manually formed scale, and the films were stretched manually until they broke. The EAB of the samples was calculated as follows:

$$\text{Elongation at break (\%)} = \frac{A - B}{A} \times 100$$

Where,

A = initial point on scale; and

B = final point on scale after stretching of edible film.

(f) Color of film

The color of film was measured using a Lovibond Tintometer (Model F, Greenwich, UK). Samples were cut with the help of scissors to the inner diameter of sample holder and secured against the viewing aperture. The sample colour was matched by adjusting the red (a*) and yellow (b*) units, while keeping the blue unit fixed at 3. The corresponding colour units were recorded. The hue and chroma values were determined by using the formulae, $\tan^{-1}(b/a)$ and $(a2 + b2)^{1/2}$, respectively, where a = red unit and b = yellow unit.

(g) pH of the film

The pH of edible packaging film was determined. 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).

3.3.Experiment No.2

3.3.1.Assessment of shelf life of paneer packed with edible film treated with clove and oregano essential oil.

(a) Preparation of paneer

Paneer was prepared by the method as suggested by Lamdande *et al*., (2012) with slight modifications and the paneer cubes were packed in edible packaging film treated with clove and oregano essential oil. Fig.3.1.

Fresh buffalo milk

⇩

Standardized to 6% fat

⇩

Heat it to 90˚C for 5min

⇩

Cool it to 70˚C

⇩

Add GDL solution (1% solution at 70˚C)

⇩

Allow the coagulum to set

⇩

Fill the coagulum into muslin cloth lined in cylindrical hoop and press for 10-15min

⇩

Immerse in chilled water for 2hrs

Fig.3.1 Preparation of paneer

Paneer was cut into cubes and packed in edible packaging film treated with different concentrations of clove and oregano essential oil viz. 0.25%, 0.37%, 0.5% and 0.75%. The treated samples were analyzed for sensory evaluation. Based on the results, 0.5% clove and 0.5% oregano essential oils treated edible packaging film were selected for further studies. The treated and control paneer were packed in LDPE was analyzed for physico-chemical and microbiological attributes at an interval of 3 days till spoilage at (4±1˚C).

(b) Sensory evaluation

The freshly prepared control and treated paneer sample were organolepticaly evaluated by semi-trained panel of 6 judges and it was repeated at 5 days interval till 15 days . The samples were judge for various sensory attributes using nine point descriptive scales (Keeton, 1983). The score of 6 judges were averaged and recorded as mean value for sensory score. Each panelist evaluated 5 samples (identified by codes) in a balanced sequential order.

(c) Physico-chemical analysis of paneer

Freshly prepared control and treated paneer was subjected to sensory evaluation by the panel of judges and zero day analysis 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 3 days till spoilage.

(d) pH

The pH of fresh and stored paneer sample (control and 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).

(e) Thiobarbituric Acid Value

TBA number of fresh and stored paneer sample (control and 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 grams 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 hour. After cooling the test tubes, the absorbance (A) at 530nm was measured in a spectrophotometer. (Model no. EQ 820 with wavelength range of 350-950 nm, INDIA).

(f) Tyrosine Value

The 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 g of sample by referring to a standard graph, which was prepared as per the procedure described by Pearson (1968).

(g) Titratable acidity

The titratable acidity of the control and treated sample were determined by AOAC (1995). In which 1gm of sample is added to 10 ml of D.W and mixed well then 1ml of phenolphatalin indicator is added and titrated it against N/9 NaOH (end point- pink colour)

(h) Proximate composition

The moisture, protein and fat contents of control sample were determined as per the standard procedures of Association of Official Analytical Chemists (AOAC, 1995).

(i) Moisture

Around 10g accurately weighed sample was placed in hot air oven at 100±1˚C for 16-18 hrs. After cooling it in desiccator for 10 min, the loss of moisture was determined and expressed as percent moisture of sample.

Moisture was calculated by using the following formula:

$$\%\text{Moisture} = \frac{W1 - W2}{W1} \times 100$$

W1= Initial weight of sample

W2 = Final weight of sample

(j) Fat

Accurately weighed samples in thimbles were dried overnight at 50˚C in hot air oven. The fat was extracted with petroleum ether (BP 60-80˚C) in Soxhlet's apparatus. Extracts in oil flask was dried at 60˚C in hot air oven for overnight. Next day the oil flask was cooled and weighed. Ether extract was calculated by difference in weight of dried oil flask before and after extraction. Fat percentage was calculated by using the following formula:

$$\text{Crude lipid content \%} = 100 \times \frac{B-A}{C}$$

Where,

A = weight of clean dry empty flask (g)

B = weight of flask with fat (g)

C = weight of sample (g)

(k) Protein

Nitrogen content of samples was estimated by the Kjeldahl method and protein content was expressed by multiplying the nitrogen value with constant factor 6.25 and taken as the crude protein content in the sample.

Calculation:

$$\%N = \frac{0.0014 \times V \times 100}{Z \times W}$$

Where,

V= volume of 0.1N H_2SO_4 neutralized by NH_3

W= weight of the sample

Z= volume of aliquot taken for distillation

Crude Protein %= N% x 6.25

(l) Microbiological analysis

Total plate count, psychrophilic count and yeast and mold count of paneer samples were estimated following the standard method of APHA (1992).

(m) Preparation of serial dilution

10 g of samples were made into paste in a sterile mortar and fine suspension was prepared by adding 90 ml of sterile Normal saline solution using a sterile pestle 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.

(n) Estimation of Total Plate Count (TPC)

All the petri plates were labeled with sample numbers and 15-20 ml of pre-sterilized molten total plate count agar was poured and allowed to solidify. Then 1 ml of appropriate dilution was transferred in petri dish in duplicate. The inoculum was streak with L-shaped spreader, and the plates were incubated at 37˚C for 24 hrs.
TPC was calculated by using the following formula:

$$\text{CFU/gm} = \frac{\Sigma c}{[n1 + (0.1 \times n2)] \times d}$$

Where:
Σc = Total no. of colonies developed on all the plates
n1= number of plates of lower dilution
n2=number of plates of higher dilution
d= dilution factor corresponding to lower dilution
CFU= colony forming unit

(o) Psychrophillic count

The procedure outlined for total plate count was followed for psychrophillic count except incubation, where the plates were incubated at 4˚C for 5-7 days. Colonies were counted and results were expressed in a similar way.

(p) Coliform count

All petri plates were labeled with sample number and 15-20 ml of pre-sterilized molten VRBA was poured and allowed to solidify. Then 1 ml of 10^{-2} and 10^{-3} dilution was transferred in petri dish in duplicate. The inoculum was streak with L-shaped spreader, and the plates were incubated at 37˚C for 24 hrs.
Coliform count was calculated using the following formula:

$$\text{CFU/gm} = \frac{\sum_c}{[n_1 + (0.1 \times n_2)] \times d}$$

Where:

$\sum_c$ = Total no. of colonies developed on all the plates

n_1 =No. of plates retained in lower dilution

n_2 = No. of plates retained in higher dilution

d = Dilution factor corresponding to lower dilution

(q) <u>Yeast and mold count</u>

All petri plates were labeled with sample number and 15-20 ml of pre-sterilized molten Sabouraud's dextrose agar was poured and allowed to solidify. Then 1 ml of appropriate dilution was transferred in petri dish in duplicate. The inoculum was streak with L-shaped spreader, and the plates were incubated at 25˚C for 5-7 days. The colonies were counted by following formula:

Yeast and mold count was calculated using the following formula.

$$\text{CFU/gm} = \frac{\sum_c}{[n_1 + (0.1 \times n_2)] \times d}$$

Where:

$\sum_c$ = Total no. of colonies developed on all the plates

n_1 =No. of plates retained in lower dilution

n_2 = No. of plates retained in higher dilution

d = Dilution factor corresponding to lower dilution

(r) <u>Statistical analysis</u>

The data obtained during the experiment was analyzed by Analysis of Variance following standard procedure (Snedecor and Cochran, 1989).

Results and Discussions

4. RESULTS AND DISCUSSION

The present study was planned to assess the stability of paneer packed with edible packaging film treated with clove and oregano essential oil at refrigeration storage temperature. The study was divided into three phase. In, first phase standardization of CMC and starch based film were prepared by analyzing different concentration of CMC over starch based film. Finally 15% CMC w/w of starch were selected for the preparation of the film. Then, the parameters like thickness of film, water vapour permeability , water solubility , elongation at break , colour of film, moisture of film and pH of film were analyzed. In second, phase of the study the film was treated with different concentration of clove and oregano essential oils viz,.0.25%, 0.37%, 0.50% and 0.75%.After the sensory evaluation, 0.50% of clove and 0.5% of oregano oil were selected for further packaging of paneer. In third, stage of study the paneer separately wrapped with clove treated edible film and oregano treated edible film and evaluated the storage of paneer against freshly prepared paneer. The control and two treated sample was packed in food grade LDPE bags and analyzed at the interval of 3 days till spoilage.

The present research work was carried out in the Department of Livestock Products Technology of Mumbai Veterinary College, Parel, Mumbai.

The Results obtained in the present investigation are discussed here under.

4.1. Parameters of the edible packaging film

Thickness, Water Vapour Permeability, Film Solubility, Moisture Content, Elongation at Break, pH of the edible packaging film were0.09±0.01mm, $2.07\pm0.02\times10^{-7g}$/m.h.Pa,84.33±1.45,22.51±1.48,22.60±1.48,5.67±0.02 respectively, concluded after three basic trial values are in accordance with Ghanbarzadeh *et. al.,* (2010),Soni *et al.,* (2016), Salgado *et al* (2013) and Navarro-Cruz *et al.* (2018). Thickness, WVP, Solubility, Moisture Content, Elongation at Break, pH of edible

film were 0.08mm, 2.34×10−7 g/m.h.Pa, 80.42±3.1.22.51±1.48,20.83±4.76 and 5.67±0.02 respectively.

4.2.Colour of film

The Hunter color values (L, a and b), total color difference (X), yellowness index (Y),and whiteness index (Z) of starch films as a function of CMC content. The value of L* was 12.91±0.1, a* was -0.01±0.03, b*-1.72±0.07,X was 1.47±0.03,Y was 1.55±0.03 and for Z was 1.84±0.03 similar result was reported by Ghanbarzadeh *et. a.l.* (2010) and Soni *et. al.*,(2016*).*

4.3.Proximate analysis of paneer

Freshly obtained paneer samples were analysis for proximate composition. The value of Moisture content of paneer was 51.67±1.42 %, fat content was 24.43±0.8 and protein content was 17.70±0.49. The moisture content of paneer was found similar by Masud *et al.,* (2007) and Kumar *et al.,* (2008). Desale *et al*., (2009) stated that the fat% of paneer ranges from 16-28%. Desale *et al.,* (2009) stated that protein% of paneer ranged from 15.06 to 20.33%.

Table 4.1 Proximate composition of paneer

Treatments	Moisture %	Fat %	Protein %
C	51.67±1.42	24.43±0.8	17.70±0.49

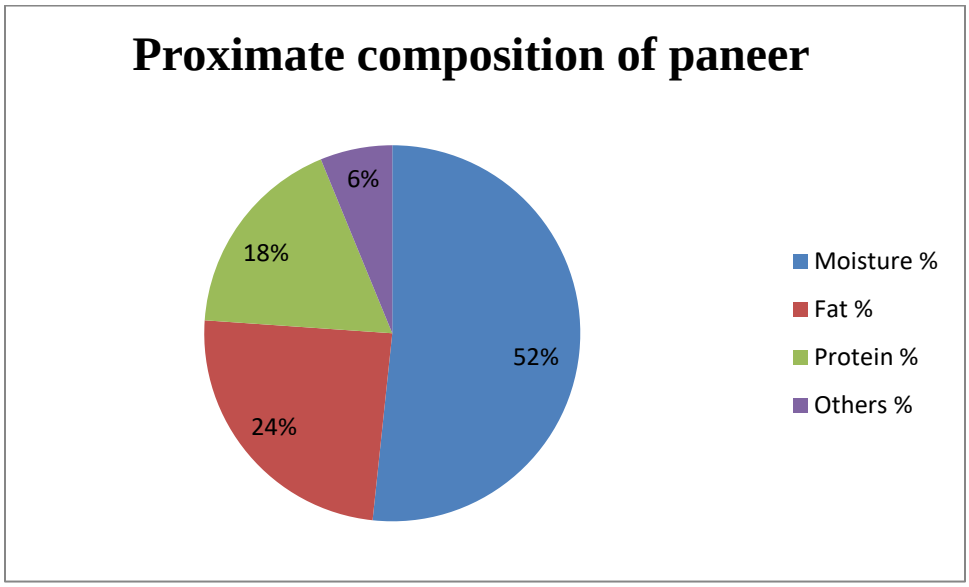

Fig 4.1 Proximate composition of paneer

4.4. Physico-chemical parameters of paneer

Physico-chemical parameters like pH, titratable acidity, TBA and Tyrosine were evaluated for control and treated paneer at the interval of 3 days till spoilage.

4.4.1. Changes in pH of control and treated paneer

The changes in pH values during storage of control and essential oil treated edible film packed paneer are tabulated in the Table 4.2 and depicted in the Fig. 4.2.

The average pH values during entire storage period for control were 5.85±0.3, 5.83±0.04, 5.77±0.02, 5.76± 0.01, 5.56±0.01 and 5.55±0.03. The pH values for day 0 day, 3 day, 6 day and day 9 were non-significant but values of 0 day, 3 day 6 day and 9 day were significantly different from values of day 12 and day 15. The average pH values during entire storage period for T1 were 5.85±0.03, 5.75±0.01, 5.74±0.03, 5.65±0.02, 5.64±0.03 and 5.63±0.04. The pH values for day 0 , day 3 and day 6 were non-significant, as well as on day 9, day 12 were non-significant but there were significant difference among the values of day 0, day 3, day 6 with day 9, day 12 and day 15 and there were significant difference among the values of day 9 and day 12 with the value of day 15. pH values for T2 were 5.87±0.02, 5.77±0.02, 5.75±0.03, 5.69±0.01, 5.71±0.04 and 5.69±0.03. The pH values for day 0, day 3 and day 6, day 9, day 12 were non-significant but the values were significantly different with day 15.

The average pH values for control, T1 and T2 were 5.85±0.03, 5.85±0.003, and 5.87±0.02 respectively on day 0. No significant difference was found in between control and treatments at ($p<0.05$). On day 3, the average pH values for control, T1 and T2 were 5.83±0.04, 5.75±0.01 and 5.77±0.02 respectively. No significant difference was found in between control and treatments at ($p<0.05$). On day 6, the average pH values for control, T1 and T2 were 5.77±0.02, 5.74±0.03 and 5.75±0.03, respectively. There was no significant difference observed between control and

treatments at ($p<0.05$). The average pH values for control, T1 and T2 were 5.76±0.03, 5.65±0.02 and 5.69±0.01 respectively on day 9th. There was no significant difference observed between control and treatments at ($p<0.05$). On day 12, the average pH values for control, T1 and T2 were 5.56±0.01, 5.64±0.03 and 5.71±0.04, respectively. There was no significant difference observed between control and treatments at ($p<0.05$). On day 15, the average pH values for control, T1 and T2 were 5.55±0.03, 5.63±0.03 and 5.69±0.03, respectively. There was significant difference observed between control and treatments at ($p<0.05$). Similar results were reported by Rai *et al.,* (2008) for pH of paneer and Makhal *et al.,* (2014) for pH of cottage cheese during the storage period.

Table 4.2 Changes in pH of control and treatment paneer during storage

TREATMENT	C	T1	T2
0 DAY	$_{a}5.85\pm0.03^{A}$	$_{a}5.85\pm0.03^{A}$	$_{a}5.87\pm0.02^{A}$
3 DAY	$_{a}5.83\pm0.04^{A}$	$_{a}5.75\pm0.01^{A}$	$_{a}5.77\pm0.02^{A}$
6 DAY	$_{a}5.77\pm0.02^{A}$	$_{a}5.74\pm0.03^{A}$	$_{a}5.75\pm0.03^{A}$
9 DAY	$_{a}5.76\pm0.03^{A}$	$_{b}5.65\pm0.02^{B}$	$_{a}5.69\pm0.01^{A}$
12 DAY	$_{b}5.56\pm0.01^{B}$	$_{b}5.64\pm0.03^{B}$	$_{a}5.71\pm0.04^{A}$
15 DAY	$_{b}5.55\pm0.03^{A}$	$_{c}5.63\pm0.04^{B}$	$_{b}5.69\pm0.03^{C}$

*Note: 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 ($p<0.05\%$).

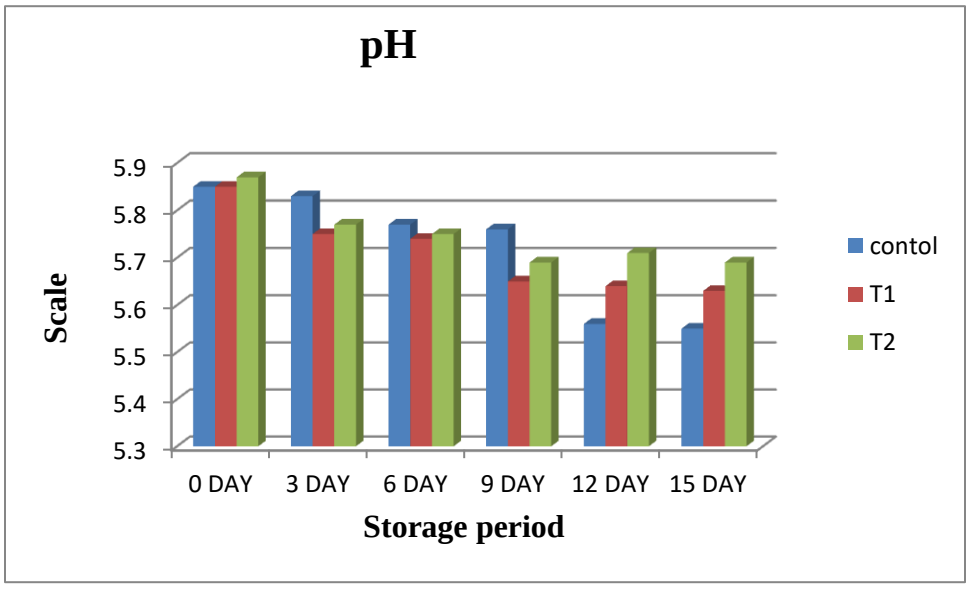

Fig 4.2 Changes in pH of control and treatment paneer during storage

4.4.2. Changes in tyrosine value

The tyrosine value of freshly prepared paneer and changes during storage are highlighted in Table 4.3 and depicted in Fig. 4.3.

Table 4.3 shows that the average tyrosine values during entire storage period for control were 12.52±0.06, 15.38±0.05, 19.78±0.05, 31.14±0.02, 40.53±0.03, 47.54±0.25. A significant ($p<0.05$) difference was observed between 0^{th}, 3^{rd}, 6^{th}, 9^{th} , 12^{th} and 15^{th} day of storage. During entire storage period, average tyrosine values for T1 were 12.47±0.01, 15.88±0.05, 17.90±0.04, 27.35±0.12, 31.51±0.13 and 37.66±0.08 which shows a significant difference at the level ($p<0.05$). During the entire storage period, the average tyrosine values for T2 were 12.45±0.01, 15.84±0.04, 17.08±0.01, 25.16±0.02, 30.20±0.05 and 35.65±0.22. A significant ($p<0.05$) difference was noted between 0^{th}, 3^{rd}, 6^{th}, 9^{th}, 12^{th} and 15^{th} day of storage.

The average tyrosine values for control, T1 and T2 were 12.52±0.06, 12.47±0.01 and 12.45±0.01 respectively on 0^{th} day. These values showed a non-significant difference on 0 day ($p<0.05$). On 3^{rd} day of storage, the average tyrosine values for control, T1 and T2 were 15.38±0.05, 15.88±0.05 and 15.84±0.04 respectively. A significant ($p<0.05$) difference was noted in all the samples. On 6^{th} day of storage, the average tyrosine values were 19.78±0.05, 17.90±0.04 and 17.08±0.01 for control, T1 and T2 respectively. All these values showed a significant difference at ($p<0.05$). The average tyrosine values on 9^{th}day 31.14±0.02, 27.35±0.12 and 25.16±0.02 for control, T1 and T2 respectively. A significant ($p<0.05$) difference was found in all the paneer samples. On 12^{th} day of storage, the average tyrosine values 40.53±0.03, 31.51±0.13 and 30.20±0.05 for control, T1 and T2 respectively. All these values showed a significant difference in relation to control and treatment ($p<0.05$). The average tyrosine values on 15^{th} day 47.54±0.25, 37.66±0.08 and 35.65±0.22 for control, T1and T2 respectively. A significant ($p<0.05$) difference was found in all the paneer samples.

The results for tyrosine values are similar as reported by Pal *et al.,* (1993) and Rai *et al.,* (2008) for paneer. At the end of storage, the tyrosine value for control was much higher as compared to T1 and T2, which indicates less proteolysis in treated paneer samples.

Table 4.3 Changes in tyrosine value of control and treatment paneer during storage

TREATMENT	C	T1	T2
0 DAY	$_{a}12.52\pm0.06^{A}$	$_{a}12.47\pm0.01^{A}$	$_{a}12.45\pm0.01^{A}$
3 DAY	$_{b}15.38\pm0.05^{A}$	$_{b}15.88\pm0.05^{B}$	$_{b}15.84\pm0.04^{C}$
6 DAY	$_{c}19.78\pm0.05^{C}$	$_{c}17.90\pm0.04^{B}$	$_{c}17.08\pm0.01^{A}$
9 DAY	$_{d}31.14\pm0.02^{C}$	$_{d}27.35\pm0.12^{B}$	$_{d}25.16\pm0.02^{A}$
12 DAY	$_{e}40.53\pm0.03^{C}$	$_{e}31.51\pm0.13^{B}$	$_{e}30.20\pm0.05^{A}$
15 DAY	$_{f}47.54\pm0.25^{C}$	$_{f}37.66\pm0.08^{B}$	$_{f}35.65\pm0.22^{A}$

*Note: 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 ($p<0.05\%$)

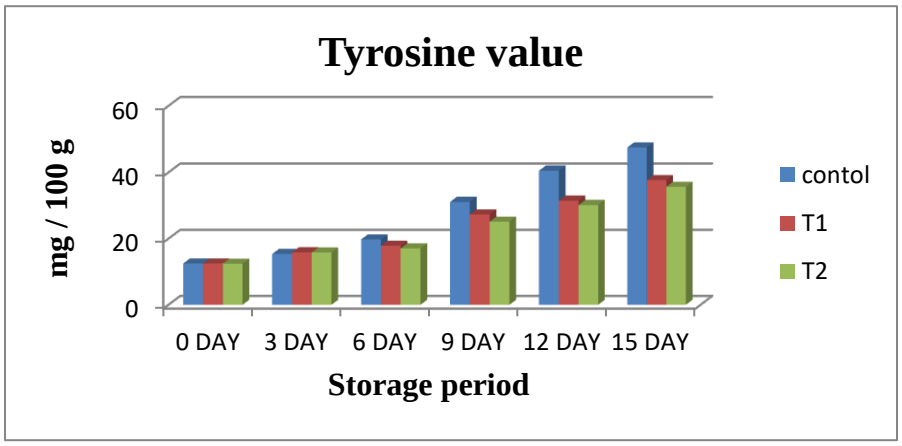

Fig 4.3 Changes in tyrosine value of control and treatment paneer during storage

4.4.3. Changes in TBA value of control and treated paneer

Table 4.4 depicts the values for TBA of freshly prepared paneer changes during storage period, the same are depicted in Fig.4.4.

It is clear from the Table 4.4, that the average TBA values during the entire storage period for control were 0.22±0.01, 0.36±0.01, 0.48±0.1, 0.68±0.02, 0.93±0.02 and 1.16±0.06 which indicates that there is a significant ($p<0.05$) difference between 0, 3 ,6 ,9 ,12 and 15 days of storage. The average TBA values during the entire storage period for T1 were 0.22±0.02, 0.31±0.02, 0.36±0.2, 0.61±0.02, 0.87±0.03 and 0.94±0.07 indicating a significant ($p<0.05$) difference between 0, 3, 6, 9, 12 and 15 days of storage. During the entire storage period, the average TBA values for T2 were 0.23±0.02, 0.26±0.02, 0.32±0.06, 0.32±0.06, 0.55±0.02, 0.74±0.03 and 0.86±0.08 showing a significant ($p<0.05$) difference between 0, 3, 6, 9, 12 and 15 days of storage.

The average TBA values for control, T1, and T2 were 0.22±0.02, 0.22±0.02, 0.23±0.02 respectively on day 0. No significant difference was found in between control and treatments at ($p<0.05$). On day 3 the average TBA values for control, T1 and T2 were 0.36±0.01, 0.31±0.02, 0.26±0.02 respectively . There was significant difference observed in between control and T1 and T2. However, there was no significant ($p<0.05$) difference was found in between T1 and T2. On 6^{th} day of storage, the average TBA values of control, T1 and T2 were 0.48±0.1, 0.36±0.2, 0.32±0.06 respectively. A significant ($p<0.05$) difference was noted in between control, T1 and T2. However, there was no significant ($p<0.05$) difference reported between T1 and T2. The average TBA values of control, T1 and T2 were 0.68±0.2, 0.61±0.02, 0.55±0.2, respectively on 9^{th} day of storage. There was a significant ($p<0.05$) difference between control, T1 and T2. The average TBA values of control, T1 and T2 were 0.93±0.2, 0.87±0.03,0.74±0.3, respectively on 12^{th} day of storage. There was a significant ($p<0.05$) difference between control, T1 and T2. On 15^{th} day control, T1, T2 value for TBA was 1.16±0.06, 0.94±0.07 and 0.86±0.08 respectively .There was significant difference was noted in all the samples at ($p<0.05$).

The results were in agreement with the reports of Shan *et al.,* (2011) for cheese. Pal *et al.,* (1993) also reported an increase in TBA values of processed paneer at refrigeration temperature. At the end of storage period, the TBA value for control was much higher as compared to T1 and T2.

Table 4.4 Changes in TBA value of control and treated paneer during storage

TREATMENT	C	T1	T2
0 DAY	$_{a}0.22\pm0.01^{A}$	$_{a}0.22\pm0.02^{A}$	$_{a}0.23\pm0.02^{A}$
3 DAY	$_{b}0.36\pm0.01^{B}$	$_{b}0.31\pm0.02^{A}$	$_{b}0.26\pm0.02^{A}$
6 DAY	$_{c}0.48\pm0.01^{B}$	$_{c}0.36\pm0.02^{A}$	$_{c}0.32\pm0.06^{A}$
9 DAY	$_{d}0.68\pm0.02^{C}$	$_{d}0.61\pm0.02^{B}$	$_{d}0.55\pm0.02^{A}$
12 DAY	$_{e}0.93\pm0.02^{C}$	$_{e}0.87\pm0.03^{B}$	$_{e}0.74\pm0.03^{A}$
15 DAY	$_{f}1.16\pm0.06^{C}$	$_{f}0.94\pm0.07^{B}$	$_{f}0.86\pm0.08^{A}$

*Note: 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 ($p<0.05\%$).

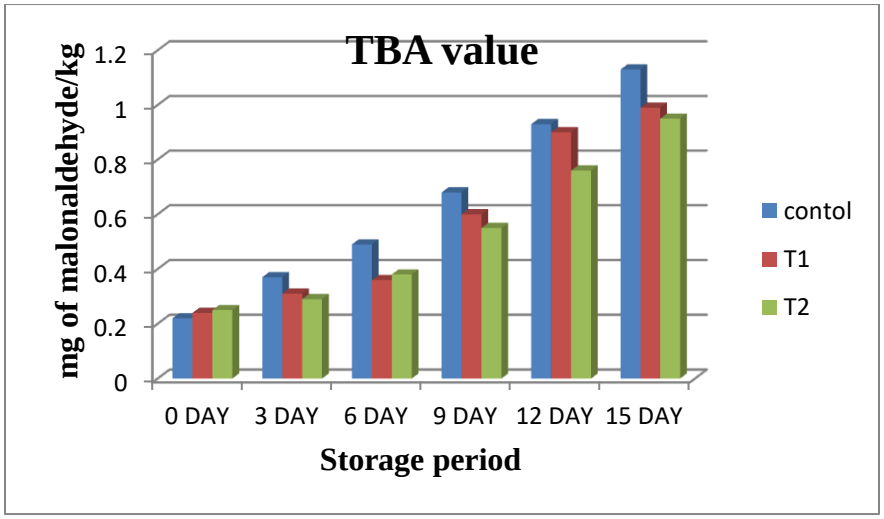

Fig 4.4 Changes in TBA value of control and treated paneer during storage

4.4.4. Changes in Titratable Acidity value of control and treated paneer

Table 4.5 depicts the values for Titratable Acidity of freshly prepared paneer and changes during storage period, the same are depicted in Fig.4.5.

It is clear from the Table 4.5, that the average titratable acidity values during the entire storage period for control were 0.29±0.01, 0.43±0.01, 0.55±0.02, 0.62±0.02, 0.74±0.01 and 0.79±0.02 which indicates that there is a significant ($p<0.05$) difference between 0, 3 ,6 ,9 ,12 and 15 days of storage. The average titratable acidity values during the entire storage period for T1 were 0.29±0.01, 0.41±0.01, 0.49±0.02, 0.55±0.02, 0.63±0.02 and 0.70±0.01 indicating a significant ($p<0.05$) difference between 0, 3, 6, 9, 12 and 15 days of storage. During the entire storage period, the average titratable acidity values for T2 were 0.30±0.01, 0.40±0.02, 0.49±0.02, 0.53±0.01, 0.59±0.01 and 0.71±0.01 showing a significant ($p<0.05$) difference between 0, 3, 6, 9, 12 and 15 days of storage.

The average titratable acidity values for control, T1, and T2 were 0.29±0.01, 0.29±0.01, 0.30±0.01 respectively on day 0. No significant difference was found in between control and treatments at ($p<0.05$). On day 3 the average titratable acidity values for control, T1 and T2 were 0.43±0.01, 0.41±0.01, 0.40±0.02 respectively. There was no significant difference observed in between control and T1 and T2. On 6^{th} day of storage, the average titratable acidity values of control, T1 and T2 were 0.55±0.02, 0.49±0.02, 0.49±0.02 respectively. A significant ($p<0.05$) difference was noted in between control, T1 and T2. However, there was no significant ($p<0.05$) difference between T1 and T2. The average titratable acidity values of control, T1 and T2 were 0.62±0.02, 0.55±0.02, 0.53±0.01 respectively on 9^{th} day of storage. There was a significant ($p<0.05$) difference between control, T1 and T2. However, no significant difference was found in T1 and T2. The average titratable acidity values of control, T1 and T2 were 0.74±0.01, 0.63±0.02, 0.59±0.01respectively on 12^{th} day of storage. There was a significant ($p<0.05$) difference between control, T1 and T2. On 15^{th} day control, T1, T2 value for titratable acidity were 0.79±0.02, 0.70±0.01 and

0.71±0.01 respectively. There was significant difference was noted in all the samples at ($p<0.05$).

The results were in agreement with the reports of Khatkar *et al.*, (2017) for paneer.

Table 4.5 Changes in Titratable acidity value of control and treatment paneer during storage

TREATMENT	C	T1	T2
0 DAY	$_{a}0.29\pm0.01^{A}$	$_{a}0.29\pm0.01^{A}$	$_{a}0.30\pm0.01^{A}$
3 DAY	$_{b}0.43\pm0.01^{B}$	$_{b}0.41\pm0.01^{B}$	$_{b}0.40\pm0.02^{B}$
6 DAY	$_{c}0.55\pm0.02^{B}$	$_{c}0.49\pm0.02^{A}$	$_{c}0.49\pm0.02^{A}$
9 DAY	$_{d}0.62\pm0.02^{B}$	$_{d}0.55\pm0.02^{A}$	$_{d}0.53\pm0.01^{A}$
12 DAY	$_{e}0.74\pm0.01^{C}$	$_{e}0.63\pm0.02^{B}$	$_{e}0.59\pm0.01^{A}$
15 DAY	$_{f}0.79\pm0.02^{C}$	$_{f}0.70\pm0.01^{B}$	$_{f}0.71\pm0.01^{A}$

*Note: 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 ($p<0.05\%$).

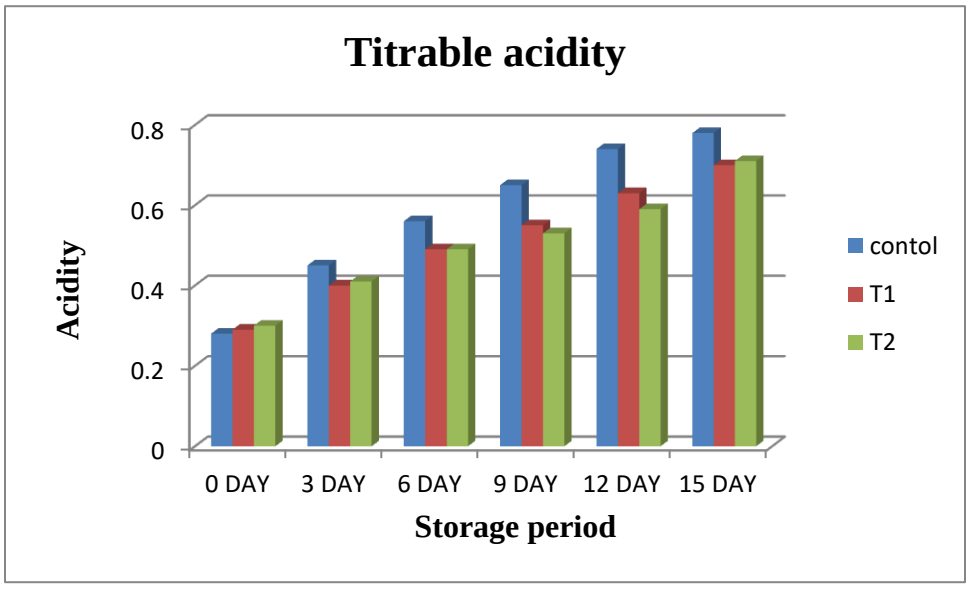

Fig 4.5 Changes in Titratable acidity value of control and treatment paneer during storage

4.5. Microbial Changes in control and treated paneer

4.5.1. Yeast and mold count

Table 4.6 and Fig. 4.6 depict the change in yeast and mold count of the paneer during refrigeration storage. During the storage period from day 3 to day 15 the average yeast and mold count for control were 2.53±0.18, 3.28±0.17, 3.37±0.01, 3.45±0.02, 3.52±0.01, which differs significantly at ($p<0.05$). But the value for day 6^{th} to day 12 were non significantly difference. The average yeast and mold count for T1 were 2.73±0.07, 3.14±0.01, 3.31±0.02, 3.43±0.02 during the storage period from day 6^{th} to day 15^{th}. These values were significantly different at ($p<0.05$). The average yeast and mold count during the storage period from day 6^{th} to day 15^{th} for T2 were 2.53±0.07, 3.06±0.01, 3.18±0.01, 3.31±0.02 which indicates a significant ($p<0.05$) difference during storage but shows non significant difference between 9^{th} and 12^{th} day.

On day zero, the average yeast and mold count for control T1 and T2 were Nil. Whereas on 3^{rd} day sample only control shows the growth of 2.53±0.18. On day 6th, the average yeast and mold count for control T1 and T2 were 3.28±0.17, 2.73±0.07 and 2.53±0.07. Control was significantly ($p<0.05$) different from T1 and T2. However, no significant difference was observed between T1 and T2 . The average yeast and mold count for control, T1 and T2 were 3.37±0.01, 3.14±0.01 and 3.06±0.01 respectively on 9^{th} day of storage. A significant difference was noted among the control and treatments at ($p<0.05$). The average yeast and mold count for control, T1 and T2 were 3.45±0.02, 3.31±0.02 and 3.18±0.01 respectively on 12^{th} day of storage. All samples was significantly ($p<0.05$) different. On 15^{th} day of storage the average yeast and mold count for control, T1 and T2 were 3.52±0.01, 3.43±0.02 and 3.31±0.02respectively. All sample was significantly ($p<0.05$) different.

The subsequent decrease in pH values during the storage period might have aided in providing an optimum growth environment for yeast and molds. The results are in agreement with Pal (1998) and Singh *et al.,* (2014). Owing to the antifungal

action, the garlic extract was observed to exhibit a significant inhibitory effect against growth of yeast and molds during the entire storage period. Similar findings were reported by Makhal *et al.*, (2014).

Table 4.6 Yeast and mold count of control and treatments of paneer during storage period

TREATMENT	CONTROL	T1	T2
0 DAY	NIL	NIL	NIL
3 DAY	$_{a}2.53\pm0.18$	NIL	NIL
6 DAY	$_{b}3.28\pm0.17^{B}$	$_{a}2.73\pm0.07^{A}$	$_{a}2.53\pm0.07^{A}$
9 DAY	$_{b}3.37\pm0.01^{C}$	$_{b}3.14\pm0.01^{B}$	$_{b}3.06\pm0.01^{A}$
12 DAY	$_{b}3.45\pm0.02^{C}$	$_{c}3.31\pm0.02^{B}$	$_{b}3.18\pm0.01^{A}$
15 DAY	$_{b}3.52\pm0.01^{C}$	$_{d}3.43\pm0.02^{B}$	$_{c}3.31\pm0.02^{A}$

*Note: 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 ($p<0.05\%$).

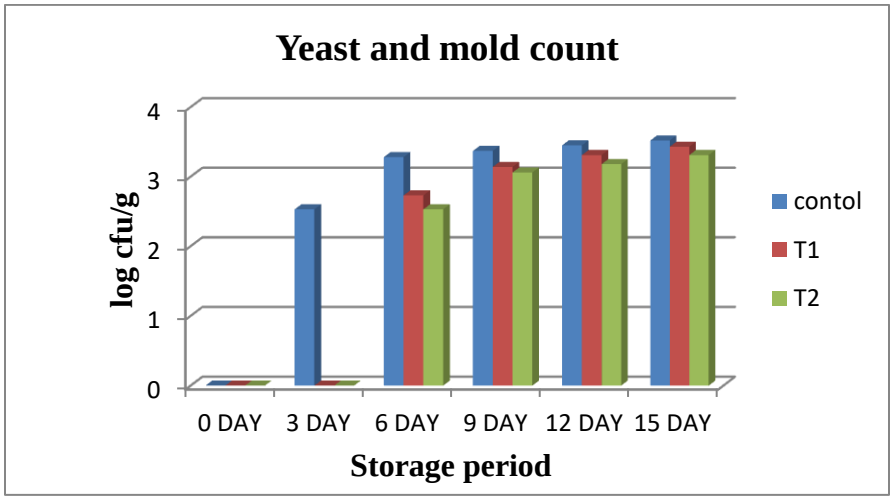

Fig 4.6 Yeast and mold count of control and treatments of paneer during storage period

4.5.2. Psychrophilic count

Table 4.7 and Fig. 4.7 depict the change in psychrophilic count of the paneer during refrigeration storage. During the storage period from day 6th to day 15th the average psychrophilic count for control were 4.63±0.01, 5.03±0.01, 5.18±0.00 and 5.20±0.01 which differs significantly between the storage period day 6th and day 9th but there were no significant difference between 6th, 12th and 15th days at ($p<0.05$). The average psychrophilic count for T1 were 3.67±0.02, 4.84±0.02, 5.17±0.02 during the storage period from 9th day to 15th day. These values were significantly different at ($p<0.05$). The average psychrophilic count for T2 during the entire storage period for T2 3.57±0.02, 4.81±0.02, 5.00±0.01 which indicates a significant ($p<0.05$) difference during storage.

Day 0 and day 3rdthe average psychrophilic count for control T1 and T2 were Nil. Whereas on 6th day only control shows the growth of 4.63±0.01. On day 9th, the average psychrophilic count for control T1 and T2 were 5.03±0.01, 3.67±0.02 and 3.57±0.02. Significant different were observed between control and treatments. The average psychrophilic count for control, T1 and T2 were 5.18±0.00, 4.84±0.02 and 4.81±0.02 respectively on 12th day of storage. There was a significant difference was noted in between control, T1 and T2 at ($p<0.05$). However, no significant difference was found between T1 and T2. On 15th day of storage the average psychrophilic count for control, T1 and T2 were 5.20±0.01, 5.17±0.02 and 5.00±0.01 respectively. Control was significantly ($p<0.05$) different from T2 and there were non significant different between control and T1 however there was significant difference between T1 and T2at ($p<0.05$).

The subsequent decrease in pH values during the storage period might have aided in providing an optimum growth environment for psychrophilic count. The results were close to the observation on cottage cheese by Makhal*et al.,* (2014) and Agnihotri and Pal (1996) on goat milk paneer.

Table 4.7 Psychrophilic count of control and treatments of paneer during storage period

TREATMENT	CONTROL	T1	T2
0 DAY	NIL	NIL	NIL
3 DAY	NIL	NIL	NIL
6 DAY	$_{a}4.63\pm0.01^{A}$	NIL	NIL
9 DAY	$_{b}5.03\pm0.01^{C}$	$_{a}3.67\pm0.02^{B}$	$_{a}3.57\pm0.02^{A}$
12 DAY	$_{a}5.18\pm0.00^{B}$	$_{b}4.84\pm0.02^{A}$	$_{b}4.81\pm0.02^{A}$
15 DAY	$_{a}5.20\pm0.01^{B}$	$_{c}5.17\pm0.02^{B}$	$_{c}5.00\pm0.01^{A}$

*Note: 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 ($p<0.05\%$).

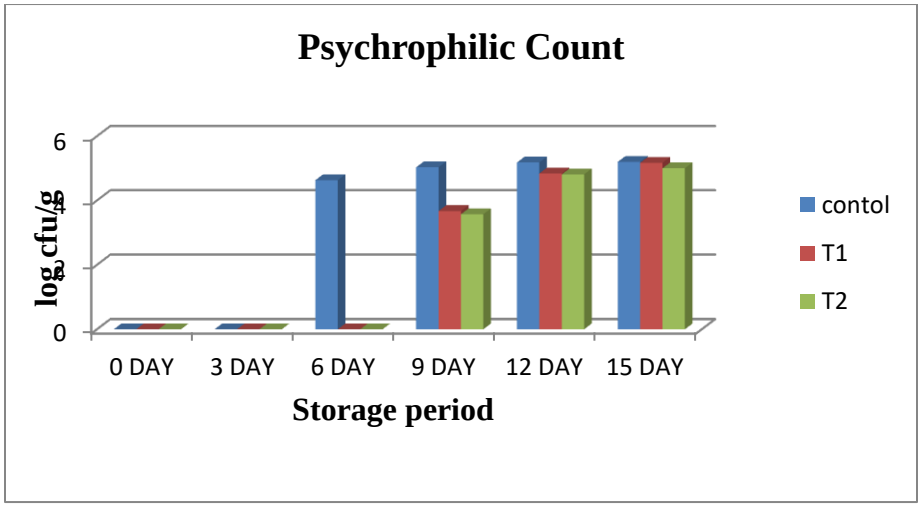

Fig 4.7 Psychrophilic count of control and treatments of paneer during storage period

4.5.3. Total Plate Count

The paneer with and without edible packaging film were subjected for Total Plate Count (TPC log10cfu/g) at day zero and subsequently at 3rd , 6th, 9th , 12th and 15th day of storage. The values for TPC are highlighted in Table 4.8 and depicted in Fig. 4.8.

It is clear from the Table 4.8, that the average Total plate count values during the storage period from 3rd to 15th day for control were 1.97±0.09, 3.30±0.15, 5.00±0.10, 5.80±0.12 and 6.77±0.03 which indicates that there is a significant ($p<0.05$) difference between 3 ,6 ,9 ,12 and 15 days of storage. The average Total plate count values during the storage period from day 3rd to day 15th for T1 were 1.27±0.18, 2.47±0.18, 3.60±0.12, 4.77±0.09 and 5.47±0.09 indicating a significant ($p<0.05$) difference between 3, 6, 9, 12 and 15 days of storage. During the storage period from 3rd day to 15th day, the average Total plate count values for T2 were 1.2±0.12, 2.20±0.12, 3.40±0.12, 4.57±0.12 and 5.37±0.09 showing a significant ($p<0.05$) difference between 3, 6, 9, 12 and 15 days of storage.

The average Total plate count values for control, T1, and T2 were nil on day 0. On day 3rdthe average Total plate count values for control, T1 and T2 were 1.97±0.09, 1.27±0.18, 1.2±0.12 respectively. There was significant difference observed in between control, T1 and T2. However no significant difference was found between T1 and T2. On 6th day of storage, the average Total plate count values of control, T1 and T2 were 3.30±0.15, 2.47±0.18, 2.20±0.12respectively. A significant ($p<0.05$) difference was noted in between control, T1 and T2 . However no significant difference was found between T1 and T2.The average Total plate count values of control, T1 and T2 were 5.00±0.10, 3.60±0.12, 3.40±0.12 respectively on 9th day of storage. There was a significant ($p<0.05$) difference between control and T1 and T2 . The average Total plate count values of control, T1 and T2 were 5.80±0.12, 4.77±0.09, 4.57±0.12 respectively on 12th day of storage. There was a significant ($p<0.05$) difference between control, T1 and T2 was observed. However, no significant difference was found between T1 and T2. On 15thday control, T1, T2

value for Total plate count was 6.77±0.03, 5.47±0.09 and 5.37±0.09 respectively. There was significant difference was noted between control, T1 and T2 samples at ($p<0.05$). However, no significant difference was found between T1 and T2.

The results were close to the investigations on cottage cheese by Makhal *et al.,* (2014) and Agnihotri and Pal (1996) on goat milk paneer.

Table 4.8 Total plate count of control and treatments of paneer during storage period

TREATMENT	C	T1	T2
0 DAY	NIL	NIL	NIL
3 DAY	$_{a}1.97\pm0.09^{B}$	$_{a}1.27\pm0.18^{A}$	$_{a}1.20\pm0.12^{A}$
6 DAY	$_{b}3.30\pm0.15^{B}$	$_{b}2.47\pm0.18^{A}$	$_{b}2.20\pm0.12^{A}$
9 DAY	$_{c}50.0\pm0.10^{C}$	$_{c}3.60\pm0.12^{B}$	$_{c}3.40\pm0.12^{A}$
12 DAY	$_{d}5.80\pm0.12^{B}$	$_{d}4.77\pm0.09^{A}$	$_{d}4.57\pm0.12^{A}$
15 DAY	$_{e}6.77\pm0.03^{B}$	$_{e}5.47\pm0.09^{A}$	$_{e}5.37\pm0.09^{A}$

Note: Row wise Mean bearing subcript indicates significant and non significant difference at ($p<0.05\%$).Column wise mean bearing superscript indicates significant and non significant difference at ($p<0.05\%$).

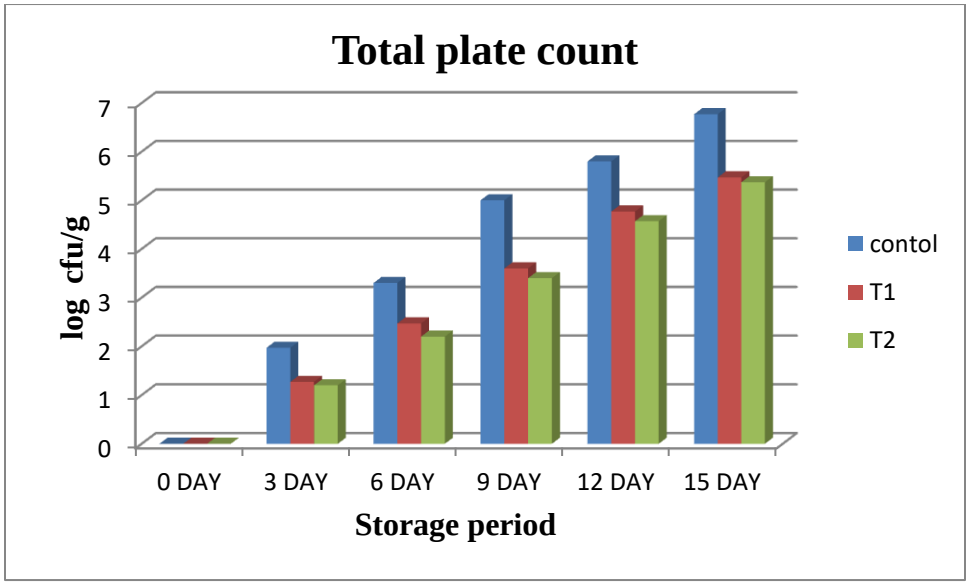

Fig 4.8 Total plate count of control and treatments of paneer during storage period.

4.5.4. Coliform count

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

4.6. Sensory evaluation of control and treated paneer

The freshly prepared control an treated paneer were evaluated organoleptically by the panel of judges on day zero and at subsequent intervals of 5 days using nine point descriptive scale. Table 4.9 and Fig 4.9, depict the change in sensory attributes at 0 day. On day zero sensory attributes of control like appearance, juiciness, flavor and texture were 8.98±0.02, 8.46±0.14, 7.95±0.18 and 8.98±0.02 respectively. The overall acceptability was 8.59±0.04 and it was significantly different from T1 and T2.Sensory characters appearance, juiciness, flavor and texture of T1 was 8.98±0.15, 8.42±015, 8.43±0.17 and 8.97±0.02 respectively and overall acceptability was 8.7±0.07 which shows significant difference from control but non significance from T2. Appearance, juiciness, flavor and texture of T2 was 8.97±0.02, 8.35±0.10, 8.72±0.16, 8.95±0.03 respectively and overall acceptability was 8.75±0.06. There was no significant difference from T1 but shows significant difference from control.

Table 4.10 and Fig 4.10, depict the change in sensory attributes at 5^{th} day. On day fifth sensory attributes for control like appearance, juiciness, flavor and texture were 7.97±0.12, 7.54±0.18, 7.32±0.16 and 8.12±0.18 respectively. It shows significant difference from T1 and T2. Sensory characters appearance, juiciness, flavor and texture of T1 was 8.43±0.01, 7.61±0.12, 7.66±0.17 and 8.42±0.14 respectively. It shows significant difference from control but non significant difference from T2 .Whereas appearance, juiciness, flavor and texture of T2 was 8.60±0.12, 7.76±0.15, 7.50±0.23, 8.36±0.16respectively. There was no significant change on the sensory evaluation of T1 and T2 but shows significant difference from control.

Table 4.11and Fig 4.11, depict the change in sensory attributes at 10^{th} day. On day 10^{th} only T1 and T2 sensory evaluation were done because microbiologically control was spoiled on 9^{th} day of storage. On day 10^{th} Sensory characters appearance , juiciness, flavor and texture of T1 was 7.35±0.07, 6.11±0.22, 7.52±0.30, 7.02±0.1 and respectively .Whereas appearance, juiciness, flavor and texture of T2 was 7.52±0.10, 6.49±0.14, 7.68±0.24, 7.14±0.15 respectively. T1 and T2 shows significant difference.T2 was considered best in terms of sensory evaluation (appearance, juiciness, flavour and texture) by the panel of judges.

The scores for all the samples were in line with Sanyal *et al.*, (2006) and Singh *et al.*, (2014) for paneer.

Table 4.9 Sensory scores of control and treatment at 0 day storage period

DAY 0			
TREATMENT	C	T1	T2
APPEARANCE	$_{a}8.98\pm0.02^{A}$	$_{a}8.98\pm0.15^{B}$	$_{a}8.97\pm0.02^{B}$
JUICINESS	$_{a}8.46\pm0.14^{A}$	$_{a}8.42\pm015^{B}$	$_{a}8.35\pm0.10^{B}$
FLAVOUR	$_{a}7.95\pm0.18^{A}$	$_{a}8.43\pm0.17^{B}$	$_{a}8.72\pm0.16^{B}$
TEXTURE	$_{a}8.98\pm0.02^{A}$	$_{a}8.97\pm0.02^{B}$	$_{a}8.95\pm0.03^{B}$
OVERALL ACCEPTABILITY	$_{a}8.59\pm0.04^{A}$	$_{a}8.7\pm0.07^{B}$	$_{a}8.75\pm0.06^{B}$

Note: 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 ($p<0.05\%$).

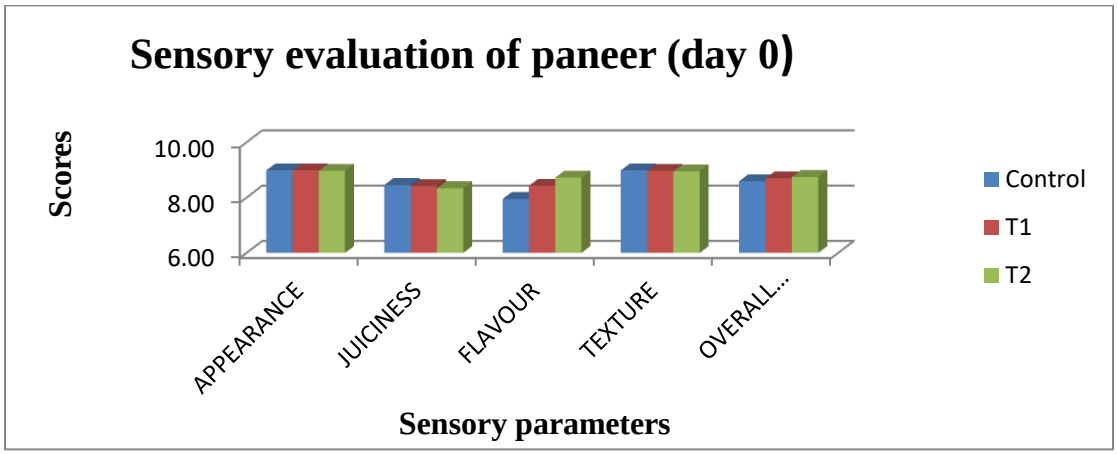

Fig 4.9 Sensory scores of control and treatment at 0 day storage period

Table 4.10 Sensory scores of control and treatment at 5thday storage period

DAY 5			
TREATMENT	C	T1	T2
APPEARANCE	$_{b}7.97\pm0.12^{A}$	$_{b}8.43\pm0.01^{B}$	$_{b}8.60\pm0.12^{B}$
JUICINESS	$_{b}7.54\pm0.18^{A}$	$_{b}7.61\pm0.12^{B}$	$_{b}7.76\pm0.15^{B}$
FLAVOUR	$_{b}7.32\pm0.16^{A}$	$_{b}7.66\pm0.17^{B}$	$_{b}7.50\pm0.23^{B}$
TEXTURE	$_{b}8.12\pm0.18^{A}$	$_{b}8.42\pm0.14^{B}$	$_{b}8.36\pm0.16^{B}$
OVERALL ACCEPTABILITY	$_{b}7.74\pm0.08^{A}$	$_{b}8.03\pm0.11^{B}$	$_{b}8.05\pm0.08^{B}$

Note: 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 ($p<0.05\%$).

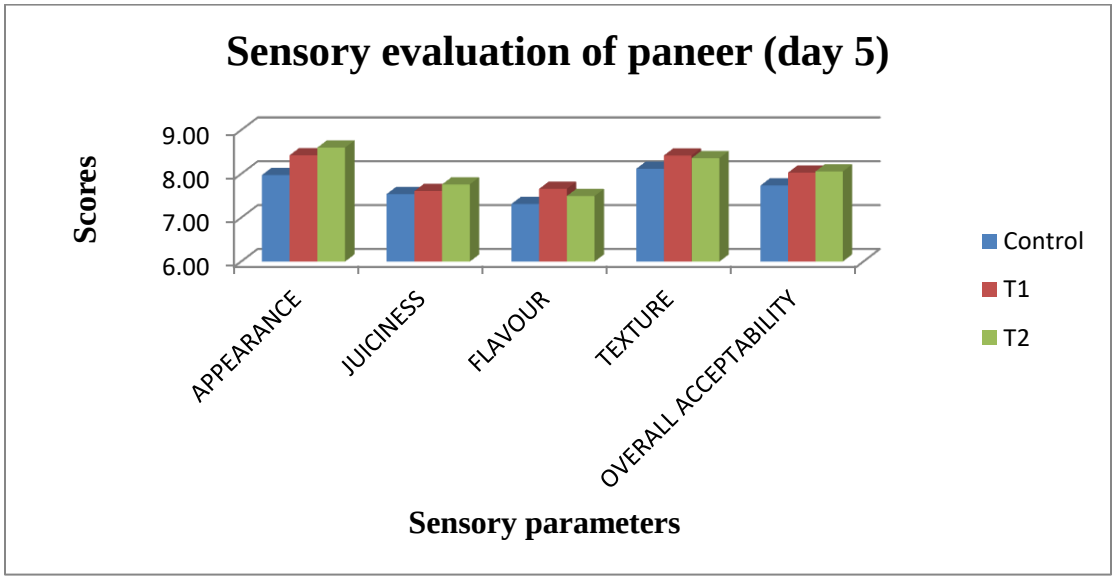

Fig 4.10 Sensory scores of control and treatment at 5 day storage period

Table 4.11 Sensory scores of control and treatment at 10th day storage period

DAY 10			
TREATMENT	C	T1	T2
APPEARANCE	NIL	$a7.35\pm0.07^{A}$	$b7.52\pm0.10^{B}$
JUICINESS	NIL	$a6.11\pm0.22^{A}$	$b6.49\pm0.14^{B}$
FLAVOUR	NIL	$a7.52\pm0.30^{A}$	$b7.68\pm0.24^{B}$
TEXTURE	NIL	$a7.02\pm0.12^{A}$	$b7.14\pm0.15^{B}$
OVERALL ACCEPTABILITY	NIL	$a7.00\pm0.08^{A}$	$b7.20\pm0.08^{B}$

Note: 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 ($p<0.05\%$).

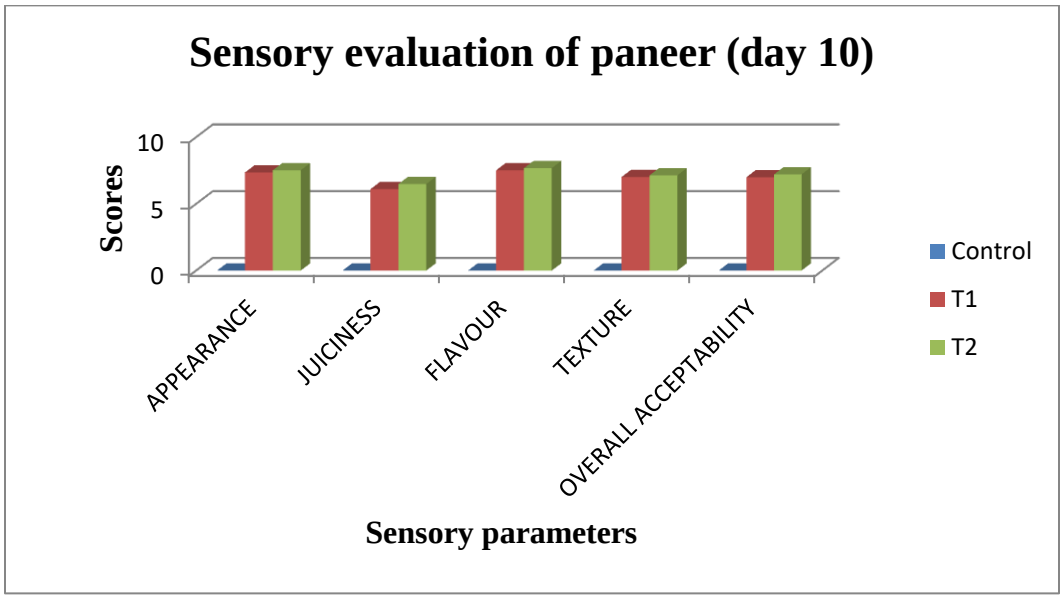

Fig 4.11 Sensory scores of control and treatment at 10th day storage period

4.7. Cost of production for edible packaging film treated with essential oil for packaging paneer

The cost of production for edible packaging film treated with essential oil for packaging paneer in table 4.21.

The cost of production for edible packaging film treated with essential oil for packaging paneer was Rs/-11.07 for 100 ml of solution. However addition of essential oil provides additional antimicrobial property to paneer.

Table 4.12 Cost of production for edible packaging film treated with essential oil for packaging paneer (per 100 ml)

Ingredients		
	Quantity required	**Amount Rs.**
Starch	5 gm	8.19
CMC	0.75gm	1.27
Glycerol	1.2 ml	1.51
Citric Acid	0.1gm	0.10
Clove Oil	-	
Oregano Oil	-	
Total cost		11.07

Plate no:-4 Edible packaging film

Plate no:-5 Experiment performing EAB

Plate no 1:- Control Paneer Sample

Plate no 2:- T1 Paneer Sample

Plate no 3:- T2 Paneer Sample

Summary and Conclusion

5. SUMMARY AND CONCLUSION

The present study was planned to assess the stability of paneer packed with edible packaging film treated with clove and oregano essential oil at refrigeration storage temperature. The study was divided into three phase. In, first phase standardization of CMC and starch based film were prepared by analyzing different concentration of CMC over starch based film. Finally 15% CMC w/w of starch were selected for the preparation of the film. Then, the parameters like thickness of film, water vapour permeability , water solubility , elongation at break , colour of film, moisture of film and pH of film were analyzed. In second, phase of the study the film was treated with different concentration of clove and oregano essential oils i.e., 0.25%, 0.37%, 0.50%and 0.75%. Among these treatments sample with 0.50% concentration of essential oil treated film was accepted organoleptically. In third, stage paneer was prepared from buffalo milk (6% fat and 9 % SNF) and evaluated shelf life of paneer wrapped with 0.50 % concentration clove and oregano treated edible film analyzed at the interval of 3 days till spoilage. Simultaneously, sensory evaluation were also done at the interval of 5 days upto acceptable limit.

Parameters of the edible packaging film Thickness, Water Vapour Permeability, Film Solubility, Moisture Content, Elongation at Break, pH of the edible packaging film were 0.09±0.01mm, $2.07\pm0.02\times10^{-7g}$/m.h.Pa, 84.33±1.45, 22.51±1.48, 22.60±1.48, 5.67±0.02 respectively, concluded after three basic trial.

The result of proximate analysis of paneer was moisture 51.5%, fat 24.43%, protein 17.70 %. pH values for control, T1 and T2 were not significantly different from 0 day to 12^{th} day but there were significant difference on day 15^{th}

The average tyrosine values for control, T1 and T2 were found 12.52 to 31.51, TBA value for control, T1 and T2 were found 0.22 to 0.87, titratable acidity for control T1 and T2 were found 0.29 to 0.63, the results revealed pH tyrosin value, TBA value, titratable acidity were under control for treatments upto 12^{th} day.

The sample stored were also monitored for microbial analysis. Average Total plate count (log10cfu/g) value ranges from 1.97 to 4.77 for control and treatments but

values were under control for treatments upto 12th day. Average psycrophilic count were nil for control and treatments upto 3 days but values were under control upto 12th day for treatments. Average yeast and mold count for control and treatments were ranges from 2.53 to 3.31 upto 12th day and coliform count were nil throughout the storage period for control and treatments.

The sample were also tested organoleptically for the period of 15 days at the interval of 5 days. Parameters like appearance, juiciness, flavor and texture were evaluated. Treated sample were found physico-chemically and microbiologically safe upto 12th day but organoleptically accepted upto 10th day

From the above result it could be concluded that:

1) CMC and starch based film can be used as edible packaging material and as a substitute of plastic.
2) Clove and Oregano essential oil has antimicrobial property and can be used at 0.5% oragnoleptically in edible packaging film and it can increase the shelf – life of paneer by 4 days at refrigerator temperature.
3) Due to the great demand of paneer in day to day life, further study is required to develop new ideas in extending the shelf life of paneer by using edible packaging material.

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Appendices

APPENDIX – I

Sensory evaluation

Livestock Products Technology

Mumbai Veterinary College, Parel, Mumbai

Date:

Name:

Sample	Appearance	Flavour	Juiciness	Texture	Overall acceptability

Marking Criteria:-

9- Like extremely

8- Like very much

7- Like moderately

6- Like slightly

5- Neither like nor dislike

4- Dislike slightly

3- Dislike moderately

2- Dislike very much

1- Dislike extremely

Signature

APPENDIX –II

Flowchart of preparation of paneer

Fresh buffalo milk

⇩

Standardized to 6% fat

⇩

Heat it to 90˚C for 5min

⇩

Cool it to 70˚C

⇩

Add GDL solution (1% solution at 70˚C)

⇩

Allow the coagulum to set

⇩

Fill the coagulum into muslin cloth lined in cylindrical hoop and press for 10-15min

⇩

Immerse in chilled water for 2hrs

Signature:

Abstract English

Appendix – G

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	**:**	**"EXTENDING THE SHELF LIFE OF PANEER COATED IN EDIBLE FILM TREATED WITH ESSENTIAL OILS"**
b)	Full name of student	**:**	SAURABH KARUNAMAY
c)	Name and address of Major Advisor	**:**	Dr. S. R. Badhe, I/C Associate Professor and Head, Department of Livestock Products Technology, Mumbai Veterinary College, Parel, Mumbai - 400012
d)	Degree to be awarded	**:**	M.V.Sc.
e)	Year of award of degree	**:**	2019
f)	Major subject	**:**	Livestock Products Technology
g)	Total number of pages in the thesis	**:**	
h)	Number of words in the abstract	**:**	
i)	Signature of Student	**:**	
j)	Signature, Name and address of forwarding authority (HOD / SH)	**:**	Dr. S. R. Badhe, I/C Associate Professor and Head, Department of Livestock Products Technology, Mumbai Veterinary College, Parel, Mumbai - 400012
	Signature of the Associate Dean	**:**	

ABSTRACT

The present study was conducted to evaluate shelf life of paneer wrapped with essential oil treated edible packaging film. Freshly packed paneer was packed with essential oil treated edible film and evaluated for shelf life of paneer.

CMC and starch based edible packaging film was prepared and standardized using 15% CMC W/W of Starch. Different parameters like water vapour permeability, moisture percentage, film solubility, pH, Elongation at break, thickness and colour of the film were evaluated. Edible packaging film was treated with different concentration of essential oil of clove and oregano.

Paneer was prepared freshly with buffalo milk (6% fat and 9% SNF) and proximate analysis was done. Paneer cubes were cut and wrapped with clove and oregano oil treated edible film and evaluated for the shelf–life at refrigeration storage temperature (4°c) till spoilage.

During the process of storage different physico-chemical parameters like pH, titratable acidity, TBA and Tyrosine was evaluated and at the same time microbiology examination of total plate count , psychrophilic count, yeast and mould count and coliform count were done at the interval of 3 days. Sensory evaluation was also done at the interval of 5 days, on the parameters like appearance, juiciness, flavour, texture and overall acceptability.

Control sample was found unattractive in sensory evaluation on 5^{th} day of storage and microbiologically unfit on 9^{th} day of storage. The paneer packed in clove and oregano essential oil treated edible packaging film was found unattractive on 10^{th} day of sensory evaluation. But found microbiologically sound at 12^{th} day of storage. The result, shows paneer packed in edible film treated with clove and oregano essential oil were found to increase the shelf –life of paneer for at least by 4 days at refrigeration (4°c) temperature.

Abstract Marathi

प्रबंध सारांश

१	प्रबंधाचे नांव	आवश्यक तेलयुक्त खाण्यायोग्य आवरण तयार करुन पनीरची साठवणूक क्षमता वाढविण्याबाबत
२	विद्यार्थ्यांचे नांव	सौरभ करुणामय
३	मार्गदर्शकाचे नांव व पत्ता	डॉ.एस.आर.बढे, प्रभारी सहयोगी प्राध्यापक, पशुजन्य पदार्थ तंत्रज्ञान विभाग, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई-१२
४	पदवी	एम.व्ही.एस.सी.
५	पदवी प्रदान करण्याचे वर्ष	२०१९
६	मुख्य विषय	पशूजन्य पदार्थ तंत्रज्ञान विभाग
७	प्रबंधाची एकुण पाने	
८	सारांशाचे एकुण शब्द	
९	विद्यार्थ्यांची सही	
१०	प्रबंध पाठविणाऱ्या अधिकाऱ्याचे नांव, पत्ता आणि सही	डॉ.एस.आर.बढे प्रभारी सहयोगी प्राध्यापक, पशुजन्य पदार्थ तंत्रज्ञान विभाग, मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई-१२
११	सहयोगी अधिष्ठाता, मुंबई पशुवैद्यकीय महाविद्यालय, मुंबई - १२	

सारांश

सदर अभ्यासाचा उद्देश हा मसाले तेलाची प्रक्रिया करुन प्रमाणित करण्यात आलेल्या इडीबल फिल्मचा वापर पनीरची साठवणूक क्षमता टिकवून ठेवण्यासाठी करण्यात आला.

कार्बोहायड्रेट आणि स्टार्चचा उपयोग करुन इडिबल फिल्मचा वापर पनीरची साठवणूक क्षमता टिकवून ठेवण्यासाठी करण्यात आला. सदर फिल्मवर ०.२५, ०.३७, ०.५ व ०.७५ तीव्रतेचे लवंग व ऑरगॅनो तेलाची प्रक्रिया करण्यात आली. तसेच ज्ञानेंद्रियाच्या चाचणीवरुन ०.५% तीव्रतेचे लवंग तसेच ऑरगॅनो तेल प्रमाणित करण्यात आले. सदर ०.५% तीव्रतेचे लवंग व ऑरगॅनो तेलाने प्रमाणित करण्यात आलेल्या इडीबल फिल्मचा उपयोग पनीरची साठवणूक क्षमता टिकवून ठेवण्यासाठी करण्यात आला. सदर पनीर साठवणूक करताना भौतिक व रासायनिक गुणधर्माचा होणाऱ्या परिणामाचा अभ्यास १५ दिवसाच्या कालावधी दरम्याने करण्यात आला. भौतिक व रासायनिक गुणधर्म पीएच, टीबीए, टायरोसिन आणि ॲसिडीटी इ. सदर साठवणूकी दरम्यान सुक्ष्मजीव जसे की टोटल प्लेट काऊंट, सायक्रोफिलिक काऊंट, कोलायफॉम काउंट, इस्ट आणि मोल्ड काऊंट इ.चा अभ्यास करण्यात आला. सदर अभ्यासामधून असे दिसून आले की पनीरच्या साठवणूक कालावधी दरम्यान पनीर हे १२ दिवसा पर्यंत सुरक्षित आढळून आले. परंतु ज्ञानेंद्रिय चाचणीमध्ये १० दिवसा पर्यंत खाण्यायोग्य आढळून आले. सदर अभ्यासावरुन असे दिसून आले की पनीरची साठवणूक क्षमता ४ दिवसांनी वाढलेली दिसून आली.

Vita

VITA

The author, Dr. Saurabh Karunamay was born on 18th of January 1991 in Officer's Colony, District Katihar of Bihar State.

He has completed his primary and secondary education from Jawahar Navodaya Vidyalaya, kolasi, Katihar. He has scored 74.40 % marks in S.S.C in the year 2007. He has completed his H.S.C. board exam with 68.00 % marks from Jawahar Navodaya Vidyalaya, kolasi, Katihar, Bihar.

Being interested in animal welfare, he has joined Ranchi Veterinary College, Ranchi, Jharkhand and completed the B.V.Sc. and A.H. with first class in 2017. He actively participated in sports and cultural events. He represented Ranchi veterinary College in Archery, Light Vocal and Elocution in many events. He is also the B – Certificate holder of National Cadet Corp.

He joined M.V.S.c. in Livestock Products Technology in Mumbai Veterinary College in 2017 and has successfully completed his M.V.S.c. course work with first class.