EFFECT OF *CINNAMOMUM VERUM* AND *ZANTHOXYLUM ARMATUM* ESSENTIAL OIL INCORPORATED SODIUM ALGINATE EDIBLE COATING ON THE SHELF LIFE OF PANEER

by

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Effect of *Cinnamomum verum* and *Zanthoxylum armatum* Essential Oil Incorporated Sodium Alginate Edible Coating on the Shelf Life of Paneer

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Approval Letter

This *dissertation* entitled *Effect of Cinnamomum verum* and *Zanthoxylum armatum Essential Oil Incorporated Sodium Alginate Edible Coating on the Shelf Life of Paneer*, presented by Bimala Pokhrel has been accepted as the partial fulfilment of the requirements for the M. Tech. degree in Food Technology.

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Abstract

The study was carried out with objective to evaluate the effect of *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate edible coating on shelf life of paneer. Paneer samples were prepared from cow milk using citric acid (1%) as coagulant and calcium chloride at different concentrations, 0, 0.05, 0.1, 0.15, 0.2 and 0.25% (w/v) of the milk and were evaluated for composition, sensory quality and yield. The best paneer was coated with sodium alginate edible coating incorporated with 2% of *Zanthoxylum armatum and Cinnamomum verum* essential oils and their mix at different combinations given by the D-optimal mixture design. The control (without coating) and coated paneer samples were packed in LDPE bags (48.7 g/m^2 and 50µm thickness), stored at 7±1ºC and 52% RH, and changes on sensory attributes, chemical composition and microbial load during storage were studied at 4 days interval till 24 days.

Results showed that $CaCl_2$ at different concentrations had significant effect ($p<0.05$) on composition, sensory quality and yield of the paneer but not on FFA. 0.1% $CaCl_2$ added paneer was significantly different ($p<0.05$) than other samples and selected as best for further coating. Storage time and edible coatings had significant effect ($p<0.05$) on the sensory, physicochemical (acidity, FFA, tyrosine release) and microbiological characteristics of paneer. The overall sensory scores of uncoated and single essential oil coated samples were below 6 on the 8^{th} days and 16^{th} days respectively whereas Zanthoxylum *armatum* and *Cinnamomum verum* essential oil (1.75+0.25) % coated sample remained 7 till 24 days of storage. Among all the treated samples, *Zanthoxylum armatum* and *Cinnamomum verum* essential oil mix combination (1.75+0.25) at 2% of sodium alginate coated sample was significantly different ($p<0.05$) in chemical characteristis (moisture content, FFA, acidity, tyrosine released) and microbial characteristics (TPC and yeast mold count).

Contents

List of Tables

List of Figures

List of plates

List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
BIS	Bureau of Indian Standards
BSA	Bovine serum albumin
DDC	Dairy Development Corporation
DFTQC	Department of Food Technology and Quality Control
EO	Essential oil
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
GRAS	Generally Recognize as Safe
LDPE	Linearly Low Density Poly Propylene
PET	Poly ethylene Terephthalate
RFTQCO	Regional Food Technology and Quality Control Office
SEM	Standard error of mean
SNF	Solids-not-fat
TBA	Thiobarbituric Acid
TCA	Trichloro Acetic acid
TMP	Total milk protein
TPC	Total plate count
W/V	Weight by volume
W/W	Weight by weight
WHO	World Health Organization
WPC	Whey Protein Concentrate

Part I

Introduction

1.1 General introduction

Paneer is one of the most popular dairy products consumed by all group of people. It belongs to the soft cheese categories. It is popular in Asian country and is consumed by the vegetarians as meat substitutes. Total milk production in Nepal is 23,01,000 MT (Gov. N., 2019/2020). Total estimated processed milk supply by DDC and other private dairies has increase from 110,144 MT (2005/06) to 160,765 MT (2015/016), Similarly, production of paneer alone by DDC is from 50 (2005/06) MT to 123 MT (2009/010)(FAO, 2010). It indicates the increase in paneer production along with the total milk production in Nepal.

Paneer is a soft cheese variety from South Asia obtained by milk acid coagulation, trapping all the fat, casein complexes with whey protein denatured and a portion of salts and lactose. It is a type of cheese that is non-fermentative, non-renneted, non-melting and unripen (Khatkar *et al.*, 2017). It is very susceptible to microbial spoilage and subsequent biochemical degradation due to elevated moisture content and paneer nutrients. At room temperature, where the activities of microorganisms are optimum, paneer spoilage is faster. Several attempts have been made to improve the shelf life of the paneer, such as the use of antimicrobial agents, chemical preservatives, paraffining, deep fat frying, paneer dipping in treated water, modified atmosphere packaging, low temperature preservation, and implementation of hurdle technology (Rajarshibhai, 2012). Yet customers are actually more interested in food that is natural or similar to natural, minimally processed and free of artificial preservatives (Chauhan *et al.*, 2012).

Various attempts had been made to increase the shelf life of paneer. Among preservatives, essential oils of some herbs and plants were traditionally used for the preservation of wide variety of foods. Antimicrobial substances such as bacteriocins, proteins or peptides secretions, bioactive molecules from plant have also been exploited in different ways for food preservation. Herbs and spices have been recognized to possess a broad spectrum of active constituents that exhibit antibacterial, antifungal, antiparasitic, and/or antiviral activities (Delesa, 2018). Similarly, spices and herbs are rich source of antioxidants. The

antioxidant properties of herbs are due to presence of some vitamins, flavonoids, terpenoids, carotenoids and phytoestrogens (Shan *et al.*, 2011).

There has been increasing concern of the consumers about foods free of chemical preservatives because of their possible toxic effect in human beings. Consumers are also demanding safe foods and less use of synthetic preservatives. It has forced the food industry to exploit the alternative methods/components against the use of synthetic antimicrobial compounds for food preservation. Spices, being natural component offer a promising alternative for food preservation. Spices have been well known for their medicinal, preservative and antioxidant properties (Souza *et al.*, 2005).

Essential oils represent an alternative to synthetic preservatives in the food industry against spoilage yeasts and moulds. Most investigated fungi showed some (higher or lower) sensitivity to essential oils or its active components. It seems that the main target of essential oil in the cell membrane is to increase permeability and disruption of membrane integrity. Both monoterpenes and phenolics are involved in the action against the cell membrane and key enzymes important for energy regulation or synthetic pathways and mycotoxin production of moulds is also affected by the essential oils. However, spore formation and germination is sometimes accelerated by essential oils, especially in case of fungi that can attack aromatic plants or fruits. The choice of essential oils and its concentration in a particular food is important because a small amount can cause sensory alterations. Hence, combinations of essential oils with each other or with other preservation techniques could be the alternative for food preservation (Krisch *et al.*, 2011).

1.2 Statement of the problem

Buffalo milk is usually preferred for making paneer because of high fat and solid content to obtain the high yield and better quality of paneer. But improvement in cow milk can be done with added calcium chloride to increase yield as well as overall quality. In Nepal, the production of cow milk is increasing in the recent years. So, cow milk can be utilized to prepare paneer. According to Aneja *et al.* (2002). Paneer is highly susceptible to chemical and microbial changes, and emphasized that its packaging should protect against these changes, maintain quality, effective sales appeal, and add to consumer convenience. For the packaging and storage of paneer at refrigeration condition, coextruded laminates,

polyethylene (PE) sachets, heat induced shrink film and wax coated parchment paper, polypropylene (PP) films of higher gauges and retort pouches found to be recommended.

Refrigeration storage of paneer significantly decreased textural properties after 15 days. Controversially all textural properties of frozen samples consistently deteriorated (Dongare *et al.*, 2019). The deterioration of paneer during storage is mainly concern with the growth of yeast and molds. Due to high moisture content and fat content of paneer, spoilage starts after the production of 2-3 days even in refrigeration (Nabvi *et al.*, 2015). Due to this, deterioration of the finished dairy products starts quickly during storage in terms of growth of microorganisms (lactic and non-lactic) present naturally or as contaminants. Thus, there is a urgent need for methods of preservation that can be easily practiced at a minimum and cost (Barman and Roy, 2018).

The shelf life of paneer is quite low and it loses freshness after 2-3 days when stored at 10°C. Generally, surface spoilage of paneer limits its shelf life. Various preservation techniques including chemical additives, packaging, thermal processing, and low temperature storage have been tried by various workers to extend its shelf life (Goyal and Goyal, 2015). Nowadays people are more conscious about their health therefore it is necessary to develop new varieties of paneer for such health conscious people (Kinjal *et al.*, 2015). So, paneer could be made by using *Cinnamomum verum* and *Zanthoxylum armatum* essential oil mix combination incorporated in sodium alginate edible coating instead of using the chemical preservatives.

1.3 Objectives

1.3.1 General objective

The general objective of this work was to study the effect of *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated sodium alginate edible coating on the shelf life of paneer.

1.3.2 Specific objectives

The specific objectives were as follows:

i. To study the effect of calcium chloride concentration on the composition, yield and sensory attributes of paneer made from cow milk.

ii. To determine of total phenol and antioxidant activity of essential oil of *Cinnamon verum* and *Zanthoxylum armatum*.

iii. To evaluate the physicochemical properties and microbial quality of paneer coated with *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated sodium alginate.

iv. To evaluate the shelf life of the *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated sodium alginate coated paneer.

1.4 Significance of the study

Spices could be used to decrease the possibility of food poisoning and spoilage, to increase the food safety and shelf-life of products, and to treat some infectious diseases. The combinations of several spices were proven to possess higher inhibitory effects on specific bacteria than those of individual spices (Liu *et al.*, 2017). Since, the combination of spices has better inhibitory capacity, cinnamon essential oil has been identifies to possess outstanding antioxidant activity as well as high antimicrobial activity against a wide range of spoilage and pathogenic micro-organism (Raju and Sasikala, 2016). Similarly, *Zanthoxylum armatum* essential oil shows an excellent antioxidant activity with moderate antimicrobial properties. These two essential oil were chosen in this study for incorporating in sodium alginate coating for the preservation of paneer (Choubey *et al.*, 2020). Originally, spices and herbs are used to change or improve taste but at the same time they enhance the shelf life because of their antimicrobial and anti -oxidant properties. The components of plants essential oil are also known to contribute to the self-defense of plants against infectious organisms (Kim, 2001). Plants essential oils occur in edible, medicinal and herbal plants, which minimize questions regarding their safe use in food products. Essential oils along with their constituents have been extensively explored as flavoring agents in foods since the ancient time and it is well established that essential oil form many herbal plants have wide spectra of antimicrobial action (Packiyasothy and Kyle, 2002).

Similarly, sodium alginate is chosen as edible coating because it has unique colloidal properties and can form strong gels or insoluble polymers through cross –linking with Ca^{2+} by post treatment of $CaCl_2$ solution. Such biopolymer-based films can keep good quality and prolong shelf life of foods by increasing water barrier, preventing microbe contamination, maintaining the flavor, reducing the degree of shrinkage distortion and retarding fat oxidation; also alginate is a generally recommended as safe (GRAS) substance by Food and

Drug Administration (FDA). Thus the incorporation of the essential oil from two spices to the sodium alginate edible coating may acts as hurdle to protect the paneer from spoilage and prolong the shelf life (Raju and Sasikala, 2016).

To prolong the shelf life of paneer several preservation techniques could be adopted. Spices and herbs have been used since ancient times, because of their antimicrobial properties increasing the safety and shelf life of the food products by acting against food borne pathogens and spoilage bacteria (Nabvi *et al.*, 2015). In addition, spices and herbs are great sources of natural antioxidants for food preservation. Therefore, use of spices and herbs could also be better choice than synthetic antioxidants (Kapadiya *et al.*, 2016).

Furthermore, most of packaging materials used now a day is non- biodegradable which ultimately contributing environmental pollution. Edible films and coatings being bidegradable, have been extensively studied in recent years. Edible films and coatings improve shelf life and food quality by providing a protective barrier against physical and mechanical damage, and by creating a controlled atmosphere and acting as a semipermeable barrier for gases, vapor, and water. Edible films and coatings are produced using naturally derived materials, such as polysaccharides, proteins, and lipids, or a mixture of these materials. These films and coatings also offer the possibility of incorporating different functional ingredients such as nutraceuticals, antioxidants, antimicrobials, flavoring, and coloring agents (Ana *et al.*, 2018).

Thus, this study was focused mainly on application of spices essential oils incorporated edible coating to enhance shelf life of paneer and enhance the value addition as qualitative factor.

1.5 Limitations of work

The limitations of the study are:

- This study gives the shelf life of paneer only under the refrigeration storage.
- Textural properties of paneer under study could not be determined.

Part II

Literature review

2.1 Dairy industry in Nepal

2.1.1 History

With the establishment of a Yak cheese factory in Langtang of Rasuwa district under Food and Agriculture Organization (FAO) assistance in 1953, coordinated milk production activities in Nepal started in 1952. In 1954, under the Department of Agriculture (DoA), a Dairy production unit was established and a small-scale milk processing plant was also initiated in Tusal, a district of Kavre village. In 1955, a Commission on Dairy Production was established.

The first five year plan (1956-61) emphasized the need for a new milk industry to be developed. Thus, a central dairy plant with an average milk processing capacity of 500 l / h was built in Lainchaur in 1956, with financial assistance from New Zealand and technical assistance from the FAO.

A second mini milk processing plant was set up at Kharipati, in the district of Bhaktapur, about the same time. The plant began milk production and marketing operations in 1958. The history of milk cooperatives dates back to the first five year plan (1956-61) in the district of Kavre, Tusal village, when the milk cooperatives were founded. Demand for milk was satisfied by raising cows / buffaloes by the citizens themselves or by the direct supply from experienced milk farmers in previous days when there were no organized dairies. These producers used to go house by house and supply households with the necessary quantity of milk (FAO, 2010).

2.1.2 Status

Production of milk in Nepal in 2019 was 23,01,000 MT. Between 1970 and 2019, production of milk of Nepal grew substantially rising at an increasing annual rate that reached a maximum of 9.0% in 2018 (Gov. N., 2019/2020). 20% of the total production is sold in the organized sector and 80% is sold in local markets and used for household use. Out of the total milk production, 70% comes from buffaloes, and 30% from cows. The

production of buffalo milk is very seasonal, while cow's milk is available throughout the year. Milk supply is currently 70 L per head per year and the Ministry of Agriculture and Livestock Development is committed to increasing milk production within 3 years to hit 91 L per head per person. The dairy industry accounts for nearly 9% of the overall gross domestic product and 26.8% of the agricultural GDP (MOALD, 2016/17).

2.2 Socio-economic impacts

Dairy farming is an important part of rural livelihoods, reflecting the notion of a cooperative approach to meeting farmers' shared goals. Dairy cooperatives have brought farmers together in a culture that has made them more social. The dairy cooperative is a popular place where farmers meet every day in the morning and evening during the distribution of milk. Thus, frequent meetings allowed them the chance to express their socio-economic effect and express shared peace. Dairy cooperatives make community cohesive and welcoming. Dairy cooperatives help to raise awareness of farmers' hygiene, sanitation, and education. Milk and livestock farming sales have improved them economically, such as decent homes, hygienic sanitation, bio-plants, television and schooling. Livestock farming, especially dairy farming, is the villages' backbone of income. Animal and animal by-products, such as food sales, milk currency, manure, draught, and biogas, retain economic value and typically have socio-economic meaning (Chaudhary and Upadhyaya, 2013).

2.3 Paneer

According to Food Safety and Standard Act of India, (2011), paneer is the product obtained from the cow and buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 70.0% moisture and the milk fat content shall not be less than 50.0% of the dry matter.

Paneer is an indigenous coagulated milk product prepared by the addition of permitted organic acids to hot milk and subsequent drainage of whey. Paneer consists of entire milk casein, part of denatured whey proteins, almost all fat, colloidal salts and soluble milk solids in proportion to the moisture content retained. The characteristics features of paneer is a typical mild acidic flavor with slightly sweet taste and has a firm, cohesive and spongy body and a close knit smooth texture. Paneer, like other indigenous dairy products, is a highly perishable product and suffers from limited shelf-life, largely because of its high moisture content. When fresh paneer is stored at higher temperatures, particularly prevailing during

summer months, its shelf-life gets reduced to less than one day. Its shelf-life is reported to be only 6 days under refrigeration (10°C), though its freshness is lost within three days (Khatkar *et al.*, 2017).

According to the mandatory standard of DFTQC (2021) - "Paneer is a solid product prepared from healthy cow or buffalo's pasteurized milk or combination thereof, precipitated by sour milk or lactic acid or citric acid". It can be prepared by using powder milk. It should be free from rancid smell or molds and free from added color and other matters. The minimum mandatory standard of paneer is as shown in Table 2.1.

Table 2.1 Mandatory standard for paneer

Parameters	Requirements
Moisture (%)	Not more than 70.00
Milk fat (% dry basis)	Not less than 50.00

Source: DFTQC (2021)

According to BIS (1983)-"Paneer is an important indigenous milk product prepared by the combined action of acid coagulation and heat treatment of buffalo or cow milk or a combination thereof". The BIS standard for the different parameters of paneer is as shown in the Table 2.2.

Table 2.2 BIS standards for paneer

Parameters	Requirements
Moisture (% by mass)	60.00 max
Milk fat (% by mass on dry matter basis)	50.00 min
Titratable acidity (% lactic acid)	0.50 max
Standard plate count per g	5×10^5 max
Coliform per g	90 max

Source: BIS (1983)

2.3.1 Paneer characteristics and composition

Typically paneer is marble to light creamy white in appearance. It must have firm and cohesive body with slight sponginess or springiness. The texture should be more compact

(close-knit), smooth and velvety. The flavour should be pleasing mild acidic, slight sweet and nutty (Gupta, 1985). It is mainly consumed in raw form or used in preparation of several varieties of culinary dishes and snacks. The deep frying ability of paneer make it further acceptance for making snacks, pakoras or fried paneer chunks (Aneja, 2007).

Paneer is a rich source of animal protein available at a comparatively lower cost and forms an important source of animal protein for vegetarians. Biological value of protein in paneer is in the range of 80 to 86 (Shrivastava and Goyal, 2007). In addition, paneer is a valuable source of fat, vitamins and minerals like calcium and phosphorus.The composition of paneer varies greatly according to the initial composition of milk as shown in Table 2.3.

Table 2.3 Proximate composition of paneer from different milk.

Type of milk used for paneer making	Constituents (%)				
	Moisture	Fat	Protein	Lactose	Ash
Buffalo milk (6% fat)	50.98	27.97	14.89	2.63	2.08
Whole buffalo milk	51.52	27.49	17.48	2.28	2.18
Cow milk (3.5%)	55.97	18.98	20.93	2.01	1.45
Cow milk (4.5%)	55.26	24.15	18.43	-	-
Buffalo and soya milk (50:50)	54.60	18.33	19.81	-	1.68

Source: Kumar *et al.* (2011)

2.3.2 Microflora of paneer

Due to high fat and moisture content, paneer is prone to microbiological spoilage leading to rancidity (due to fat breakdown) and mouldy surface (due to mould growth). Storage of packaged paneer at low temperature (5-10^{o}C) can check the rancid defect (Raju and Sasikala, 2016).

The microbiological quality of paneer, like other indigenous milk products, chiefly depends on the conditions of manufacture, subsequent handling, storage and sale of the product. The possible sources of contamination might be air, water, utensils, cutting knife, muslin cloth as well as persons handling the product. Hence, the number and types of microorganisms and their distribution in the product may vary depending on the location of the halwai shop, extent of exposure of the product to the atmosphere, temperature and period of storage etc. According to a survey conducted on the market quality of paneer obtained

from Karnal and Delhi samples were found to contain heavy load of total bacterial population, coliforms and yeasts and moulds (Rajorhia, *et al.,* 1984). Microbial quality of paneer is also a prime factor for the improved shelf life. Proper maintenance of hygienic coditions during preparation of paneer in laboratories shows the absence of coliform (Naraya et al., 2016), But the paneer produced by the local vendor showed a higher load of coliform due unhygenic practices during the preparation (Dhole, 2009 ; Agnihotri, 1996).

2.1.2 Principle of paneer making

The basic principle of paneer making is physical and chemical changes in casein and whey protein due to the combine action of heat and acid coagulation. When milk is acidified, the colloidal calcium phosphate in the casein micelles progressively solubilises and aggregation of the casein occurs as the isoelectric point is approached. In milk of normal pH, the casein micelles are stablized by hydration and steric repulsion due to their negative charges. On acidification, the micelles become unstable and aggregate as a result of charge neutralization, leading to the formation of chains and clusters that are link together to give three-dimensional network. In milk preheated at high temperatures (90ºC), gelation occurs more rapidly at higher pH than in unheated milk. Interaction of whey proteins with casein micelles on heating milk at its natural pH may increase the hydrophobicity of the micellar surface and reduce the hydraton barrier against aggregation, thus allowing aggregation and gelation to occur at a higher pH than in unheated milk. Heating milk also results in dissociation of κ-casein from micelles, and this could further sensitize the α_s-casein framework to calcium induced aggregation. The development of typical reohological characteristics of paneer could be due to the intensive heat induced protein-protein intractions. Paneer manufacture essentially involves the formation of co-precipitates due to complexing of whey proteins denatured by the heat and the casein. Serum proteins , particularly β–lactoglobulins, are bounded to ƙ-caseins via disulphide bridges and calcium linkages. The higher the degree of co-precipitation, the greater was the total solids recovery and yield of paneer (Pal and Khan, 2011).

2.3.3 Method of manufacturing

The general flow chart for manufacturing paneer is presented in Fig. 2.1 (Sachdeva and Singh, 1988b).

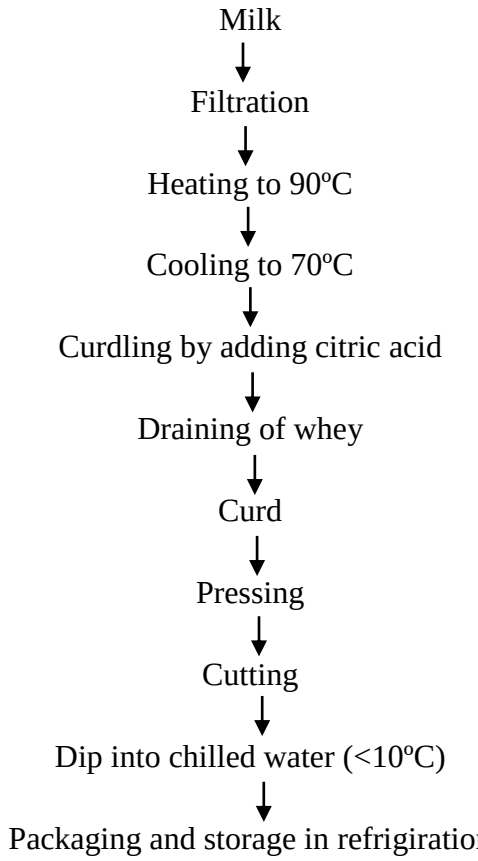


Fig. 2.1 General flow chart for preparation of paneer

Milk having or standarized to 4.5% fat and 8.5% is heated to 90ºC without holding. Thereafter, the temperature of milk is brought down to 70ºC and coagulated at this temperature using 1% citric acid solution. The temperature of citric acid solution is also maintained at 70ºC. Citric acid solution is added with continuous stirring till clear whey separate out. After complete coagulation, stirring is stopped and the coagulated mass (curd) is allowed to settle down for about 5 min. The temperature of the content is not allowed to drop below 63ºC until this stage. The curd is collected and filled in hoops (with holes on all its sides to facilitates the expultion of whey) lined with clean fine muslin cloth. The hoops containing curd is pressed for 10-20 min. Thereafter the pressed block of curd is removed and immeresed in chilled water of <10˚C for about 2 h. Dipping of paneer facilitates cooling the product and also it absorbs moisture and improves the body and texture of paneer. The chilled paneer is then removed from water and cut into desirable size and packaged in suitable packaging material. Finally, it is stored under refrigeration till marketing and consumption.

2.3.4 Improvement in quality of paneer

Paneer made from cow milk results in soft, fragile and weak body and texture due to lower amount of calcium and different make up of casein. Good quality paneer could be manufactured from cow milk by using calcium salt that help to improve cross linking during curd formation. The addition of 0.08-0.15% calcium chloride helped to produce better quality paneer from cow milk (Sachdeva *et al.*, 1991). The addition of 0.1% calcium chloride has reported to increase total solids, yield and sensory characteristics of paneer (Singh and Kanawjia,1988). By adding 0.15% calcium chloride, the yield of paneer made from recombined milk, and diluted milk can also be improved.

The yield of low fat paneer can be increased by adding 0.1% sodium citrate or 0.5% sodium chloride (Chawla *et al.*, 1987). Improvement on overall sensory score including body and texture, flavor, color and appearance was observed by Arora *et al.* (1996) when 0.05% $CaCl_2$ in milk. Addition 0.5% common salt in milk improves yield, textural properties as well as enhanced the shelf life of paneer for about 2 days at room temperature (Pal and Kapoor, 2000). The paneer cubes dipped in 1-5% brine solution decreased moisture content and water activity as well as increased flavour and overall acceptability (Kaur and Bajwa, 2003). Various emulsifing salts like monosodium phosphate, disodium phosphate, sodium hexametaphosphate, tetrasodium pyrophosphate, trisodium citrate and sodium tripolyphosphate at 1-3% could be used for the preparation of processed paneer (Pal and Kapoor, 2000). Pal *et al.* (1993), observed that paraffining of low-fat paneer cubes (1 inch cubes) increased the shelf life by over 10 days compared to un-paraffined ones. The use of sour butter milk with 0.15% titrable acidity by addition sodium bicarbonate and washing of curd with hot water at 72ºC before pressing to mitigate the problems of self-coagulation of milk during heating, development of acidic smell, sour taste and grainy texture in paneer (Pal and Garg, 1989).

The yield of paneer has shown to be increased when sodium alginate, carrageenan or pre-gelatinized starch was used at different levels as due to retention of moisture (Sachdeva and Singh, 1988a). Similarly, addition of pre-gelatinized starch at 0.1% with higher coagulation temperature (90ºC) improved the body texture and yield of filled paneer (Roy and Singh, 1994). The physicochemical and storage characteristics of paneer made from cow milk using calcium sulphate(0.02 v/v) and disodium hydrogen phosphate (0.05) was studied and found the influenced of additives on the composition, quality, yield and shelf life of paneer (Mistry

et al., 1990). Effect of variation of $CaCl_2$ (0-0.15%) and NaCl (0 to 1%) on the yield of paneer made from cow milk was studied and the yield was found between 14.79% to 15.5% (Kinjal *et al.*, 2020).

2.3.5 Preservation of paneer

A study on effect of low temperature storage of paneer at 10ºC, -13ºC and -32ºC has reported no change in moisture, total nitrogen, non-protein nitrogen (NPN) and pH up 6 days of storage at 10ºC, but the pH decreased significantly on the 7th day. Further the sensory quality of frozen paneer was acceptable upto 90 days, after that the body and texture became crumbly and fluffy (Arora and Gupta, 1980). Paneer stored at -9ºC and -15ºC has shown to be a shelf life of 80 days without affecting on textural and sensory attributes except surface drying of paneer (Vishweshwaraiah, 1987). Dried extruded paneer at 75ºC for 2 h could be stored well upto 2 months as against control which had only 3 days at 10ºC but has a poor rehydration characteristics and cohesiveness (Vishweshwaraiah, 1987). Paneer cubes packed in tin cans, followed autoclaving at 15 psi for 15 min result in shelf life of 50 days at ambient temperature but after have developed mouldy flavour, slight browning and cooked flavour (Sachdeva, 1983).

It has been reported that paneer treated with 20 ppm lactoferrin resulted in 14 days keeping quality at refrigation temperature (4±1ºC) and 6 days at room temperature (30±1ºC) (Shashikumar and Puranik, 2012). Paneer dipped in 5% brine solution has enhanced the shelf life of paneer up to 22 days at refrigeration temperature (Sachdeva, 1983). Modification on the method of production and its shelf life was studied by Bhattacharya *et al.* (1971). An extension 6 days in shelflife at room temperature has been reported, when raw paneer was dipped in 18% salt solution for 30 min. Overnight dipping of paneer in brine solution followed by packaging and refrigeration has observed to be increased the keeping quality of paneer up to 12 days (Singh and Kanawjia, 1988).

The effect of delvocid (a non toxic antibiotic produced by *Streptomyces natalensis*) in combination with hydrogen peroxide (0.2%) has been studied for enhancing the shelf life of paneer. Delvocid (0.5%) in combination with hydrogen peroxide (0.2%) has shown to extend shelf life of paneer till 32 days, while paneer immersed only in delvocid solution (0.5%) resulted in deterioration of paneer on 8th day of refrigeration storage (Sachdeva, 1983).

Similarly, It has been shown that paneer containing potassium sorbate (0.1%) could be preserved up to 13, 3, and 1 day at 7, 22 and 37ºC respectively (Thakral, 1986). Addition of sorbic acid to milk (0.10%) before preparation of paneer followed wrapping in sorbic acid coated paper (2 g/m^2) result in shelf life extension of up to 6 days at ambient temperature and 36 days at 5ºC (Singh *et al.,* 1989). The use of bacteriocin (from *Enterococcus facecium* BS 13) alone or in combination with sorbic acid has also shown to increase the shelf life of the paneer up to 15 days at refrigerated temperature (Bakkali *et al.*, 2008). The combined treatment of sorbic acid (0.01%) in milk and paneer irradiated at 2.5 KGy resulted in shelf life of 30 days at ambient temperature (Singh *et al.*, 1991). An extended shelf life of paneer up to 40 and 20 days at refrigerated and room temperature (37°C) temperatures, respectively by using benzoic acid (1,200 ppm) has also been reported (Modi and Jain, 1988).

Modified atmospheric packaging (MAP) with carbon dioxide (CO_2) was found to be effective in extending shelflife of paneer as compared to MAP with control (atmospheric air), nitrogen(N_2) with less reduction of moisture content and increment of free fatty acid . A maximum loss in moisture was observed in control (atmospheric air) as followed by vaccum, 100% nitrogen and 100% carbon dioxide along with free fatty acid content (as % oleic acid) increased from 0.18 to 0.24, 0.21, 0.20 and 0.21 in atmospheric air, vacuum, 100% CO_2 and 100% N_2, respectively (Rai *et al.*, 2008). Some biochemical changes related to microorganism during storage of paneer was studied and yeast and molds in brine paneer and spiced paneer on the 5^{th} day of storage was observed by Pal (1998).

Thippeswamy *et al.* (2011) studied the shelf stability of paneer by combined application of hurdle technology and MAP, and reported the use of sodium chloride 3% and citric acid 0.1% reduced a_w from 0.994 to 0.970 and pH from 5.6 to 5.1, respectively. While mixture of CO_2 and N_2 (50:50) resulted in incresaed shelf life up to 12 days at ambient temperature (30±1°C) and 20 days at refrigeration (7±1°C) temperature. Microwave heated followed by MAP packaging of paneer has shown increased shelf life to 35 days at 7±1ºC (Karthikeyan, 2005).

Several researcher have studdied the effect of spices essential oil in enhancement of shelf life of paneer and other perishable products. Paneer containing cardamom, clove, cinnamom and ginger each for low, minimum, and high doses shows shelf life of 28, 32 and 36 days; 24, 28 and 32 days; 32, 36 and 40 days respectively at 5±1°C (Makhal and Kanawjia, 2005).

Bhatt (2012) studied the use of oleoresins and essential oils of cardamom, cinnamon and clove directly in milk at 40°C, among these the essential oil of cardamom and cinnamon were found most effective to enhance the shelf life of paneer. Amongst the essential oils of cardamom, cinnamon and their combination; essential oil of cardamom was most effective to control microbial growth and deteriorative chemical changes in paneer. Shelf life of paneer prepared with 0.01% essential oil of cinnamon, 1:1 combination of essential oil of cinnamon and cardamom, and essential oil of cardamom alone and packed in PET/LDPE film was reported to be 10, 15 and 15 days respectively while only 5 days was reported for control paneer storage at 7°C. The incorporation of cinnamon essential oil into sodium alginate-calcium formulation to extend the shelf life of paneer was studied at refrigeration temperature and the author has shown that the shelf life of paneer incorporated with 2.5% cinnamon essential oil extend shelf life up to 13 days compared to 5-6 days only for control sample (Raju and Sasikala, 2016). However, uses of some selected spices in paneer to extend its shelf-life have been carried out.

Seven different spices like garlic, ginger onion, cinnamon, black peeper, cardamom and cloves were used directly to the coagulum. Black pepper, cardamom and clove used at the rate of 0.6% while cinnamon at the rate of 0.4% have shown extended shelf life of paneer to 21 and 28 days under refrigeration storage at 7°C in PET/LDPE film. Among the seven different spices, cardamom and cinnamon was found to be effective to extend the shelf life of paneer (Eresam *et al.*, 2015). Shelf life of paneer treated with cinnamon essential oil packed in different packaging material (LDPE, Nylon and Metalized polyester) was carried out and the treated paneer packed in the metalized polyester was found to have the shelf life of 18 days at refrigeration storage of 8±1ºC (Anju *et al.*, 2017).

Shelf life of paneer could be significantly increased by using packaging materials. The type of packaging material also plays an important role in enhancement of shelf life. Normally, paneer blocks of required size/weight are packaged in polyethylene pouches, heat sealed and stored under refrigeration conditions. Alternatively, they are vacuum packaged in laminated or co-extruded films. The shelf life of tin-packaged paneer was reported to be 3 months at 35°C and paneer packaged in laminated pouches had a shelf life of about 30 days at refrigerated storage (6 ± 1°C) (Sachdeva *et al.*, 1991).

2.4 Edible film and coatings

Edible films or coatings have provided an interesting and often essential complementary means for controlling the quality and stability of numerous food products. There are many potential uses of edible films (e.g., wrapping various products, individual protection of dried fruits, meat and fish, control of internal moisture transfer in pizzas, pies, etc.). The effectiveness of such coating are based on the films properties (e.g., organoleptic, mechanical, gas and solute barrier). Polysaccharide (cellulose, starch, dextrin, vegetable and other gums) and protein (gelatin, gluten, casein,) based films have suitable mechanical and organoleptic properties, while wax (beeswax, carnauba wax, etc) and lipid or lipid derivative films have enhanced water vapour barrier properties. The film forming technology, solvent characteristics, plasticizing agents, temperature effects, solvent evaporation rate, coating operation and usage conditions of the film (relative humidity, temperature) can also substantially modify the ultimate properties of the film (Guilbert and Gontard, 1995).

Considerable interest in edible films and coatings has been reviewed due to their environment friendly nature and compatibility to use in food industries. Traditional synthetic polymeric materials can not be consumed, whereas, edible film and coatings can be consumed with food products. Edible films and coatings have long been used for food protection and shelf life improvement (Guilbert and Gontard, 1995). Earlier works reported that in twelth century, wax coating of citrus fruits reduced the rate of dehydration and in sixteenth century, fat coating on meat reduced shrinkage. Soy protein films were used in Asia to improve appearance and preservation of some foods in fifteenth century, while sucrose was used to prevent oxidation of nuts such as almonds since nineteenth century (Debeaufort *et al.*, 1998).

There is no compositional difference between edible film and coating, however they differ in their thickness. Films are formed separately by casting process and then are applied on food surface, but the coatings are formed directly on food surface either by spraying, dipping, and spreading. A film can be separated from food surface but the coating generally is considered as an integral part of final product. Both film and coating can be applied on food surface or in-between the food components. They help in reducing moisture loss, fat migration, flavor loss and to incorporate of antimicrobials, antibrownings agents, antioxidants, colors, flavors, sweeteners, nutraceuticals, vitamins, minerals there by enhancing shelf life and nutritional quality. The other possible application is multilayer

packaging i.e. the edible film and coating of food material packed in non-edible packaging materials. Although they are unable to replace synthetic polymeric materials completely as of now but they can have huge potential application in new product development (Tharanathan, 2003).

2.5 Types of biopolymers used in edible film and coating

Edible films and coatings are prepared from different types of biopolymers, they are proteins, lipids, polysaccharides and composites.

2.5.1 Proteins

Protein films are generally prepared by heat denaturation of protein in a suitable solvent and then evaporation of solvent. Generally, water or ethanol are used as solvents in most of the protein film preparations. Milk protein, corn protein, gelatin, soy protein, wheat gluten protein were investigated for film forming properties and applied in food packaging (Ramos *et al.*, 2013).

2.5.1.1 Milk protein

Film can be prepared from either total milk protein (TMP) or from different types of milk proteins. The formation of TMP films was complicated due to the presence of lactose, which crystallized during film formation and leads to non homogeneous film and film adhesion to surfaces. Lactose can be extracted from non-fat dry matter by ultra-filtration (UF) or suspension in ethanol followed by a filtration. Addition of potassium sorbate also inhibits crystallization (Maynes and Krochta, 1994).

UF-TMP films are more flexible and with better water moisture barrier, but denaturation by ethanol may reduce film water solubility. Heating TMP solution up to 135°C produces insoluble films, and films are stronger and less brittle due to the dissociation of micelles and intermolecular bonds (Singh and Fox, 1989).

Milk contains two major protein viz. casein and whey protein. Casein consists of β-casein, κ-casein and α-casein. Caseins have more open structure and high amount of proline contributing to its better emulsification property than whey protein. Commercially casein is available as calcium caseinate, magnesium caseinate, sodium caseinate and potassium caseinate. Caseinate films are less water vapor barrier due their hydrophilic nature. Among

all other milk proteins, β-casein film and coating shows lower water vapor and gas permeabilities (McHugh and Krochta, 1994).

The films prepared from caseinates are water soluble and the water insoluble films can also be manufactured by treating such films with a buffer at the isoelectric point of casein. Whey proteins are the protein that remains soluble after casein is precipitated at pH 4.6. They are globular and heat labile in nature, consist of several component proteins, including α-lactalbumin (α-La), β-lactoglobulin (β-Lg), bovine serum albumin (BSA), immunoglobulins (Ig), and proteosepeptones (Kinsella and Whitehead, 1989). Industrially produced whey protein concentrates (WPC) have a protein content of 25–80%. Whey protein isolates (WPI), which have a protein content of about 90%, are prepared from WPC by adding an ion-exchange step. The most commonly used methods today for the production of WPC are ultrafiltration because of their advantages of cost reduction, high process speed, and the absence of protein denaturation (Morr and Ha, 1993).

2.6 Spices and spices extracts

Plant, animal, and microbes represent an unlimited source of compounds with medicinal properties (Gottardi *et al.*, 2016). According to the U.S. Food and Drug Administration (FDA), spice is an "aromatic vegetable substance in the whole, broken, or ground form, the significant function of which in food is seasoning rather than nutrition" and from which "no portion of any volatile oil or other flavoring principle has been removed" (Sung *et al.*, 2012). More than 100 varieties of spices are produced throughout the world. Asia is the main leader for the production of spices, particularly of cinnamon, pepper, nutmeg, cloves, and ginger, while Europe grows mainly basil, bay leaves, celery leaves, chives, coriander, dill tips, thyme, and watercress. In America, pepper, nutmeg, ginger, and sesame seed are mainly produced (Prasad *et al.*, 2011).

Spices exert antimicrobial activity in two ways either by preventing the growth of spoilage microorganisms or by inhibiting/regulating the growth of pathogenic microorganisms (Tajkarimi *et al.*, 2010). Spices are storehouse of many chemically active compounds that also impart flavor, fragrance and piquancy. Flavor of most of the spices is due to its volatile oils, fixed oils oleoresins (Parthasarathy *et al.*, 2008).

Phytochemicals in spices are secondary metabolites, which are originated for the protection from herbivorous insects, vertebrates, fungi, pathogen, and parasites. Most

probably, no single compound is responsible for flavor; but a blend of different compounds such as alcohols, phenols, esters, terpenes, organic acids, resins, alkaloids, and sulphur containing compounds in various proportions produce the flavors. Besides these flavoring components every spice produce contains the usual components such as proteins, carbohydrates, fiber, minerals, tannins or polyphenols (Raghavan, 2007).

Spice extracts are highly concentrated forms of spices; contain the volatile and non-volatile oils that give each spice its characteristic flavor. The volatile portions of spice extractives give the particular aroma of the spice. The non-volatiles include fixed oils, gums, resins, antioxidants and hydrophilic compounds and they contribute to the taste of a spice (Raghavan, 2007).

Spice extracts are prepared as natural liquids and dry encapsulated oils. The spice extracts are standardized for color, aroma, and taste. They are more concentrated than dried or fresh spices and so are used at much lower levels. These extractives provide more consistency than dried spices in prepared foods. The spice extracts are available as essential oils, oleoresins and modified extract etc. (UNIDO and FAO, 2005).The volatile portions of spice extracts is referred to as essential oils, most spices owe their distinctive “fresh” character to their essential oil content that generally ranges from 1 to 5% but even goes up to 15% in certain spice (Raghavan, 2007). Essential oils are produced by grinding, chopping, or crushing the leaf, seed, stem, root or bark, followed by extraction through cold expression, dry distillation or hydro (water, steam or steam and water) distillation and recovering the distillate oil. Depending upon the method of extraction, the nature of the volatiles can differ with the same type of spice (UNIDO and FAO, 2005).

Essential oils are the major flavoring constituents of a spice. They are soluble in alcohol or ether and are only slightly soluble in water. They provide more potent aromatic effects than the ground spices. Essential oils lose their aroma with age. They are more concentrated (~75 to 100 times) than the fresh spice. They do not have the complete flavor profile of ground spices, but they are used where a strong aromatic effect is desired. Essential oils are used at a level of 0.01 to 0.05% in the finished product (Rudolfi *et al.*, 1988).

Essential oil can be irritating to the skin, toxic to the nervous system if consumed as such and can cause allergic reactions and even miscarriages. The mixture of non-volatile and volatile flavor components of spices is referred to as oleoresins. They are produced by

grinding or crushing the spices, extracting with a solvent and then removing the solvent. Oleoresins have the full flavor, aroma, and pungency of fresh or dried spices because they contain the high boiling volatiles and non-volatiles, including resins and gums that are native to spices (Raghavan, 2007).

The non-volatile components create the heat and or pungency of spice. Oleoresins are viscous oils and thick pastes; more difficult to handle than essential oils. Oleoresins are used at very low concentrations because they are highly concentrated. They have greater heat stability than essential oils. Oleoresins give more uniform flavor and color with less variability than their ground spice counterparts. They are typically used in high heat applications such as soups, salad dressings, processed meats, and in dry mixes and spice blends. They are convenient to use because of the ease with which they disperse into water-based foods such as soups, sauces, pickles, or gravies (UNIDO and FAO, 2005).

Spices essential oils have extensively studied for their antimicrobial activity. Gram-positive bacteria were shown to be more sensitive to the spice essential oils than Gram-negative bacteria. *Staphylococcus aureus* and *Bacillus subtilis* were the most sensitive bacterial strains tested; where as a strain of *Escherichia coli* (MTCC-118) was the least sensitive (Patel *et al.*, 2007). It showed that spice essential oils may prove useful in inhibiting bacteria of food spoilage and health significance. Among the essentials oils of aniseed, mustard, cardamom, cinnamon, dill, mace, zedoary, prikhom, and bitter ginger; cinnamon oil had the highest antibacterial activity (Nanasombat and Wimuttigosol, 2011). Commonly used spices like garlic, cumin and cinnamon imparts higher total phenol and radical scavenging activity (Wijewardhana *et al.*, 2019). The most sensitive bacteria was reported to be *Bacillus cereus* (0.5mg/mL minimum inhibitory concentration, MIC). Anise, cinnamon, dill, and prikhom exhibited strong antifungal activity against *Rhodotorulaglutinis*, *Aspergillus ochraceus*, and *Fusarium moniliforme*. Two oil combinations: i) cinnamon and mace oils and ii) cinnamon and prikhom oils showed a synergistic effect against *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Salmonella rissen* [(0.32- 0.38 mg/ml fractional inhibitory concentration index (FICI)]. Cinnamon, mace, and prikhom oils had also strong antioxidant activity generally ranges from 0.29 to5.66 mg/ml (IC_{50} value), from 61.46% to 68.52% antioxidant activity, from 0.22 to 2.19 mM/mg reducing capacity, and from78.28 to 84.30% inhibition based on 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching, ferric reducing (FRAP), and superoxide anion

scavenging activity assays, respectively. These oils contained high amount of total phenolic (51.54 - 140.9 μg gallic acid equivalents/mg).

Herbs and spices containing essential oils in the range of 0.05–0.1% has demonstrated activity against pathogens, such as *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*, in food systems. Application of herbs, spices and essential oils with antimicrobial effects comparable to synthetic additives but is still remote for three major reasons; limited data about their effects in food, strong odor, and high cost. Combinations of techniques have been successfully applied in several foods and in vitro experiments (Tajkarimi *et al.*, 2010).

2.6.1 Essential oils

Essential oils are plant derived volatiles with a hydrophobic character. They are extracted from various plant organs; leaves, fruits, flowers, bulbs, seeds, roots, wood and bark of aromatic plants. Essential oils are known to possess antiviral, antibacterial, antifungal and insecticidal properties (Burt, 2004). They can have more than 50 components, of which 2-3 are the main components representing 85-95% of the whole volume, while the others are the minor components, sometimes below 1%. The chemical character of the compounds influences their antimicrobial efficacy and the mechanism of action of the target organisms. The two main groups of essential oils are terpene and terpenoids, and the aromatic and aliphatic constituents (Bakkali *et al.*, 2008).

Essential oils have several targets in the cell and cellular activities. Essential oils can have direct effect on degradation of cell wall, and weakening the membrane causing enhance permeability, lead to the loss of the intracellular components (Cox *et al.*, 2000). Essential oils can also react with important cell membrane proteins depleting their function (Burt, 2004). The hydroxyl groups of phenolic and alcoholic components present in essential oil can form hydrogen bonds with amino acid residues in the active site of enzymes (Cristani *et al.*, 2007). Monoterpenes, pinene and limonene were inhibited the respiratory activity in intact yeast cells and also in isolated mitochondria (Uribe *et al.*, 1985).

Spore formation of *Aspergillus species* was found to be reduced by essential oil from lemon grass and cassia, cinnamon or clove (Paranagama *et al.*, 2003). It seems that they cause disruption of ergosterol biosynthesis in fungi as similar to the action of azole fungicides (Daferera *et al.*, 2000).

2.6.2 Anti- yeast and molds activity of essential oil

Essential oils contains the phenolic compounds as main constituents with membrane disrupting ability, is responsible for inhibitory effects. Some essential oil, containing non-phenolic main compounds have been also found to show high toxicity against yeasts. In general, MIC of the main components of the essential oils found to be higher than the MIC for the parent essential oils, suggesting the synergistic effect of essential oil's components. It seems that monoterpenes (α-pinene, β-pinene, and α-terpinene) beside phenolics play a considerable role in disturbing the membrane function in yeasts (Elgayyar, 2001; Sachetti *et al.*, 2005; Tserennadmid *et al.*, 2011).

The most frequently investigated species in the antifungal tests are strains from the *Aspergillus, Penicillium* and *Fusarium*. A broad spectrum of essential oils and essential oil components has been used against molds. In mycotoxigenic fungi, not only the growth inhibition but also the reduction of toxin production by essential oil was also reported. Environmental factors such as water activity and temperature have described to play important role in the anti-fungal and anti-mycotoxigenic effect of essential oils (Krisch *et al.*, 2011).

2.6.3 Cinnamon (*Cinnamomum verum- dalchhini*) essential oil

Cinnamon powder in meat and cheese exhibited bacteriostatic action on *Listeria monocytogenes*. Foods treated with 6% cinnamon showed 1 to 2 $\log_{10}$ reduction in *Listeria* counts per g than in controls on holding the foods at 30ºC for 7 days. Treatment with 3% cinnamon also slow down the growth of the microorganisms significantly in meat but to a lesser extent in cheese at 30ºC (Menon, 2000).

Antimicrobial activity of spices were well investigated in meat products against *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Mycobacterium smegmatis, Micrococcus luteus*, and *Candida albicans* test strains by using disc diffusion method (Agaoglu *et al.*, 2007). In a study, it has been found that cinnamon was the most effective spice against tested microorganisms. The antimicrobial activity of cinnamon may be explained by its volatile oil components. The most important active substances found in cinnamon oil are cinnamic aldehyde and eugenol. Water extract of cinnamon at 600 µg/ml concentration was found to have 95% inhibitory effect towards *Escherichia coli* , while other spices extracts (turmeric, asafetida, thyme, tulsi,

clove) have shown to have 100% inhibitory effect at the same concentration (Venugopal *et al.*, 2009).

2.6.4 Timur (*Zanthoxyllum armatum*) essential oil

Timur (*Zanthoxylum armatum*), a main non-timber forest product is a major indigenous spice of Nepal. This spice is mostly cultivated in the mid-western region of Nepal. Annually, about 850-1100 MT of *Timur* is harvested in Nepal (Tiwary *et al.*, 2007). The ripe fruit follicles are usually reddish in color and 4 to 5 mm in diameter. The dried fruit also contain an aroma that is present in brown fruit wall (pericarp-shell). It may be able to develop numbing or anesthetic feeling on the tongue. Seeds are solitary, globes, shining and have bitter taste (Brijwal *et al.*, 2013). The seeds and barks of *Zanthoxylum armatum* are highly used in indigenous system of medicine for the cure of carminative, stomachic and anti-helminthic. The seeds are used as an aromatic tonic for fever and dyspepsia. The seed extract has been reported to be effective against roundworms. The seeds taste great on addition in pickles making it a household preference in Nepalese kitchen (Dhakal and Sharma, 2020).

Timur is not only used as flavorings in cooking but also its seed oil and crushed seeds are added to cereal seeds and legumes to protect them against damages caused by stored grain pests. The hydro distilled essential oil of *Zanthoxylum armatum* was found inhibitory effects against five fungi, i.e. *Aspergillus* sp., *Alternia* sp., *Penicillium* sp., *Cladosporium* sp., and *Helminthosporium* sp. (Prajapati *et al.*, 2015). *Zanthoxylum armatum* was found to have potential antibacterial activity against 10 medically important bacterial strains, namely *Bacillus subtilis, B. cereus, B. thuringiensis, Staphylococcus aureus, Pseudomonas* spp, *Proteus* spp, *Salmonella typhi*, *Escherichia coli*, *Shigella dysentriae*, *Klebsiella pneumonia* (Joshi *et al.*, 2009).

2.7 Safety issues of spices essential oil

Starting from the food preparation, spices can affect both food spoilage microorganisms (food preservation) and human pathogens (food safety) due to the antimicrobial and antifungal activity of their natural constituents. Spices are provided from natural herbs and plants and generally recognized as safe (GRAS) by the American Food and Drug Administration (FDA). However, the need of high amount of natural compounds represents the main limitation for effective performance against microorganisms. Mostly, their organoleptic characteristics may impact the results of *in vitro* and *in vivo* trials. For this

reason, combinations of spices or their pure natural compounds, applied with or without additional technologies, represent a promising alternative to avoid this problem. Synergistic effects can lead to a reduction of both natural compounds used and treatment applied (Rubio *et al.*, 2013). Use of spices as preservatives has been assessed in multiple foods: meat, fish, dairy products, vegetables, rice, fruit, and animal food (Tajkarimi *et al.*, 2010).

2.8 Use of spices in dairy products to extend shelf life

Use of spice has also been reported in dairy products viz. cheese, butter, traditional Indian dairy products, ice cream, etc. to extend their shelf life.

2.8.1 Cheese

The extracts of cinnamon stick, oregano, clove, pomegranate peel and grape seed has been reported to be effective against *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica* with reduced lipid oxidation (TBA-reactive substances) of cheese stored at room temperature (Shan *et al.*, 2011).

2.8.2 Butter

Farag *et al.* (1989) used thyme and cumin essential oils to prevent butter deterioration during storage at room temperature. Butter oxidation and lipolysis were followed by measuring the acid, peroxide and thiobarbituric acid (TBA) values. Use of thyme and cumin essential oils to prevent deterioration of butter and extracts of oregano and sage to prevent microbial growth in butter; and the effects of some herbs on growth and acid production of cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Sharma and Verma, 2006).

2.8.3 Traditional Indian dairy products

It has been reported that the cardamom powder added at the rate 0.1% enhance the shelf life of *sandesh* to 90 days as against 60 days for control sample at 7^{o}C. Similarly cardamom powder and saffron has also studied as a flavor enhancer and preservative effect in *sandesh* (Sen and Rajorhia, 1996).

2.8.4 Ice cream

Pinto (2004) has evaluated the suitability of ginger juice as a flavoring agent in developing ginger ice cream and studied its effect on certain physico-chemical and sensory characteristics of ice cream.

2.8.5 Fermented dairy products

Dahi, yoghurt, acidophilus milk and garlic extract were assayed for antimicrobial activity against *Micrococcus flavus*, *Enterococcus faecalis*, *Bacillus cereus* and *Salmonella veltevreders* (Ghodekar and Gandhi, 1988). All 4 organisms were found to be inhibited by garlic extract and to a lesser extent by acidophilus milk however only *M. flavus* was inhibited by yoghurt and none of the organisms was inhibited by *dahi*. Titrable acidity as % lactic acid of *dahi*, yoghurt and acidophilus milk were reported to be respectively 0.77, 0.95 and 1.44 in the absence of garlic extract; 0.67, 0.80 and 1.19 with 1% garlic extract, and 0.25, 0.35 and 0.54 with 3% garlic extract. The author concluded that antimicrobial activity of the cultured milks was marginally improved by addition of garlic extract (Ghodekar and Gandhi, 1988). Food spoilage is important consideration, so excess of food can be processed and stored in a practical manner without or little deterioration (Patton, 1955).

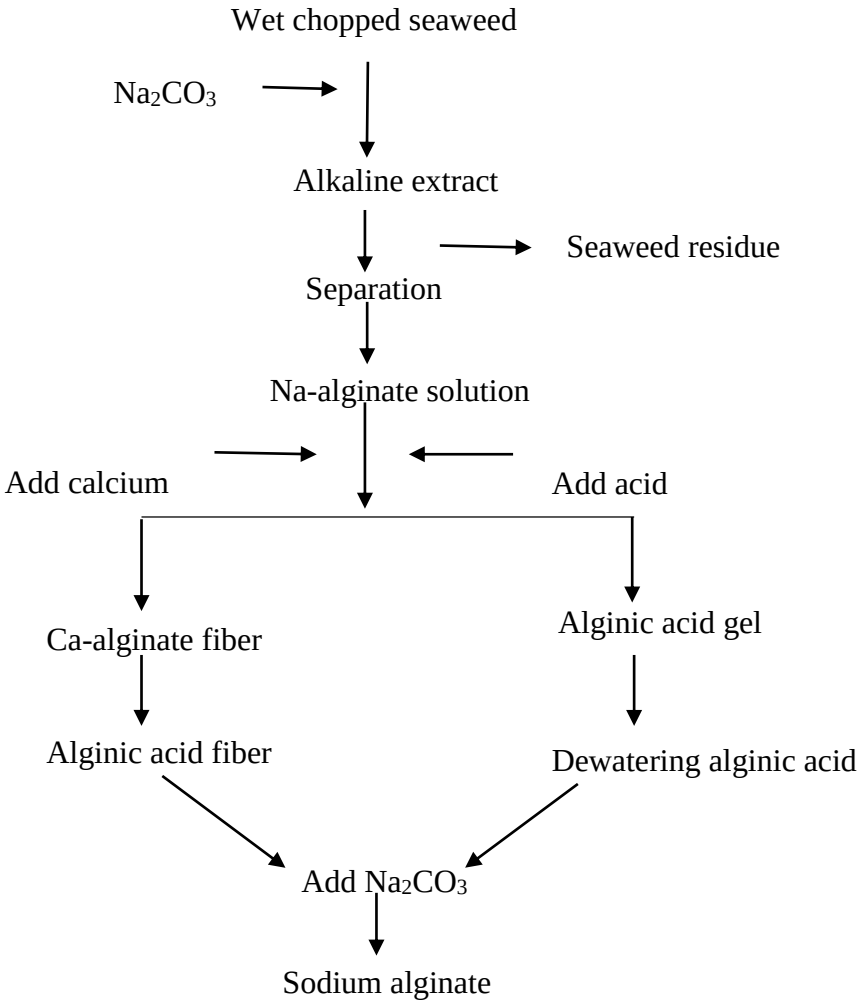
2.9 Sodium alginate

Alginate, an anionic polysaccharide produced from brown algae (*Phaeophyceae*) and bacteria (*Azobacter vinelandii*), has been proposed as a potential fat replacer (Brownlee *et al.*, 2009). Alginate contains linear polymers of (1, 4) linked β-D- mannuronic acid (M) and α-L- guluronic acid (G) residues (Draget, 2009). Due to its high water retention capacity, alginate helps to improve the texture, organoleptic qualities and acceptability of low fat processed food products, which in turn increases consumer acceptance (Brownlee *et al.*, 2009).

Sodium alginate is a commonly used alginate and is readily soluble in water. Sodium alginate along with other salts such as potassium, calcium and aluminum as well as an ester derivative of propylene glycol alginates have been permitted in food. Alginate is also used widely in ice cream to avoid crystallization and shrinkage, and in the beer and fruit beverages industries to increase foam levels (Mancini *et al.*, 2002). Moreover, it is also used in many dairy desserts, meat and vegetable products, bakery items, marmalades, jams, salad dressings

(to prevent separation) and various canned foods (Mancini *et al.*, 2002; Yilmazer *et al.*, 1991).

Sodium alginate ($NaC_6H_7O_6$) is a linear polysaccharide derivative of alginic acid composed of 1, 4 β –mannuronic (M) and α - L- guluronic (G) acids. Sodium alginate is a cell wall component of marine brown algae, and contains approximately 30-60% alginic acid. The conversion of alginic acid to sodium allows its solubility in water, which assists its extraction. Bacterial alginates are synthesized by only two bacterial genera, *Pseudomonas* and *Azotobactor*, and is used for protection from the environment and synthesis of biofilms in order to adhere to surfaces (Urtuvia *et al.*, 2017). The biggest advantage of alginates is that it has liquid-gel behavior in aqueous solutions. When monovalent, ions (sodium alginate) are exchanged for divalent ions (especially calcium), there action proceeds almost immediately changing from a low viscosity solution to gel structure. There are two different ways of recovering the sodium alginate. The detail outline of the process is given in Fig. 2.2.



Source: Mchugh (2003)

Fig. 2.2 Method for manufacturing sodium alginate

2.10 Use of calcium salt as food additive

Calcium helps in building the cross linkages during the formation of curd and thus favorable impacts yield, body and texture and overall acceptable of paneer. In order to produce good quality paneer from cow, use of calcium chloride ($CaCl_2$) at the rate of 0.08-0.15% has been suggested. Incorporation of $CaCl_2$ to milk resulted in increased fat and protein content and increased total solid recovery in paneer (Suthar, 2015).

Part III

Materials and methods

3.1 Materials

For the preparation of paneer cow milk of 3.7% fat and 8.3% SNF was collected from local Dairy farm of Biratnagar. Essential oil of *Cinnamon verum* and *Zanthoxylum armatum* were collected from Thapa Jadibutti and Processing Center Udayapur. LDPE bags (48.70 g/m^2 and 50 µm thickness) were used for packaging of samples during storage. List of other materials used for this work including instrument, glassware and chemicals requirement are listed in Appendix N and Appendix O.

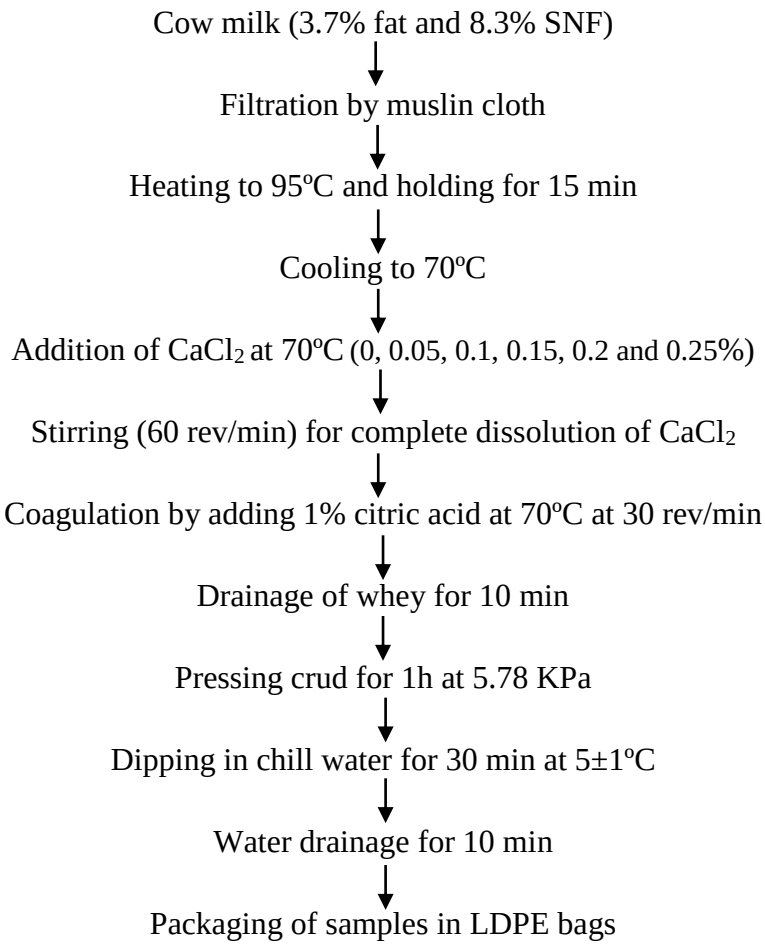
3.2 Methods

3.2.1 Preliminary study

A preliminary study was done to study the effects of calcium chloride concentrations on cow milk to observe the effect on sensory attributes (body and texture, flavor, color and appearance, and overall acceptability), physicochemical compositions (moisture content, fat content, protein content, total ash, free fatty acid and acidity) and yield of paneer. Samples with different concentration of calcium chloride are coded as, control (0.00%), C1 (0.05%), C2 (0.10%), C3 (0.15%), C4 (0.20%) and C5 (0.25%). Sample with best sensory attributes, physicochemical properties and higher yield was selected for further study.

3.2.2 Preparation of paneer

Paneer was prepared with slight modification as per method given by Sachdeva and Singh, (1988b). Sterilization of utentiles and muslin cloth were achieved by keeping them in boliling water for 5 min (Rajarshibhai, 2012). The process flow chart used for paneer making is presented in the Fig. 3.1.



Source: Sachdeva and Singh, (1988b)

Fig. 3.1 Preparation of paneer

3.2.3 Experimental design

D-optimal mixture design using Design of Expert (DOE) software, (version 10) developed by Stat- Ease was used to determine the different combination of *Zanthoxylum aramatum* and *Cinnamon verum* essential oil to be incorporated in sodium alginate edible coating for the paneer. Total output were found to be eight formulations for the two components and the proportion of each components were varied from 0% to 2%, with the total sum of incorporation of essential oils (components) as 2% of the coating as shown in Table 3.1.

Table 3.1 Essential oil combinations given by D-optimal mixture design

Sample code	Alginate gel (g)	Essential oil (%)		Final weight (g)
		Zanthoxylum armatum	*Cinnamon verum*	
T1	98	0	2	100
T2	98	2	0	100
T3	98	1	1	100
T4	98	0.5	1.5	100
T5	98	1.5	0.5	100
T6	98	1.75	0.25	100
T7	98	0.25	1.75	100
T8	98	1.25	0.75	100

3.2.4 Preparation of edible coating

Sodium alginate edible coatings were prepared as per the method described by (Raju and Sasikala, 2016), with slight modification. Two grams of sodium alginate was taken and dissolved in 100 ml of sterile distilled water at 70°C to form stock solutions of alginate (2% w/v). This solution was stirred for 10 min for complete dissolution. Glycerol as plasticizer and $CaCl_2$ to strengthen the film was added at the rate of 0.3 g/g and 0.05 g/g respectively. Final alginate concentration of 1% w/v in the film-forming mixture was achieved by adding required amount of distilled water.

Essential oil and their mix (10 ml) was prepared as shown in Table 3.2 and added at the rate of 2% (w/w) on the sodium alginate coating solution to achieve the required proportion of essential oils for each of combination treatment (T1, T2, T3, T4, T5, T6, T7 and T8) as mentioned in Table 3.1. Sodium alginate coating solution containing each of the essential oil mixture prepared for individual combinations were homogenized by model HL7707/00 of Philips mixer grinder at 7000 rpm for 5 min. The mixture was stirred for 5 min, cooled to room temperature and degassed by holding into refrigerator for 12 h before applying as coating in the paneer (Raju and Sasikala, 2016).

Table 3.2 Preparation of essential oil mixture

Sample code	Essential oil (g)		Final weight (g)
	Zanthoxylum armatum	*Cinnamomum verum*	
T1	0	10	10
T2	10	0	10
T3	5	5	10
T4	2.5	7.5	10
T5	7.5	2.5	10
T6	8.75	1.25	10
T7	1.25	8.75	10
T8	6.25	3.75	10

3.2.5 Coating of paneer cube with edible coating solution

As per the procedure described by Raju and Sasikala (2016), the edible coating at the rate of 0.6 kg/m^2 was applied onto the surface of paneer under aseptic conditions by gentle brushing of the paneer surface until the entire surface was covered by the coating. The samples were allowed to stand for some time so that the residual coating could drop off. The samples were left for drying in cabinet drier at 50±2°C for 30 min. After drying they were cooled to 15°C, packed in LDPE (48.70 g/m^2 and 50 µm thickness) and stored in refrigerator at a temperature of 7±1^{o}C and 52% RH.

3.2.6 Sensory analysis

Paneer was cut in to cubical pieces of approximately 2 cm x 2 cm x 2 cm size and was subjected to sensory evaluation by semi-trained panel of nine judges using 9 point hedonic scale (Singh and Maharaj, 2014).

3.2.7 Determination of shelf life of paneer

Shelf-life of paneer was determined by studying changes in sensorial acceptability (Body and texture, taste and flavor, color and appearance, and overall acceptability), physicochemical parameters (moisture content, acidity content, FFA, and tyrosine released) and microbial load (coliform, standard plate count, and yeast and mold count). The analysis was carried out till 24 days at the interval of 4 days.

3.3 Analytical procedure

3.3.1 Analysis of milk for proximate composition

Moisture, fat, protein, SNF, specific gravity of milk was as per the methods given by AOAC (2005).

3.3.2 Analysis of essential oil

3.3.2.1 Determination of total phenolic content

Total phenolic contents of the extracts were determined by folin-ciocalteu colorimetric method as described by Genwali *et al.* (2013). Briefly, 0.1 ml of essential oil and 0.5 ml of folin-ciocalteau's phenol reagent was taken in test tube and then 0.4 ml of 7.5% Na_2CO_3 was added. The resulting solution was incubated for 1 h at room temperature and absorbance was measured at 765 nm. Gallic acid was taken as standard and the results was expressed as mg of gallic acid equivalents (GAEs) per g of essential oil.

3.3.2.2 Determination of DPPH radical scavenging activity

DPPH free radical scavenging activities (antioxidant activities) of essential oil were determined by method described by Vignoli *et al.* (2011) with slight variation. Different dilutions of the extracts were made using 80% methanol. Then 1 ml of the extract was mixed with 2 ml of 0.1 mM DPPH solution. The absorbance was read at 517 nm after 30 min incubation in the dark. Finally, % scavenging activity was determined using following equation.

$$\% \text{ Scavenging activity} = \left(A_c\text{-}A_s\right) \times \frac{100}{A_c}$$

Where A_c is the absorbance of control and A_s is the absorbance of test sample. The IC_{50} value was determined as the concentration required to give 50% scavenging activity using linear regression equation.

3.3.3. Analysis of paneer for proximate composition

3.3.3.1 Determination of moisture

Moisture content in paneer was determined as per methods given by AOAC (2005).

Calculation of moisture content

$$\% \text{ Moisture content} = \left(\frac{W_1 - W_2}{W_1 - W_0} \right) \times 100$$

Where,

W_0 = Weight in g of the empty dish

W_1 = Weight in g of the dish with paneer sample before drying

W_2 = Weight in g of the dish with paneer sample after drying

3.3.4 Analysis of paneer for chemical characteristics during storage

The chemical changes taking place in the paneer were analyzed by using different methods as described in the following sections.

3.3.4.1 Determination of acidity

The acidity of the paneer sample was determined as per methods given by AOAC (2005).

Calculation of acidity:

$$\text{Acidity}\left(\text{as \% lacticacid}\right) = \frac{\left(10 - V\right) \times MW}{10 \times W}$$

Where,

V = Volume of HCl consumed.

N= Normality of HCl.

MW = Molecular weight of lactic acid.

W = Weight of sample.

3.3.4.2 Determination of free fatty acid

The free fatty acid of paneer samples were determined as per methods given by AOAC (2005).

Calculation of free fatty acids:

$$\text{Free fatty acid}\left(\%\ \text{oleic acid}\right)=\frac{2.82\times T}{5\times W}$$

Where,

T = Volume in ml of 0.02N NaOH required for titration, and

W = Weight in g of the sample taken

3.3.4.3 Determination of tyrosine release

Proteolytic changes in terms of tyrosine release in stored paneer samples were estimated by the procedure suggested by Kunitz (1946) with slight modification.

One gram of properly mixed and ground sample of paneer was taken and to that 100ml of distilled water was added. Thus prepared solution was filtered through the Whatman No. 41 filter paper. 5 ml of the filtrate was taken in a test tube and 5 ml of 10% TCA was added. The sample was then centrifuged for 10 min at 4000 rpm. The clear supernatant was taken and measured absorbance at 280 nm in UV- VIS spectrophotometer. A standard curve for tyrosine was used to convert 280 nm absorbance unit to µg tyrosine released per 100 g of sample.

3.3.5 Microbiological analysis

Microbial testing was done to find safety aspect of paneer for consumption and important aspect, which determines the shelf life of the product.

3.3.5.1 Preparation of sample

The samples for microbiological analyses was prepared under aseptic conditions. Paneer sample of about 10 g was taken aseptically by sterile spatula and mixed in stomacher bag by adding 90 g of Buffer Peptone Water (first dilution 1:10). Sample was mixed homogeneously and diluent was prepared in 9 ml BPW from the first dilution as per requirements (Aneja, 2003).

3.3.5.2 Enumeration of microorganisms

The pour plate method was adopted for the enumeration of different groups of microorganisms in paneer as described by Aneja (2003).

3.3.5.3 Total plate count (TPC)

Total plate count of paneer was determined as method described by Aneja (2003).

3.3.5.4 Coliform count

Coliform count of paneer was determined by the method as described by Aneja (2003).

3.3.5.5 Yeast and mold count

Yeast and molds count was determined as method described by Aneja (2003).

3.4 Statistical analysis

The sensory analysis data was analyzed by using one way and two- way ANOVA using IBM-SPSS Statistics version 20 at a significance level of ($p<0.05$). Upon significant difference means was separated by using Tukey HSD test. The data obtained during physicochemical was first processed and then analyzed by Microsoft Excel 2016.

Part IV

Results and discussion

Paneer samples were prepared from cow milk using citric acid (1%) as coagulant and calcium chloride at different concentrations; 0 (control), 0.05, 0.1, 0.15, 0.2 and 0.25% w/v of the milk. Compositional, sensory quality and yield of the paneer were determined. The best paneer was selected, was coated with *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated sodium alginate edible coating. The results of changes on sensory attributes, chemical composition and microbial load during storage have been discussed in this chapter.

4.1 Chemical composition of milk

The proximate composition of milk used for the preparation of paneer is shown in the Table 4.1

Table 4.1 Composition of milk used for paneer preparation.

Constituents	Content*(% wb)
Moisture	88.39±0.12
Fat	3.70±0.08
SNF	8.30±0.05
Protein	2.97±0.03
Acidity (as % lactic acid)	0.16±0.00
pH	6.58±0.02
Ash	0.69±0.01
Specific gravity	1.03±0.00

*Values represent the mean ± standard deviation of triplicate samples

Cow milk taken for the preparation of paneer was found to have 86.57% moisture, 3.5% fat, 9.9% SNF, 0.178 acidity (as %lactic acid) and 0.69% ash (Mistry *et al.,* 1990). Minimum mandatory standard for cow milk as specified by government of Nepal is fat and SNF not

less than 3.5% and 7.5% respectively (DFTQC, 2021). Mishra *et al.*, (2020), observed % fat ranged from 3.69 to 3.98, % protein 2.97 to 3.03 and SNF 8.29 to 8.25 for cows of Chitwan. The results of the composition of cow milk in this study showed within the rage found by the authors.

4.2 Estimation of total phenolic and radical scavenging activity of essential oil

The total phenolic content and IC_{50} concentration of the essential oil are shown in the Table 4.2. It was found that the essential oil of *Zanthoxylum armatun* had higher total phenol content and IC_{50} concentration than that of the *Cinnamomum verum* for *Zanthoxylum armatum* essential oil with a strong antioxidant property.

Table 4.2 Total phenol content and radical scavenging activity of *Zanthoxylum armatum* and *Cinnamon verum*

Essential oil	Total phenolic content (mg GAE/g)*	IC_{50} concentration
Zanthoxylum armatum	20.077±0.078	81.13 µl/ml
Cinnamomum verum	18.60±0.112	467.0 µl/ml

*Values represent the mean ±standard deviation of triplicate sample.

The values obtained for *Zanthoxylum armatum* and *Cinnamomum verum* essential oil were similar to the results obtained by Dhakal and Sharma (2020). They found the total phenol content of *Zanthoxylum armatum* ranges from 20.05 to 23.36 mg GAE/g and the radical scavenging activity ranged from 66.40 to 384.03 µl/ml in different extract solutions. Similarly, Wijewardhana *et al.* (2019) found that the total phenolic content and radical scavenging activity (IC_{50} concentration) of cinnamon (*Cinnamomum zeylanicum*) bark extract were 18.94 mg GAE/mg and 0.009 mg/ml respectively.

4.3 Effect of calcium chloride on chemical and sensory attributes of paneer

The results obtained for effect of added calcium chloride at different concentration in cow milk (3.7% fat and 8.3% SNF) on composition and sensory attributes of paneers are discussed on the following headings.

4.3.1 Effect of calcium chloride on moisture content of paneer

The moisture content of the paneers prepared from cow milk added with different concentration of calcium chloride is depicted in Fig. 4.1. Addition of calcium chloride had significant ($p<0.05$) effect on moisture content. Moisture contents of paneer samples were found to increase up to 0.1% $CaCl_2$ concentration and then decreased with increase in concentration of $CaCl_2$. The minimum and maximum moisture content were found to be 53.16 and 54.42% for control and 0.1% $CaCl_2$ added samples respectively. Paneer sample with 0.1% added calcium chloride had higher moisture retention and may be due to increment in water holding capacity. Increment in moisture content of paneer with addition of 0.1% sodium citrate and 0.5% calcium chloride was also reported by Chawla *et al.* (1987). Mistry *et al.*, (1990) also found that 0.02% calcium sulphate and 0.05% disodium hydrogen phosphate added paneer had increased moisture content. The variation in moisture content could be due to several reasons viz. pressing condition, scale of production, difference in voluminosity and hydration properties of casein micelles of milk of different breed.

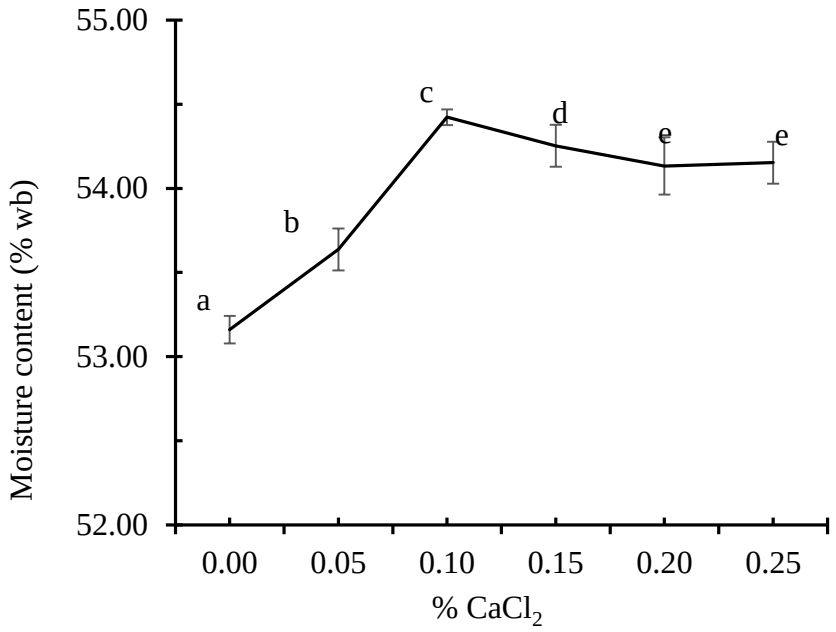


Fig. 4.1 Effect of calcium chloride on moisture content of paneer

4.3.2 Effect of calcium chloride on fat content of paneer

The fat contents of the paneers prepared from cow milk added with different concentration of calcium chloride are depicted in Fig. 4.2. Statistically there was significant ($p<0.05$) effect

on the fat content with the added $CaCl_2$ at different concentration. The minimum and maximum values were found to be 19.36 and 21.20 for control and 0.1% $CaCl_2$ added panner respectively. Fat content of paneer depends upon the fat content of the milk used for making paneer. Paneer made from buffalo milk has been reported to be higher as the level of fat is high in buffalo milk. Increase in fat content of salt added paneer sample was also reported by Mistry *et al.*, (1990). This study also showed that the retention of fat was higher in the paneer treated with 0.1% $CaCl_2$ concentration. This may be due to better entrapment of fat particles in the casein network during coagulation there by less loss of fat in the whey.

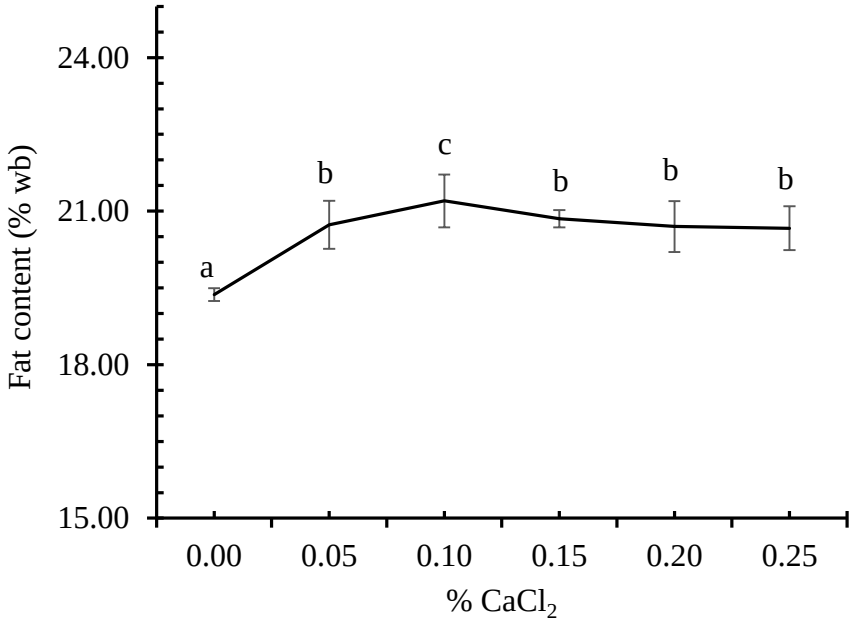


Fig. 4.2 Effect of calcium chloride on fat content of paneer

4.3.3 Effect of calcium chloride on the protein content of paneer

The protein contents of paneers prepared from cow milk added with different concentrations of calcium chloride is shown in Fig. 4.3. A statistically significant effect on the protein content was shown by $CaCl_2$ addition. The minimum and maximum values were found to be 18.16% and 19.56% for control and 0.1% $CaCl_2$. Similar trend was also shown on paneer made from calcium sulphate and disodium hydrogen phosphate, and values of protein content observed in this study were also in between the value obtained by Rajorhia *et al.* (1984). The reported protein content for control paneer ranged from 17.57% to 17.99% while that of calcium sulphate and disodium hydrogen phosphate added paneer ranged from 21.18% to 22.15%. It could be said that the addition of $CaCl_2$ to milk could facilitate the

binding of soluble protein and thus improving the total protein content. In addition, variation of the protein content may partly be due to the variation in the moisture and fat content of the paneer samples and may be also due to losses of these constituents in whey. Addition of salts to milk appeared to slightly improve the protein content of paneer.

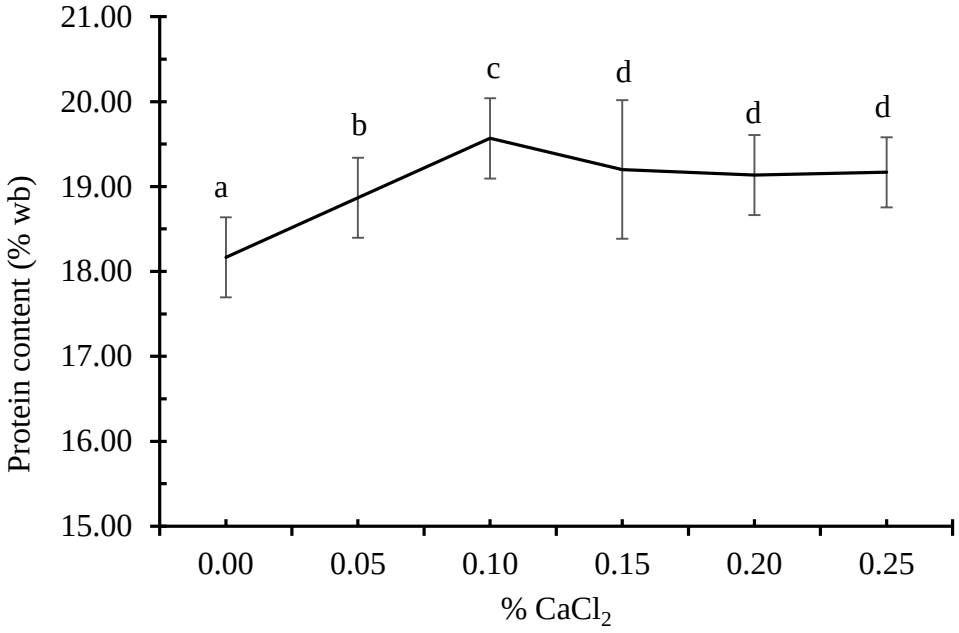


Fig. 4.3 Effect of calcium chloride on protein content of paneer

4.3.4 Effect of calcium chloride on the total ash content of paneer

The changes in total ash contents of the paneers prepared from cow milk added with different concentration of calcium chloride are depicted in Fig. 4.4 Addition of $CaCl_2$ had significant effect ($p<0.05$) on the total ash content of the paneer samples. An increment in total ash content was found with the increase in concentration of the $CaCl_2$. The value ranged from a minimum of 1.86% to maximum 2.16% for control and 0.25% $CaCl_2$ added samples respectively.

Addition of $CaCl_2$ to milk had oblivious influence on ash content of paneer. During acidification of milk colloidal calcium phosphate moves into the soluble phase (Patton, 1955). During this process most of the salts would lost in the whey but some might be combined with proteins or with other chemical combinations in the coagulum while part of it may be held in the watery portion of paneer. Ash content of the treated samples were higher than the control sample. Ash content of paneer thus depends upon the actual amount of salts

in salt solution (whey) in the paneer and chemical linkage of salt to proteins. Increase in concentration of calcium chloride in milk resulted in higher ash content of paneer.

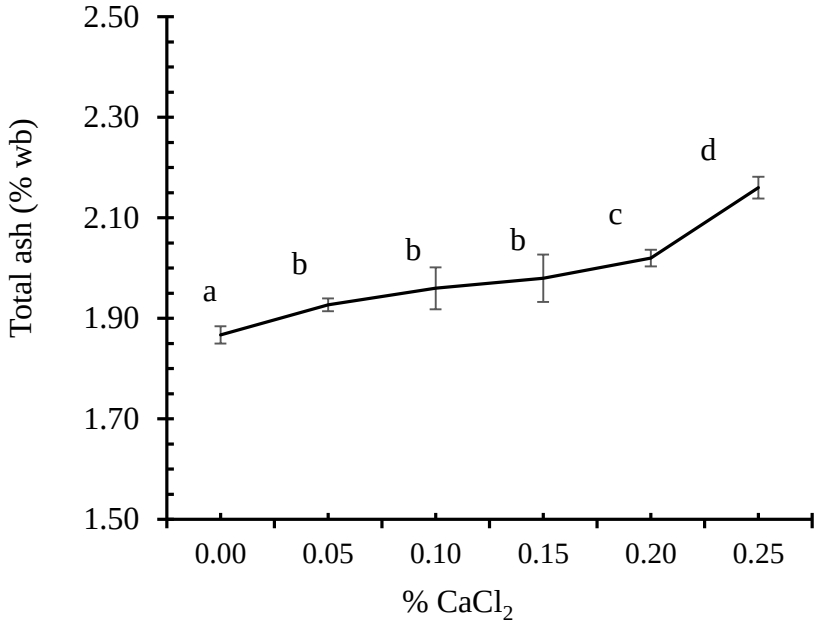


Fig. 4.4 Effect of calcium chloride on total ash content of paneer

4.3.5 Effect of calcium chloride on the free fatty acids content of paneer

The free fatty acid (FFA) contents of paneers prepared from cow milk added with different concentration of calcium chloride is depicted in Fig. 4.5.

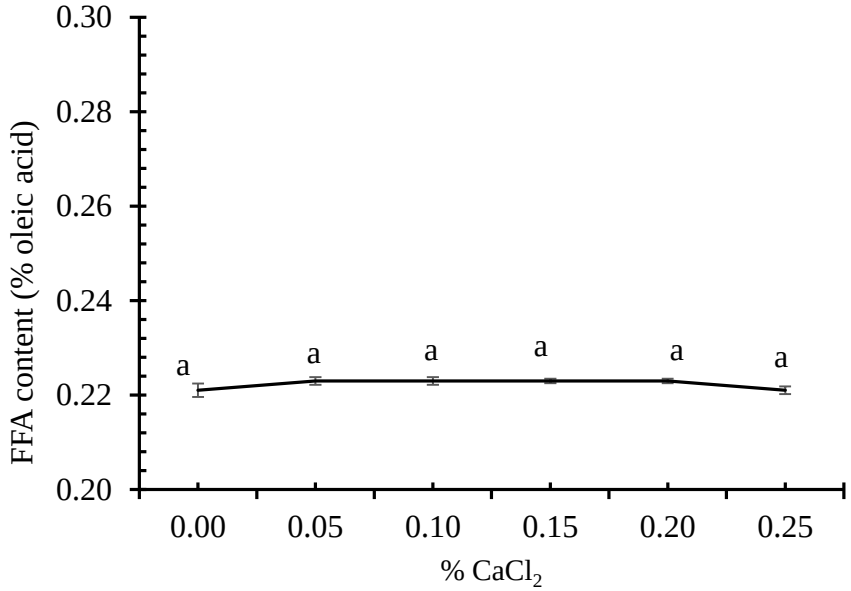


Fig. 4.5 Effect of calcium chloride on free fatty acid content of paneer

Statistically there was no significant difference ($p>0.05$) in the FFA content of the control and treated samples. This may be due to freshly prepared samples.

4.3.6 Effect of calcium chloride on the acidity of paneer

The acidity (as % lactic acid) contents of paneers prepared from cow milk added with different concentration of calcium chloride is depicted in Fig. 4.6. Statistically there was a significant difference ($p<0.05$) in the % lactic acid content of paneer samples. Acidity of the sample was found to decrease with the increase in the concentration of $CaCl_2$. The acidity of paneer possibly depend upon the amount of coagulant required for precipitation of milk, nature and concentration of additives used, amount of whey retained in the paneer after pressing. Similar results of decrease in acidity of paneer were found by Mistry *et al.*, (1990) with the addition of salt calcium sulphate and disodium hydrogen phosphate.

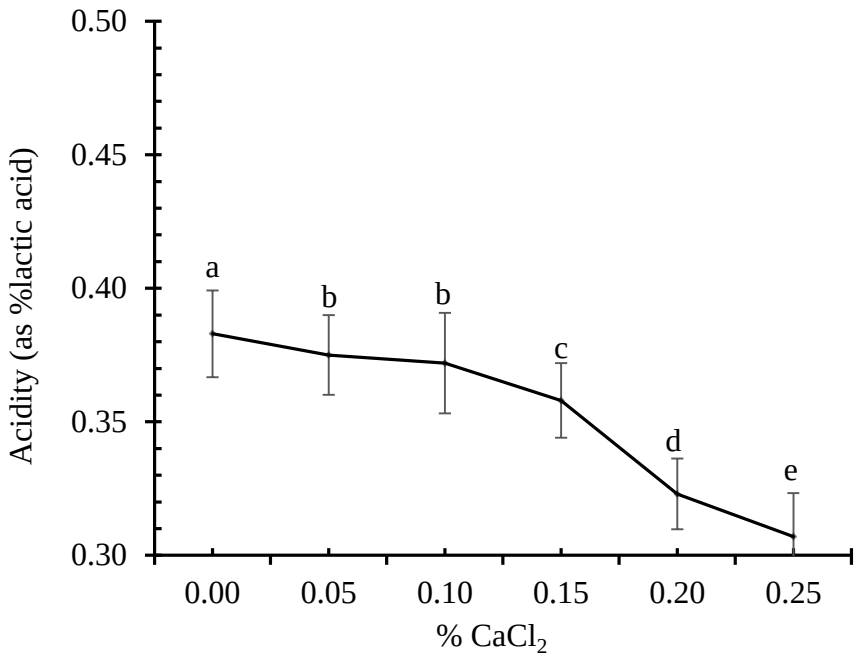


Fig. 4.6 Effect of calcium chloride on acidity of paneer

4.3.7 Effect of calcium chloride on the yield of paneer

The % yield of the paneers made from cow milk added with different concentration of $CaCl_2$ is depicted in Fig. 4.7. Statistically there was a significant difference ($p<0.05$) in the % yield of paneer with increase in concentration of $CaCl_2$. A gradual increase in % yield was found

with a minimum of 13.38% and a maximum16.65% for the control sample and 0.1% $CaCl_2$ added sample.

Kinjal *et al.* (2020) studied the addition of combination of calcium chloride and sodium chloride in different proportion with varying temperature and found that the % yield of paneer ranged from 15.46% to 20.55%. Similarly, the increase in yield of paneer and total solid recovery with the addition of $CaCl_2$ at the rate of 0.1% to milk prior to coagulation was reported by Singh and Kanawjia (1988). The increase in the yield of paneer through acidification in presence of calcium chloride at the rate of 0.15% was observed by Makhal and Kanawjia (2005).

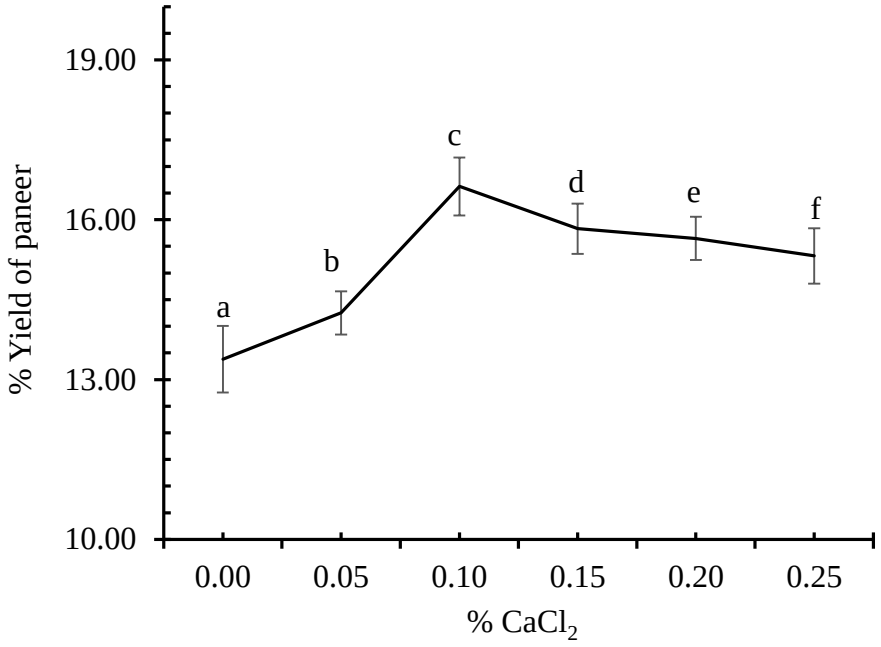


Fig. 4.7 Effect of calcium chloride on yield of paneer

4.3.8 Effect of calcium chloride on the sensory attributes of paneer

The sensory quality of product decides acceptance or rejection of the product for consumption. It is one of the most important criteria for evaluating the quality of any products. These qualities include body and texture, taste, flavor, color and overall acceptability. Samples were evaluated for the sensory quality. The mean values of sensory parameters of paneer samples are as shown in the Fig.4.8. The samples coded with control, C1, C2, C3, C4 and C5 in the Fig.4.8 represent the addition of $CaCl_2$ at the rate of 0%, 0.05%,

0.1%, 0.15%, 0.2% and 0.25 % respectively. Statistically there was significant effect ($p<0.05$) on sensory attributes of the sample with varied $CaCl_2$ concentration.

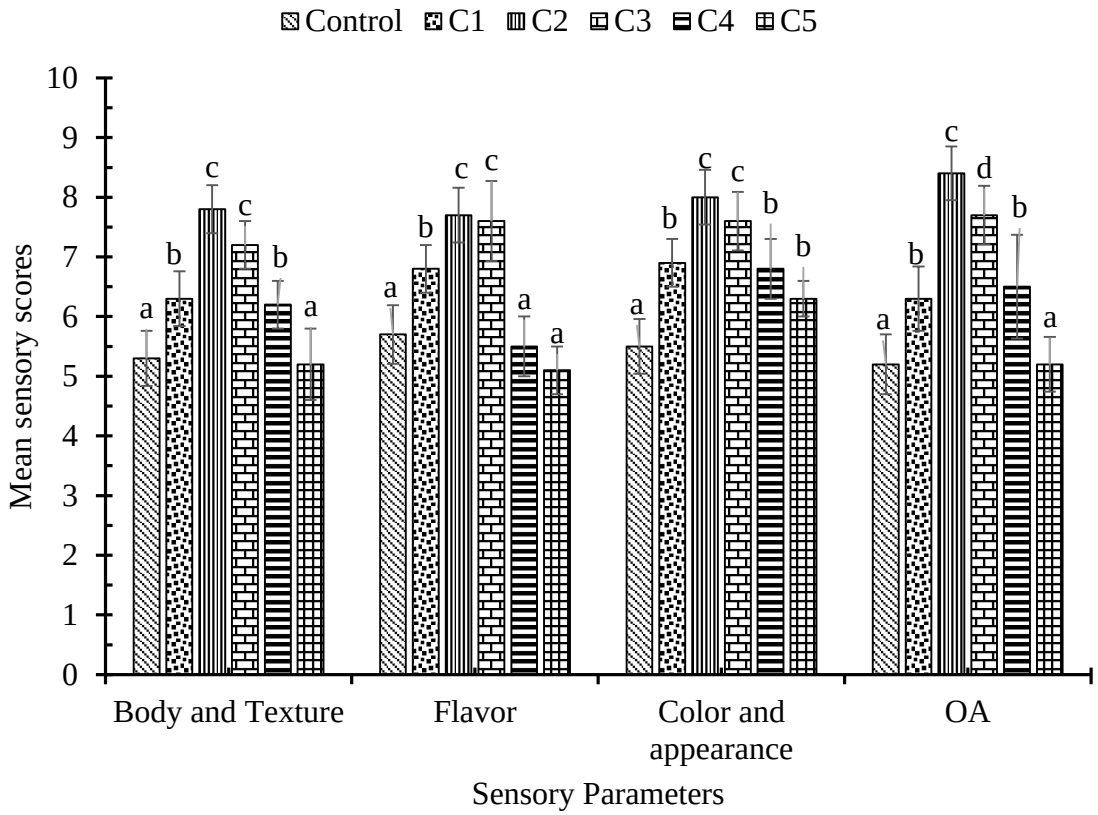


Fig. 4.8 Mean sensory score for calcium chloride treated paneer samples.

The mean sensory scores for body and texture were 5.3, 6.6, 7.8, 7.2, 6.2 and 5.2 for paneer prepared by using 0, 0.05, 0.1, 0.15, 0.2 and 0.25% $CaCl_2$ respectively. Statistical analysis showed that addition of $CaCl_2$ had a significant effect ($p<0.05$) on body and texture of the paneer. The mean score increased significantly with increasing $CaCl_2$ concentration up to 0.1% and decreased afterwards. Paneer prepared without $CaCl_2$ addition had the minimum scores while that made by adding 0.1% $CaCl_2$ had the maximum scores of all the samples. The mean scores between the paneers made by using 0.1 (C2) and 0.15 (C3) % $CaCl_2$ were not significantly different.

A significant increase in the body and texture of $CaCl_2$ treated paneer were reported by Kinjal *et al.* (2020) but Arora *et al.* (1996) when added $CaCl_2$ at 0.05% in milk, resulted in improvement of body and texture score. Finding of this study are in accordance with Singh

and Kanawjia (1988). They reported the improvement in body and texture upon addition of $CaCl_2$ at 0.1% to milk prior to coagulation while making paneer.

The mean sensory scores for flavor were 5.7, 6.8, 7.7, 7.6, 5.5 and 5.1 for paneer prepared by using 0, 0.05, 0.1, 0.15, 0.2 and 0.25% $CaCl_2$ respectively. Statistical analysis showed that addition of $CaCl_2$ had a significant effect ($p<0.05$) on flavor of the paneer. The mean score increased significantly with increasing $CaCl_2$ concentration up to C2 and decrease afterwards with a mean score below that of control at 0.25% $CaCl_2$ (C5). Paneer prepared with 0.25% $CaCl_2$ addition had the minimum scores while that made by adding 0.1% $CaCl_2$ had the maximum scores of all the samples. The mean scores between the paneers made by using 0.1 (C2) and 0.15 (C3) % $CaCl_2$ were not significantly different ($p>0.05$).

Addition of salt mixture (sodium chloride at the rate of 0.5% and calcium Chloride at the rate of 0.08) had shown a significant positive correlations for flavor (Kinjal *et al.*, 2020). Similarly, addition of sodium chloride increased the flavor score of experimental paneer was reported by Yadav *et al.* (1994) and Kaur and Bajwa (2003).

The mean sensory scores for color and appearance were 5.5, 6.9, 8.0, 7.6, 6.8 and 6.3 for paneer prepared by using 0, 0.05, 0.1, 0.15, 0.2 and 0.25% $CaCl_2$ respectively. Statistical analysis showed that addition of $CaCl_2$ had a significant effect ($p<0.05$) on color and appearance of the paneer. The mean score increased significantly with increasing $CaCl_2$ concentration up to Tc2 and decrease afterwards with a non-significant ($p>0.05$) mean scores between the paneers made by 0.2 (C4) and 0.25 (C5) % $CaCl_2$. Paneer prepared without $CaCl_2$ addition had the minimum scores while that made by adding 0.1% $CaCl_2$ had the maximum scores of all the samples. The mean scores between the paneers made by using 0.1 (C2) and 0.15 (C3) % $CaCl_2$ were not significantly different. Upon addition of salt (sodium chloride 0.5% and Calcium chloride 0.08%) there was significant increase in color and appearance (Kinjal *et al.*, 2020).

The mean sensory scores for overall acceptance were 5.2, 6.3, 8.4, 7.7, 6.5 and 5.2 for paneer prepared by using 0, 0.05, 0.1, 0.15, 0.2 and 0.25% $CaCl_2$ respectively. Statistical analysis showed that addition of $CaCl_2$ had a significant effect ($p<0.05$) on overall acceptance of the paneer. The mean score increased significantly with increasing $CaCl_2$ concentration up to C2 and decrease afterwards with a mean score similar to control at 0.25% $CaCl_2$ (C5). Paneer prepared without and 0.25% $CaCl_2$ addition had the minimum scores while that made by adding 0.1% $CaCl_2$ had the maximum scores of all the samples. The

mean scores of the paneers made by using 0.05 (C1) and 0.2 (C4) % $CaCl_2$ were not significantly different.

Increase in sensory score for the overall acceptability with the increasing concentration of $CaCl_2$ was reported by Kinjal *et al.* (2020). Sachdeva *et al.* (1991) observed that increase in the overall acceptability of paneer when 0.08% $CaCl_2$ is added in paneer. Similar trend was observed by Singh and Kanawjia (1988) upon addition of 0.1% $CaCl_2$. Kaur and Bajwa (2003) observed a good sensory score for a paneer dipped in 3% brine solution.

Overall, paneer sample C2 with the added 0.1% $CaCl_2$ was found to be best in terms of sensory attributes including body and texture, flavor, color and appearance and overall acceptability as well as with respect to be paneer yield, retention of moisture, protein, fat and having minimum acidity (as discussed earlier).

4.4 Effect of *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate edible coating on sensory attributes of paneer during storage

The results of sensory scores obtained for the paneer samples coated with essential oil mix incorporated sodium alginate during storage period are discussed. The samples with different code below represent the essential oil mixture in different proportion. The % of *Zanthoxylum armatum* and *Cinnamomum verum* essential oil were in the proportion of (0+2), (2+0), (1+1), (0.5+1.5), (1.5+0.5), (1.75+0.24), (0.25+1.75) and (1.25+0.75) respectively for sample T1, T2, T3, T4, T5, T6, T7 and T8. The paneer sample without edible coating was coded as control.

4.4.1 Body and texture

The average score for body and texture of control and all the coated paneers (T1 toT8) during storage at 7±1^{o}C for 24 days are presented in Table 4.3. The treatment, storage days and their interaction had significant effect on average scores obtained for body and texture.

Table 4.3 Sensory score for body and texture of paneers coated with essential oil mix incorporated coating

Treatments	Storage days						
	0	4	8	12	16	20	24
Control	8.40±0.48^{a}	7.90±0.30^{a}	6.3±0.46^{a}	NA	NA	NA	NA
T1	7.90±0.30^{b}	7.80±0.40^{a}	7.4±0.49^{b}	6.70±0.46^{a}	6.00±0.00^{a}	NA	NA
T2	7.90±0.30^{b}	7.90±0.40^{a}	7.4±0.49^{b}	6.70±0.46^{a}	6.00±0.00^{a}	NA	NA
T3	7.90±0.30^{b}	7.80±0.30^{a}	7.5±0.50^{b}	7.20±0.40^{ab}	6.80±0.40^{b}	6.1±0.30^{a}	NA
T4	7.90±0.30^{b}	7.90±0.30^{a}	7.5±0.50^{b}	7.20±0.40^{ab}	6.80±0.40^{b}	6.0±0.00^{a}	NA
T5	7.90±0.30^{b}	7.90±0.30^{a}	7.6±0.49^{b}	7.40±0.49^{b}	6.90±0.00^{b}	6.3±0.46^{ab}	NA
T6	7.90±0.30^{b}	7.90±0.30^{a}	7.8±0.50^{b}	7.60±0.49^{b}	7.00±0.30^{b}	6.8±0.40^{b}	6.5±0.50
T7	7.90±0.30^{b}	7.90±0.30^{a}	7.5±0.40^{b}	7.20±0.40^{ab}	6.80±0.40^{b}	6.1±0.30^{a}	NA
T8	7.90±0.30^{b}	7.90±0.30^{a}	7.6±0.49^{b}	7.40±0.49^{b}	6.90±0.30^{b}	6.3±0.46^{ab}	NA

Values represent means± standard deviation. Means with different alphabets in the same column are significantly different ($p<0.05$).

Essential oil mix incorporated edible coating has significant effect ($p<0.05$) on the body and texture scores of paneers samples at different days interval of 24 days storage periods. Paneer with different proportion of essential oil mix incorporated sodium alginate edible coating and without coating (control) were not significantly ($p>0.05$) different on the body and texture scores up to 4th days of storage, whereas after then there was significant ($p<0.05$) difference. There was highly decrease in body and texture of control sample from 8.4 to 6.3 in 8 days and become unacceptable after 12 days of Storage. It was pointed out that, none of the paneer samples except control were discarded due to any conspicuous defect in body and texture during storage up to16 days with score remain above 6. Afterward, the edible coating treated paneer T1 and T2 were observed unacceptable on day 20 and on day 24 all the treated sample except T6 were also observed unacceptable with respect to body and texture. Similar type of changes was also by Khatkar *et al.* (2017) on the body and texture score of cinnamon essential oil treated paneer during storage. The decrease in the body and texture scores might be due to the biochemical and microbiological changes during storage and these changes found slower in the treated paneers probably reflect the protective role of coatings and presence of active components in essential oil (Eresam *et al.*, 2015; Khatkar *et al.*, 2017).

The results observed were in accordance with the findings of Eresam *et al.* (2015). Based on sensory score for body and texture, T6 was only found acceptable even during 24 days of storage with average score of 6.5.

4.4.2 Flavor

The average sensory scores for the flavor of control and all the coated paneer (T1 to T8) during storage at 7±1ºC for 24 days are shown in Table 4.4. The treatment, storage days and their interaction were found to have significant effect on average scores obtained for flavor.

Table 4.4 Sensory score for taste and flavor of paneers coated with essential oil mix incorporated coating

Treatments	Storage days						
	0	4	8	12	16	20	24
Control	7.80±0.40^{a}	6.50±0.50^{a}	5.7±0.46^{a}	NA	NA	NA	NA
T1	7.60±0.48^{a}	7.60±0.49^{b}	7.4±0.49^{b}	6.70±0.64^{a}	6.22±0.46^{a}	NA	NA
T2	7.60±0.48^{a}	7.80±0.49^{b}	7.4±0.49^{b}	6.70±0.64^{a}	6.22±0.46^{a}	NA	NA
T3	7.80±0.40^{a}	7.60±0.40^{b}	7.6±0.49^{b}	7.20±0.40^{ab}	7.00±0.00^{b}	6.3±0.46^{a}	NA
T4	7.60±0.48^{a}	7.60±0.49^{b}	7.6±0.49^{b}	7.20±0.40^{ab}	7.00±0.00^{b}	6.3±0.46^{a}	NA
T5	7.70±0.45^{a}	7.70±0.46^{b}	7.7±0.46^{b}	7.20±0.40^{ab}	7.11±0.50^{bc}	6.4±0.49^{a}	NA
T6	7.80±0.40^{a}	7.80±0.40^{b}	7.8±0.50^{b}	7.70±0.64^{b}	7.44±0.30^{c}	7.1±0.30^{b}	7.0±0.40
T7	7.60±0.48^{a}	7.60±0.49^{b}	7.5±0.60^{b}	7.20±0.40^{ab}	7.00±0.00^{b}	6.3±0.46^{a}	NA
T8	7.80±0.45^{a}	7.70±0.46^{b}	7.6±0.49^{b}	7.20±0.40^{ab}	7.00±0.30^{bc}	6.4±0.49^{a}	NA

Values represent means± standard deviation. Means with different alphabets in the same column are significantly different ($p<0.05$).

The proportions of essential oils mix in edible coating of paneer had significant ($p<0.05$) effect on the flavor scores at different days interval of 24 days storage period. The flavor scores for the control sample decrease sharply from 7.8 to 5.7 during 8 days of storage and become unacceptable due the putrid odor, acidic and bitter taste. A similar trend on decrease in flavor score for sample T1 and T2 was found and reaches to 6.22 on the 16th days of storage while the other remaining samples were acceptable up to 20 days of storage, except T6 which was liked up to 24 days of storage. Paneer samples coated with the mixture of essential oils (T3, T4, T5, T6, T7 and T8) had got slow decrease in the taste and flavor score than that the single essential oil coated samples (T1 and T2). Similar changes in taste and

flavor during storage days were also reported in cinnamon essential oil treated paneer and in direct spices added paneer packed in variety of packaging materials (Eresam *et al.*, 2015; Khatkar *et al.*, 2017). Based on taste and flavor score, T6 was perceived acceptable with average score of 7 till 24 days. However in case of cinnamon essential oil incorporated paneer, the acceptability of taste and flavor has been reported to be better than the control but only for 9 (average score 6) days in LDPE packed paneer (Khatkar *et al.*, 2017). It shows that incorporation essential oil mix in edible coating had better effect in retaining the taste and flavor as a sensory attribute compared to directly treat single essential oil in paneer during storage.

4.4.3 Color and appearance

Table 4.5 shows average color and appearance scores of paneer samples during different days of storage at 7±1ºC for 24 days.

The treatment, storage days and their interaction had significant effect on average scores obtained for color and appearance. Table 4.5 shows that essential oil mix incorporated edible coating has significant effect on the color and appearance scores of paneers samples at different days interval of 24 days storage periods($p<0.05$).

Table 4.5 Sensory score for color and appearance of paneers coated with essential oil mix incorporated coating

Treatments	Storage days						
	0	4	8	12	16	20	24
Control	8.10±0.30^{a}	7.60±0.49^{a}	6.0±0.00^{a}	NA	NA	NA	NA
T1	7.90±0.30^{a}	7.90±0.30^{a}	7.4±0.49^{b}	6.80±0.40^{a}	6.50±0.50^{a}	NA	NA
T2	7.90±0.30^{a}	7.90±0.30^{a}	7.4±0.49^{b}	6.80±0.40^{a}	6.50±0.50^{a}	NA	NA
T3	7.90±0.30^{a}	7.90±0.30^{a}	7.6±0.49^{b}	7.30±0.46^{ab}	7.30±0.46^{b}	6.3±0.46^{a}	NA
T4	7.90±0.30^{a}	7.90±0.30^{a}	7.6±0.49^{b}	7.30±0.46^{ab}	7.30±0.46^{b}	6.3±0.46^{a}	NA
T5	7.90±0.30^{a}	7.90±0.30^{a}	7.7±0.46^{b}	7.40±0.49^{ab}	7.40±0.60^{b}	6.5±0.50^{a}	NA
T6	7.90±0.30^{a}	7.90±0.30^{a}	7.8±0.50^{b}	7.80±0.60^{b}	7.80±0.49^{b}	7.4±0.49^{b}	7.0±0.00
T7	7.90±0.30^{a}	7.90±0.30^{a}	7.5±0.60^{b}	7.30±0.46^{ab}	7.30±0.46^{b}	6.3±0.46^{a}	NA
T8	7.90±0.30^{a}	7.90±0.30^{a}	7.6±0.49^{b}	7.40±0.49^{ab}	7.30±0.49^{b}	6.5±0.50^{a}	NA

Values represent means± standard deviation. Means with different alphabets in the same column are significantly different ($p<0.05$).

The score for control was slightly higher than the treated samples, no significant difference ($p>0.05$) on color and appearance scores was found on the 0 day and 4^{th} days of storage. This may be due to the coating over the other samples which make them appear a bit hazy but the score decrease sharply from 8.1 to 6 during 8 days of storage and liked slightly. On the 8^{th} days of storage the control sample became yellowish tint due to rapid chemical changes. A very slow decrease in the scores for the coated paneers with the mixture of essential oils (T3, T4, T5, T6, T7 and T8) as compared to score obtained by the paneer samples coated with single essential oils (T1 and T2).

Similar changes for color and appearance during storage days were also noticed in cinnamon essential oil treated paneer and in direct spices added paneer packed in variety of packaging materials (Eresam *et al.,* 2015; Khatkar *et al.,* 2017). But in case of cinnamon essential oil incorporated paneer packed with LDPE, no any defect in color and appearance has been reported for only 9 days (average score 6) in LDPE packed paneer (Khatkar *et al.,* 2017). Compared to these result, study showed that better effectiveness of Zanthoxylum *armatum* and *Cinnamomum verum* essential oils and their mix combinations incorporated Na-alginate coating of paneer during storage as concern to loss of color and appearance. Even among the coated paneers with incorporation of the mixture of essential oils, the paneer sample T6 was observed more acceptable as denoted by its scores for color and appearance varies from 7.9 in the beginning to 7.0 at 24 days of storage period**.**

4.4.4 Overall acceptability

The average scores obtained for overall acceptability of control and different proportion essential oils mix incorporated edible coated paneer samples (T1 to T8) in different days of storage at 7±1ºC for 24 days are shown in Table 4.6.

Table 4.6 Sensory score for overall acceptability of paneer samples coated with essential oil mix incorporated coating

	Storage days						
Treatments	0	4	8	12	16	20	24
Control	8.30±0.45^{a}	6.60±0.49^{a}	5.8±0.40^{a}	NA	NA	NA	NA
T1	7.60±0.48^{b}	7.60±0.49^{b}	7.4±0.49^{b}	6.80±0.40^{a}	6.00±0.00^{a}	NA	NA
T2	7.60±0.48^{b}	7.80±0.49^{b}	7.4±0.49^{b}	6.80±0.40^{a}	6.00±0.00^{a}	NA	NA
T3	7.80±0.40^{ab}	7.60±0.40^{b}	7.6±0.49^{b}	7.50±0.50^{ab}	7.10±0.30^{b}	6.3±0.46^{a}	NA
T4	7.70±0.45^{ab}	7.70±0.46^{b}	7.6±0.49^{b}	7.50±0.50^{ab}	7.10±0.30^{b}	6.3±0.46^{a}	NA
T5	7.70±0.45^{ab}	7.70±0.46^{b}	7.7±0.46^{b}	7.60±0.49^{b}	7.30±0.64^{bc}	6.5±0.50^{a}	NA
T6	7.80±0.40^{ab}	7.80±0.40^{b}	7.8±0.50^{b}	7.80±0.60^{b}	7.70±0.46^{c}	7.2±0.40^{b}	7.0±0.45
T7	7.70±0.45^{ab}	7.70±0.46^{b}	7.5±0.60^{b}	7.50±0.50^{ab}	7.10±0.30^{b}	6.3±0.46^{a}	NA
T8	7.70±0.45^{ab}	7.70±0.46^{b}	7.5±0.49^{b}	7.60±0.49^{b}	7.10±0.46^{bc}	6.5±0.50^{a}	NA

Values represent means± standard deviation. Means with different alphabets in the same column are significantly different ($p<0.05$).

The treatment, storage days and their interaction had significant effect on average scores obtained for overall acceptability ($p<0.05$). Table 4.6 depicts that proportions of essential oils mix incorporated coating had significant effect on the overall acceptability scores of paneer during different day's interval of 24 days storage ($p < 0.05$). The result indicated that the overall acceptability scores for the control sample were highest in the beginning than the treated samples. This may be due to the coating over the other samples which make them appear a bit hazy but the score of control sample decrease sharply from 8.3 to 5.8 during 8 days of storage and become unacceptable. On the 8th days of storage the control sample became yellowish with acidic bitter taste due to rapid chemical and microbiological changes. In contrast, essential oil mix incorporated edible coated paneer samples; T1 and T2 were found to be acceptable up to 16th days of storage with average score of 6 and liked slightly. The samples; T3, T4, T5, T7 and T8 were found acceptable up to 20 days of storage while the overall acceptability score of the sample T6 remains above 7 on the same days of storage. Similar finding are also mentioned by earlier researchers (Eresam *et al.*, 2015; Khatkar *et*

al., 2017). A very slow decrease in the overall acceptability score which varies from 7.8 in the beginning to 7 at 24 days of storage period of the T6 sample was observed**.**

Based on overall acceptability including other sensory attributes (body and texture, flavor, and color and appearance) T6 paneer sample was observed best and acceptable during storage up to 24 days. On the basis of these, it can be noticeable that the combination of *Zanthoxylum armantum* essential oil (1.75%) and *Cinnamomum verum* essential oil (0.25%) incorporated Na-alginate edible coating among all other combinations of them imcluded in this study, will be most effecting in retaining all of the sensory attributes of the panner during refregerated storage up to 24 days.

4.5 Effect *of Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated sodium alginate edible coating on chemical attributes of paneer during storage

4.5.1 Effect on moisture content of paneer

The result of moisture content of control paneer and essential oil mix edible coated paneers (T1 to T8) at four days interval during 24 days of storage are given in the Table 4.7.

Table 4.7 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on moisture content.

Treatments	Storage days						
	0	4	8	12	16	20	24
Control	54.42±0.00^{a}	54.16±0.02^{a}	53.87±0.01^{a}	53.46±0.02^{a}	52.93±0.01^{a}	52.35±0.00^{a}	51.74±0.01^{a}
T1	54.43±0.02^{a}	54.33±0.02^{b}	54.02±0.01^{b}	53.92±0.01^{b}	53.67±0.01^{b}	53.38±0.01^{b}	53.03±0.01^{bc}
T2	54.42±0.01^{a}	54.32±0.01^{b}	54.02±0.01^{b}	53.91±0.01^{b}	53.63±0.01^{c}	53.37±0.01^{b}	53.05±0.00^{b}
T3	54.43±0.02^{a}	54.33±0.02^{b}	54.03±0.01^{b}	53.92±0.01^{b}	53.62±0.01^{c}	53.36±0.01^{b}	53.03±0.01^{bc}
T4	54.44±0.01^{a}	54.34±0.01^{b}	54.04±0.01^{b}	53.93±0.00^{b}	53.63±0.00^{bc}	53.38±0.01^{b}	53.02±0.01^{c}
T5	54.43±0.01^{a}	54.33±0.01^{b}	54.03±0.01^{b}	53.91±0.01^{b}	53.66±0.01^{bc}	53.37±0.00^{b}	53.02±0.00^{c}
T6	54.42±0.01^{a}	54.32±0.01^{b}	54.02±0.01^{b}	53.93±0.00^{b}	53.63±0.01^{bc}	53.37±0.01^{b}	53.01±0.00^{c}
T7	54.42±0.02^{a}	54.32±0.02^{b}	54.02±0.01^{b}	53.92±0.00^{b}	53.63±0.01^{bc}	53.38±0.00^{b}	53.03±0.00^{bc}
T8	54.44±0.01^{a}	54.34±0.00^{b}	54.03±0.01^{b}	53.93±0.01^{b}	53.66±0.02^{bc}	53.37±0.01^{b}	53.01±0.00^{c}

Values represent means± standard deviation. Means with different alphabets in the same column are significantly different ($p<0.05$).

Statistically there was significant effect ($p<0.05$) of storage days, treatment and day treatment interaction on moisture content of paneer coated with *Cinnamomun verum* and

Zanthoxylum armatum essential oil incorporated Na-alginate edible coating (Appendix D). However, no significant difference ($p>0.05$) in moisture content was observed between the treated samples in different days of storage periods. Highest decrease in moisture content from an initial 54.41% to 51.73% was observed in control sample during storage of 24 days. On the 0 day of storage there was no significant difference ($p>0.05$) in moisture content of control and all the treated samples. All the treated samples including the control had found moisture content at 24 days of storage below the level (60%) as suggested by BIS standard (BIS, 1983)

The gradual decrease in moisture content of paneer during storage was might be due to expulsion of moisture from the product to the surrounding, but in case of treated sample with the slower rate that might be due to the essential oils layer formation and edible coating on the surface of the sample which acts as a barrier. A decrease trend in moisture content during storage was also observed in spice powdered added paneer by (Mistry *et al.*, 1990; Pal *et al.*, 1993; Raju and Sasikala, 2016) and also in cinnamon essential oil incorporated sodium alginate edible coated paneer (Raju and Sasikala, 2016).

4.5.2 Effect on free fatty acid

The free fatty acid (FFA) content of control and essential oil mix edible coated paneer samples (T1 to T8) during 24 days of storage period are presented in Appendix E and the trend is depicted in Fig. 4.9

The treatment, storage days and their interaction had significant effect on FFA content of paneer ($p<0.05$) (Appendix E). Fig. 4.9 shows the effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on FFA of control paneer sample with the treated samples (T1 to T8). Statistically there was significant difference ($p<0.05$) in FFA content of control sample with the treated sample. A fast increase in FFA value of the control sample was observed than the treated samples. In contrast, the slow increase in FFA was observed in sample T5, T8 and T6.

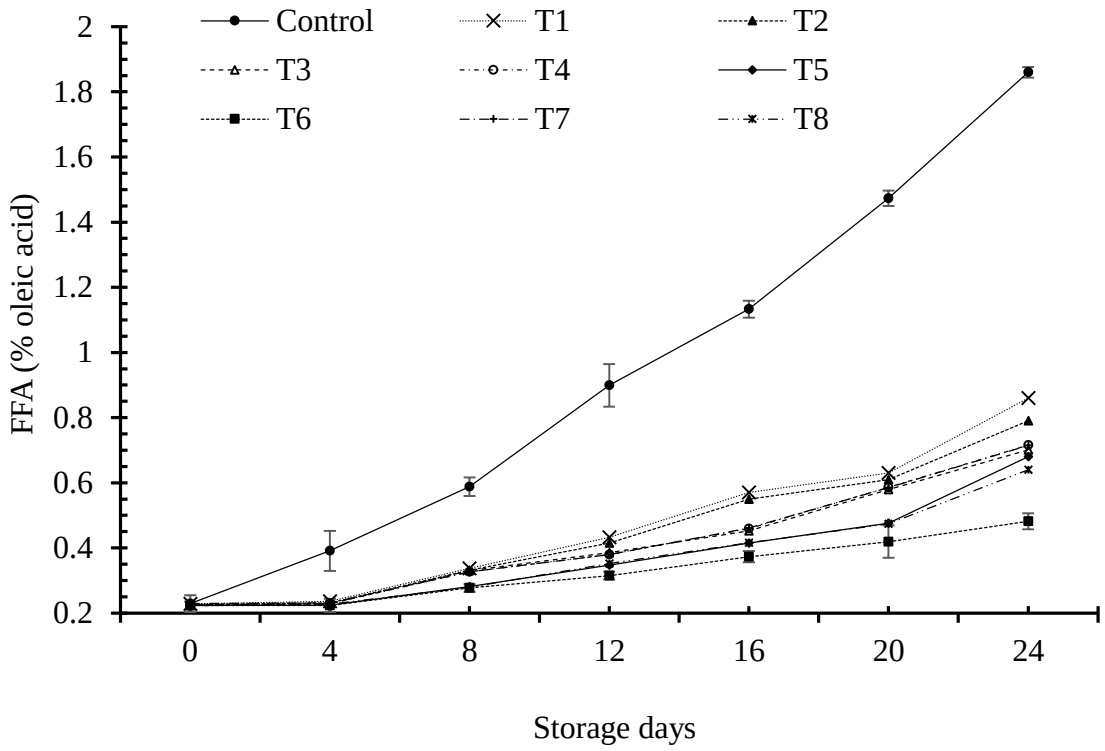


Fig. 4.9 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on FFA.

There was significant ($p<0.05$) increase in free fatty acid of all the samples with the increase in storage period whereas no significant difference ($p>0.05$) was observed on the 0 day may be due to the freshness of the sample (Fig. 4.9a). Free fatty acid of control sample rose initially from 0.22 % oleic acid to 0.588% oleic acid on 8^{th} day of storage, whereas sample T1 and T2 attained the similar value only on 16^{th} day of storage. Remaining other samples showed a very slow rate of increase in FFA values. Among all the treated samples, a slowest increase in FFA value was observed in sample T6, displaying the increament from 0.223 % oleic acid at the beginning to 0.482% oleic acid on 24^{th} day of storage.

The increase trend in FFA during storage of paneer found in this study was similar to earlier reported trend in paneer packed by sorbic acid coated butter paper by Singh *et al.* (1989) and paneer stored in modified atmospheric packaging by Rai *et al.* (2008) but the initial values were slighter higher than reported by Raju and Sasikala (2016) and Eresam *et al.* (2015) and Rajarshibhai (2012). A slow increase in FFA of the treated sample may be due to antimicrobial and antioxidant properties of essential oils. The increase in FFA with

the storage period was found very slower as compared to *Cinnamon verum* spices oil only incorporated paneer coated with sodium alginate coating obtained by Raju and Sasikala (2016). They found the increase in FFA from 0.175% oleic acid to 0.542% oleic acid on 5^{th} day of storage for control and for clove spices treated sample 0.176% oleic acid to 0.409% oleic acid on 10^{th} days of storage and for *Cinnamon verum* spices treated sample 0.176 to 0.419 on 18^{th} days of storage.

4.5.3 Effect on acidity

The acidity (as % lactic acid) of control and essential oil mix edible coated paneer samples (T1 to T8) during storage of 24 days are represented in Appendix F and the trend is depicted in Fig. 4.10

The treatments, storage days and their interaction had significant effect on acidity of the paneers (Appendix F). Fig. 4.10 shows the changes in acidity of control sample and treated samples (T1 to T8) of paneer during storage. Statistically there was significant difference ($p<0.05$) in acidity of control and treated sample (Appendix F). A fast increase in acidity of control sample was observed in comparison to all the treated samples.

The acidity of control sample increased significantly ($p<0.05$) from an initial 0.303 to 1.827 in 24^{th} days. All the treated samples showed the slow rate of increase in acidity. Among the treated samples, sample T6 showed a slow increase in acidity that ranges from 0.300 % lactic acid at the beginning to 0.491% lactic acid on the 24^{th} days of storage.

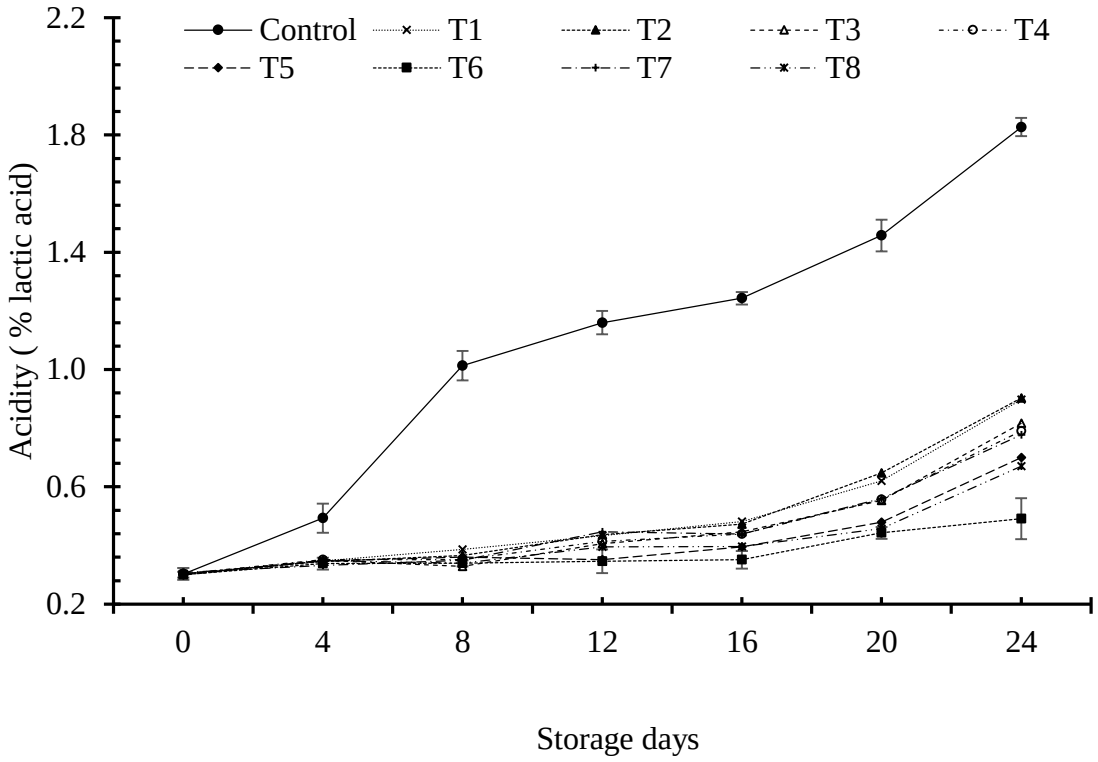


Fig 4.10 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on acidity

The increase in acidity of control paneer sample was a natural process and with higher extent. The increase in acidity of paneer during storage was also reported earlier by various research workers (Bhattacharya *et al.,* 1971; Mistry *et al.*,1990; Pal and Garg, 1989). An increase in acidity compared to control paneer at slow rate in treated samples might be due to the antimicrobial property of the essential oil incorporated into the edible coating and barrier property of coating itself.

The increasing pattern of acidity was in slower rate in case of mixture of essential oil coated paneers (T2, T3, T4, T5, T6, T7 and T8) than the single essential oil coated paneer (T1 and T2) also exhibited the interaction effect on acidity paneer of the combined essential oil coating was more profound than single essential oil coating. Among the treatment, sample T6 shows a very slow rate of increase in acidity from initial value 0.30 to 0.491on 24th days. The acidity value obtained for sample T6 was comparatively less than the value obtained for individual spice added paneers (0.99, 0.50, 0.74 and 0.75 as % lactic acid respectively for

black pepper, cardamom, cinnamon and cloves) for similar storage periods (Eresam *et al.*, 2015). It highlights combination of essential oils mix coating in paneer could be a good strategy for controlling acidity which is one of the important indicators of the microbiological activity in paneer and determinants for sensory attribute of paneer during storage.

4.5.4 Effect on tyrosine release

Fig. 4.11 demonstrates the change in tyrosine released of control and essential oils mix edible coated paneer samples (T1 to T8) during 24 days of storage.

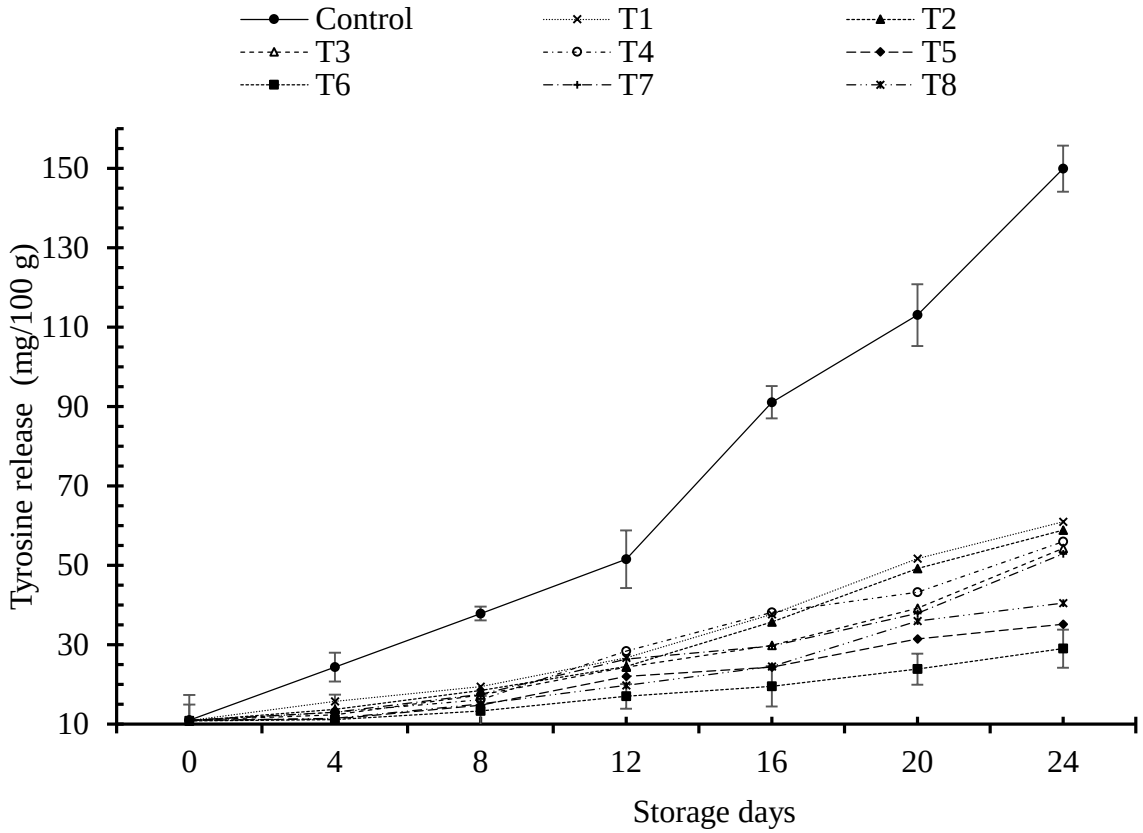


Fig. 4.11 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on tyrosine release of paneer.

Statistically there was significant difference ($p<0.05$) in tyrosine release of control and treated samples (Appendix G). A fast increase in tyrosine content of control sample was observed in comparison to all the treated samples (T1 to T8) , T3 and T4. Among the treated samples T6, T5 and T8 shows a slow rate of increase in tyrosine during 24 days of storage.

Tyrosine released is an indication of the extent of proteolysis in paneer. Although there was no significant difference ($p>0.05$) in tyrosine release of control and treated samples initially but a significant ($p<0.05$) increase in tyrosine release was noticed for all the samples with the increase in storage period. A sharp increase in the tyrosine content of control paneer was found during the storage period after 8^{th} days and onward reaching the final value of 149.97 mg/100 g on the 24^{th} days of storage. But in all treated paneers, comparatively a slower rate of increase in tyrosine release than control was found. Among all the treated samples, it was observed that a slowest rate of increase in sample T6 with the value ranges from 10.75 mg/100 g to 28.98 mg/100 g during 24 days of storage. Similar trend of proteolysis were reported for paneer made from cinnamon essential oil treated milk packed in different packaging materials by Anju *et al.* (2017).The increase in total soluble nitrogen content (as an indicator for the proteolysis) of powdered spices treated paneer was also observed by Eresam *et al.* (2015).

The sharp increase in tyrosine released in control sample indicates a high rate of proteolysis than treated samples. Slower rate of proteolysis in treated samples might be due to the anti-microbial and anti-oxidant properties of the essential oils and their combinations incorporated in edible coating. The values for the rate of increase in tyrosine release in this study were less than that observed by Khatkar *et.al.* (2017), probably due to the barrier properties of the sodium alginate edible coating and interaction of essentials oils used in coating.

4.6 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on microbiology of paneer during storage

4.6.1 Effect on total plate count

The trend of changes in Total plate count (TPC as log cfu/g) of control and essential oil mix incorporated edible coated paneers (T1 to T8) during storage is depicted in Fig. 4.12

The treatment, storage days and their interaction had significant effect on the TPC of the paneer samples (Appendix H). Statistically there was significant difference ($p<0.05$) in TPC count of control sample with the treated sample (Appendix H). The TPC count (log cfu/g) of the control sample was showed a sharp increase than that of the treated paneers (T1 to T8). Increase TPC count of sample T5, T7 and T8 were similar thereby resulting oerlapped

trend line whereas a slow increase was observed in sample T6 with initially 3.51 log cfu/g to 4.97 log cfu/g on 24th days of storage.

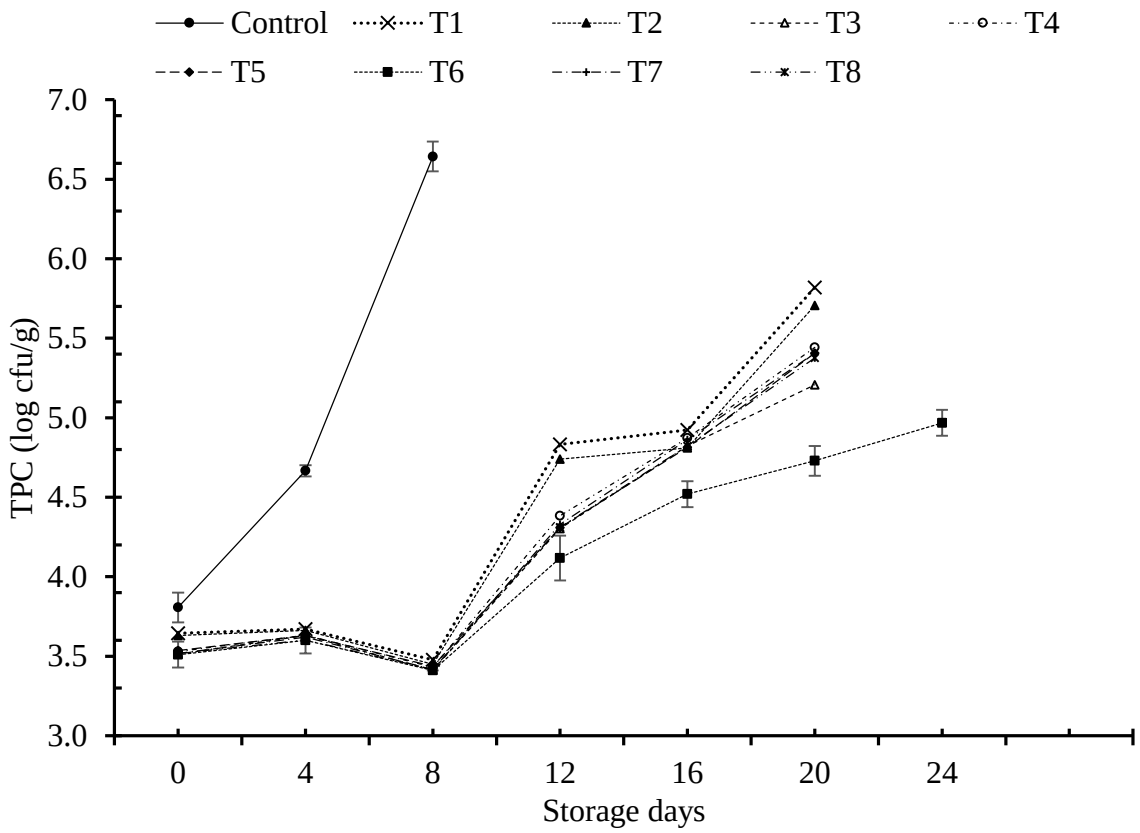


Fig. 4.12 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on TPC.

The microbial counts (log cfu/g) of the control sample increased rapidly from an initial 3.81 to 6.64 log cfu/g on 8th day of storage with sharp increment. It may be due to more availability of favorable conditions for the growth of a wide variety of organism in stored products. Gupta (1985), Ghodekar and Gandhi (1988), Pal *et al.* (1993) and Pal (1998) also observed similar trends of increase in standard plate count of fresh paneer sample upon storage. Later control sample became unacceptable due to visible mold growth. Whereas, treated samples shows a cyclic increase and decrease in microbial count with a slower rate in comparison to control sample till 12th day of storage. After that a gradual increase in TPC was observed in all the treated samples but the rate of increment was very slow in T6 sample in comparison to the other treated samples. This may be due to the antimicrobial activity of the *Zanthoxylum armatum* and *Cinnamomum verum* essential oil.

A cyclic decrease and increase up to 14th days of storage and then gradual increase in TPC with cardamom treated paneer samples was also observed by Eresam *et al.* (2015). But a gradual decrease in TPC of cinnamon essential oil treated paneer sample packed in different packaging material were observed by Khatkar *et al.* (2017). It has been mentioned that the antimicrobial potency of spices essential oil are different with respect to types of organisms (Patel, 2007) and also types of spices (Tajkarimi *et al.* 2010). According to Joshi *et al.* (2009), plant extracts and their components are hydrophobic, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable and causing leakage will lead to death of the microbes.

4.6.2 Effect on total coliform count

Total coliform per gram of paneer samples were not detected. Absence of coliform was also observed by Narayan *et al.*, (2016) in freshly prepared paneer for pickling. Dhole *et al.*, (2009) found the average coliform counts in the market samples of paneer ranges from $12.6x10^3$ to $23.2x10^3$ cfu/g. Paneer sample treated with brine solution, UV irradiation and microwave showed the presence of coliform initially 2-3 cfu/g and reached to 110 cfu/g on 25th days of storage (Barman and Roy, 2018) . Coliform counts (most probable number (MPN) presumptive) of the paneer prepared from the goat milk raises from initial value of 22.01±3.75 to 1445.40 ±584.59 on 7th day of storage (Agnihotri and Pal, 1996). The absence of coliform in paneer samples is probably due to the proper maintenance of hygienic conditions during the preparation and storage of paneer.

4.6.3 Effect on yeast and molds count

The trend of change in yeast and molds count (log cfu/g) of control and paneers coated with essential oil mix (T1 to T8) are depicted in Fig. 4.13. There was highly significant ($p<0.05$) variation in yeast and mold counts of all the samples with the increase in storage period and treatments (Appendix I).

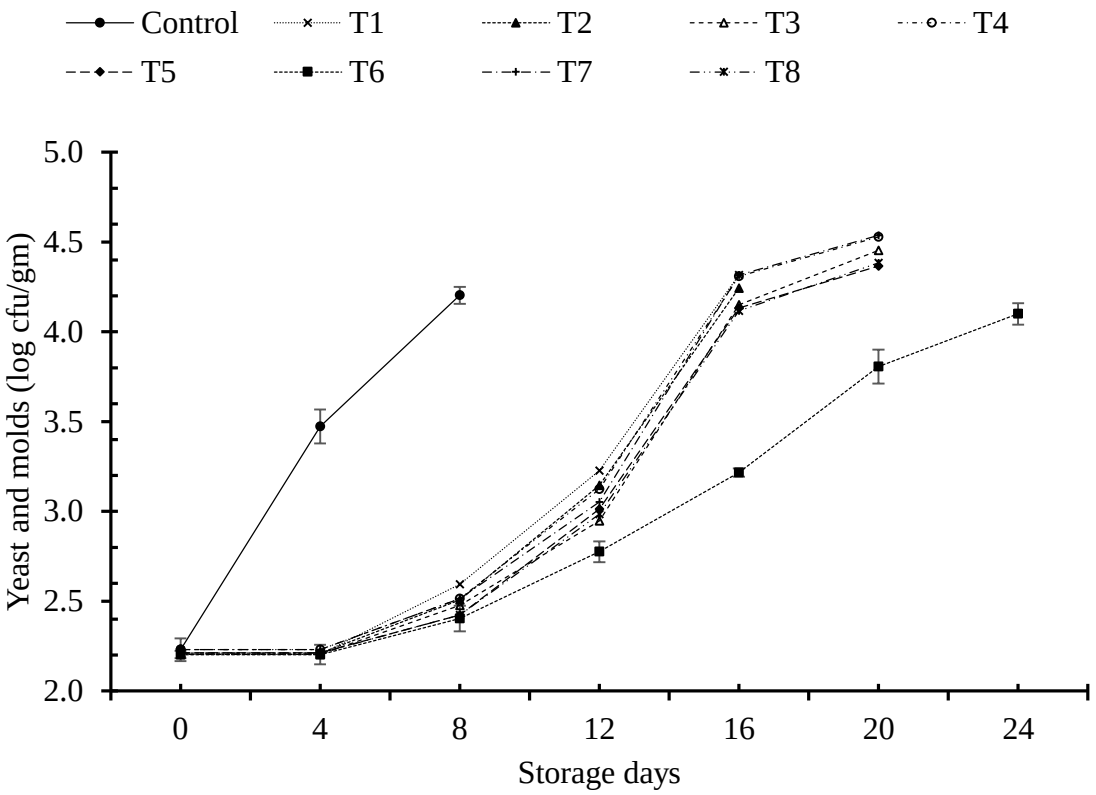


Fig. 4.13 Effect of sodium alginate edible coating incorporated with *Zanthoxylum armantum* and *Cinnamomum verum* essential oil on yeast and molds count of paneer.

The yeast and mold count of control sample increase from 2.2 log cfu/g to 4.2 log cfu/g on 8th day of storage and later on, control sample became unacceptable due to tremendous visible mold growth. The sharp increase in yeast and mold count of control sample might be due to availability of favorable conditions moisture, acidity increase for the growth. Gupta (1985), Ghodekar and Gandhi (1988), Pal *et al.* (1993) and Pal (1998) also observed similar trends of increase in yeast and mold count of fresh paneer upon storage. Compared to control, all the treated paneers (T1 to T8) showed slow rate in increasing yeast and mold count and the paneer sample T6 slowest rate of increase in yeast and mold count from 2.20 to 4.1 log cfu/g up to 24 days of storage. Similar trend of increased in yeast and mold count (2.3 to 4.51 log cfu/g was observed in paneer made after paneer curd treated with cardamom powders by Eresam *et al.* (2015).

4.7 Cost of coated paneer

The cost of the coated paneer was calculated by considering the cost of raw materials, transportation cost and processing cost used in paneer with 20% overhead cost of the final product. The cost of paneer was calculated to be NRs. 569.46/kg. The cost calculations are given in Appendix M.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the study, the following conclusions can be drawn:

- Paneer made from cow milk with addition of 0.1% $CaCl_2$ was found best in terms composition, yield and sensory quality of paneer.
- Both the essential oil of *Zanthoxylum armatum* and *Cinnamomum verum* showed a high phenolic content and high free radical scavenging activity.
- Paneer with 0.25% *Cinnamomum verum* and 1.75% *Zanthoxylum armatum* essential oil incorporated Na-alginate edible coating was found to be best and acceptable in terms of sensory attributes and physiochemical properties and microbial properties of paneer during storage up to 24 days showing longer (24 days) shelf life.

5.2 Recommendations

Based on the current work, the following recommendations can be made:

- Studies regarding incorporation of others spices essential oil and their combinations on sodium alginate based and other edible coating of paneer to extend shelf life of paneer can also be done.
- Study could also be carried out at different storage temperature and various packaging materials

Part VI

Summary

Paneer is a popular dairy product in south Asia. Extending its shelf life and storage stability is seen as a one of the leading issue regarding its market potential. One of the measures to enhance the shelf life of paneer is use of preservative. Spices and herbs; and their essential oils are well known for their medicinal, preservative and antioxidant properties; have been attempted to exploit in paneer preservation and extension of shelf life by direct addition in milk or treated in paneer curd. However, this study was conducted with objective to utilize essential oils and their mixtures incorporated in sodium alginate edible coating of paneer and to evaluate the storage stability and shelf life of the coated paneer during refrigerated storage condition.

A preliminary study was performed to study the effect of different concentration of $CaCl_2$ (0 to 2.5%) and best sample was selected based on composition, sensory attributes and yield of paneer prepared from cow milk. Essential oil of *Zanthoxylum armatum* and *Cinnamomum verum* in single and in combinations of mix of 2% in edible coating; obtained from D-optimal mixture design were incorporated in Na-alginate coatings of the paneer. All together 9 combinations; one being control (without coating and essential oil) and others containing *Zanthoxylum armatum* and *Cinnamomum verum* essential oils combinations 0 and 2% (T1); 2 and 0 % (T2); 1 and 1% (T3); 0.5 and 1.5% (T4); 1.5 and 0.5% (T5); 1.75 and 0.25% (T6); 0.25 and 1.75% (T7) and 1.25 and 0.75% (T8) were applied in sodium alginate edible coating of the paneer. The control paneer and all the treated paneer after packaging with LDPE were kept in refrigerated temperature (7±1ºC, RH 52%), and the changes in sensory attributes (body and texture, flavor, color and appearance, and overall acceptability), physicochemical properties (moisture content, free fatty acid, acidity, and tyrosine release) and microbial load (Total plate count, coliform and yeast and mold count) were monitored for ever four days interval up to 24 days to evaluate storage stability and shelf life of the paneer.

With respect to sensory attributes, all the *Zanthoxylum armatum* and *Cinnamomum verum* essential oils and their mix incorporated sodium alginate coating of the paneer showed better scores than control in terms of body and texture, flavor, color and appearance, and overall acceptability during refrigeration storage (7±1ºC, RH 52%). The perceived score are even

found better in combined essential oil mix coated edible coating compared to single essential oil incorporated edible coatings. Defect on body and texture, flavor, and color and appearance was perceived before 16 days of storage among the combined EO mix incorporated edible coating except paneer sample T6. Sample with 1.75 % *Zanthoxylum armatum* and 0.25% *Cinnamomum verum* essential oil mix incorporated sodium alginate coating (T6) was found best and acceptable with average score of (7.00) for overall acceptability of paneer till 24 days of storage period.

Regarding physicochemical and microbial changes, storage days and treatment had significant effect ($p< 0.05$) on retention on moisture content and changes on FFA, acidity, tyrosine released, microbial load (TPC and yeast and mold count) during different days interval up to 24 days of refrigerated storage (7±1ºC, RH 52%). All the essential oils and their mix incorporated sodium alginate coating of the paneer compared to control paneer showed better retention on moisture and slow increase in FFA, acidity, tyrosine released and microbial loads during the storage condition. Even among the treated paneer, the higher retention of moisture content (53.01%) and slowest increase in FFA (0.482% as oleic acid), acidity (0.49% as lactic acid), tyrosine released (28.98 mg/100 g), TPC (4.97 log cfu/g), and yeast and mold counts (4.1 log cfu/g) were found for 1.75% *Zanthoxylum armatum* and 0.25% *Cinnamomum verum* essential oils incorporated sodium alginate coating of the paneer (T6) at 24 days of storage.

On the basis of these finding, it can be concluded that 1.75% *Zanthoxylum armatum* and 0.25% *Cinnamomum verum* (T6) coating combination is effective on extension of shelf life of paneer with minimum physicochemical and microbial changes along with acceptable sensory attributes till 24 days of refrigerated storage.

References

Agaoglu, S., Dostbil, N. and Alemdar, S. (2007). Antimicrobial activity of some spices used in the meat industry. [Bull Vet. Inst. Pulawy]. pp. 53-57.

Agnihotri, M. K. and Pal, U. K. (1996). Quality and shelf-life of goat milk paneer in refrigerated storage. *J. Small Ruminant Res.* **20** (1), 75-81.

Ana, G., Abrunhosa, L., Pastrana, L. M. and Minguel., A. C. (2018). Edible films and coatings as carriers of living microorganisms: A new strategy towards biopreservation and healthier foods. *J. Food Sci. Food Safety* **17** (3), 594-614.

Aneja, K. R. (2003). "Experiments in Microbiology, Plant Pathology and Biotechnology." (4th ed.). New Age International (P) Limited. New Delhi.

Aneja, R. P. (2007). "East-west Fusion of Dairy Products"(6th ed.). A Dairy Indian Publication. New Delhi.

Aneja, R. P., Mathur, B. N., Handan, R. C. and Banerjee, A. K. (2002). Heat and acid coagulated products. *In:* "Technology of Indian Milk Products". pp. 133-158. New Delhi, India. Dairy India Year Book Publications.

Anju B.K., Aradhita R. and Kaur, A. (2017). Studies on the shelf life extension of paneer with the addition of plant essential oil and different packaging materrials. *Int. J. Curr. Microbiol. Appl. Sci.* **6**, 376-389.

AOAC (2005). Dairy Products. *In* "Official Methods of Analysis". (L. Robert and Jr. Bradley, Eds.). pp. 1-87. University of Wisconsin.

Arora, K., Sabikhi, L. and Kanawjia, S. (1996). Manufacture of paneer from substandard buffalo milk. *Indian J. Dairy Bio.Sci.* **7**, 71-75.

Arora, V. K. and Gupta, S. K. (1980). Effect of low-temperature storage on paneer. *Indian J. Dairy Sci.* **33** (3), 374-380.

Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M. (2008). Biological effects of essential oils–a review. *J. Food Chem. Toxicol.* . **46** (2), 446-475.

Barman, A. K. and Roy, P. K. (2018). Comparative study on the shelf life of treated paneer. *J. Agri. Eng. Food Technol.* **5** (2), 108-112.

Bhattacharya, D. C., Mathur, O. N., Srinivasan, M. R. and Samlik, O. (1971). Studies on the method of production and shelf life of paneer (cooking type of acid coagulated cottage cheese). *J. Food Sci. Technol.* **8** (5), 117-120.

BIS. (1983). "IS: 10484". Report No. IS: 10484. Beaureau of Indian Standards. (Ministry of Consumer Affairs, Food and Public Distribution), India. pp. 1-11.

Brijwal, L., Pandey, A. and Tamta, S. (2013). An overview on phytomedicinal approaches of *Zanthoxylum armatum*: An important magical medicinal plant. *J. Med Plants Res.* **7** (8), 366–370.

Brownlee, I. A., Seal, C. J., Wilcox, M., Dettmar, P. W. and Pearson, J. P. (2009). Applications of alginates in food. In: "Alginates: Biology and Applications." (Rehm B., Eds.) pp. 211-228. Berlin, Heidelberg. Springer.[doi.org/10.1007/978-3-540-92679-5_9].

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* **93** (3), 223-253.

Chaudhary, B. and Upadhyaya, M. (2013). Socio-economic impacts of dairy cooperative. *Econ. J. Dev.* **15** (1), 15-23.

Chauhan, R., Ruby, K. and Dwivedi, J. (2012). *Bergenia ciliata* mine of medicinal properties: a review. *Int. J. Pharm. Sci. Rev. Res.* **15** (2), 20-23.

Chawla, A. K., Singh, S. and Kanawjia, S. K. (1987). Effect of fat levels, additives and process modifications on composition and quality of paneer and whey. *Asian J. Dairy Res.* **6** (2), 87-92.

Choubey, S., Katekar, G., Pawar, P. and Godbole, S. (2020). Extraction and evaluation of anti-oxidants from fruit peels and Its utilization in paneer to increase its shelf-life. *Int. J. Adv. Sci. Res. Manage.* **5** (1), 13-21.

Conner, D. E. and Beuchat, L. R. (1984). Effects of essential oils from plants on growth of food spoilage yeasts. *J. Food Sci.* **49** (2), 429-434.

Cox, S. D., Mann, C. M., Markham, J. L., Bell, H. C., Gustafson, J. E., Warmington, J. R. and Wyllie, S. G. (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.* **88** (1), 170-175.

Cristani, M. D., Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., Venuti, V., Bisignano, G., Saija, A. and Trombetta, D. (2007). Interaction of four monoterpenes contained in essential oils with model membranes: implications for their antimicrobial activity. *J. Agri. Food chem.* **55** (15), 6300-6308.

Daferera, D. J., Ziogas, B. N. and Polissiou, M. G. (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agri. Food chem*. **48** (6), 2576-2581.

Debeaufort, F., Quezada-Gallo, J. A. and Voilley, A. (1998). Edible films and coatings: tomorrow's packagings: a review. *J. Food Sci.* **38** (4), 299-313.

Delesa, D. A. (2018). Traditional medicinal plants for industrial application as natural food preservatives. *Int. J. Adv. Res. Biol.Sci.* **5** (4), 85-94.

DFTQC (2021). “Minimum Mandatory Standard of Food and Feed Products”. Department of Food Technology and Quality Control. Retived from http://www.dftqc.gov.np/downloadsdetail/6/2020/45564816/ [Accessed 24 March, 2022].

Dhakal, D. and Sharma, K. R. (2020). Estimation of total phenol and antioxidant activity of *Zanthoxylum aramantum* of Nepalese origin. *Int. J. Curr. Pharm. Res.* **12**, 31-40.

Dhole, P. T., Deokar, D. K., Pawar, R. D. and Patil, S. J. (2009). Chemical quality of paneer sold in Ahmennagar city. *(Maharashtra). Int Dairy Industry Conference. Goa, Feb (pp. 7-9).* **XXXVII** 7-9.

Dongare, S. A., Dige, Y. P. and Syed, H. M. (2019). Storage study and textural profile analysis of paneer at different temperature. *J. Pharm. Phytochem.* **8** (2), 864-868.

Draget, K. I. (2009). Alginates. *In:* "Handbook of Hydrocolloids" (2nd ed., Vol. 29). pp. 807-828. Woodhead Publishing.

Elgayyar, M., Draughon, F. A., Golden, D. A., & Mount, J. R. (2001). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J. Food Protection.* **64** (7), 1019-1024.

Eresam, E. K. K., Pinto, S. and Aparnathi, K. D. (2015). Evaluation of selected spices in extending shelf life of paneer. *J. Food Sci. Technol.* **52** (4), 2043-2052.

FAO. (2010). ""Dairy Sector Study of Nepal". Food and Agriculture Organization of the United Nations., Nepal. pp. 1-2. Retrieved from http://nepalagritech.com.np/wp-content/uploads/2016/10/Dairy-Sector-in-Nepal-FAO.pdf. [Accessed 16 April 2021].

Farag, R. S., Badei, A. Z. M. A., Hewedi, F. M. and El-Baroty, G. S. A. (1989). Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J. Am. Oil Chemists' Soc.* **66** (6), 792-799.

Genwali, G. R., Acharya, P. P. and Rajbhandari, M. (2013). Isolation of gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. *Nepal J. Sci. Technol.* **14** (1), 95-102.

Ghodekar, D. R. and Gandhi, D. N. (1988). Antibacterial activity of garlic extract against lactic acid bacteria and contaminants of fermented milks. *Ind. J. Dairy Sci.* **41** (4), 511-512.

Gottardi, D., Bukvicki, D., Prasad, S. and Tyagi, A. K. (2016). Beneficial effects of spices in food preservation and safety. *Frontiers in Microbiol.* **7** (12), 1394-1409.

Government, N. (2019/2020) “Statistical information on Nepalese Agriculture”.(Ministry of Agriculture Livestock and Development), Nepal. Retrived from https://s3-ap-southeast-1.amazonaws.com/prod-gov-agriculture/server-assets/publication-1625998794412-f37e4.pdf[Accessed 24 March 2022]

Goyal, S. and Goyal, G. K. (2015). Maximizing shelf life of paneer - a review. *J. Food Sci.* **56** (8), 1253-1261.

Guilbert, S. and Gontard, N. (1995). Edible and biodegradable food packaging.. *In:* "Foods and Packaging Materials-Chemical Interactions". (Ackermann P, Jägerstad M and Ohlsson T., Eds.). pp. 159-168. The Royal Society of Chemistry, Cambridge.

Gupta, M. (1985). Studies on food poisoning organisms in paneer.Unpublished Ph.D. Thessis. Kurukshetra Univ., NDRI, Karnal, India.

Joshi, B., Lekhak, S. and Sharma, A. (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu University J. Sci. Eng.Technol.* **5** (1), 143-150.

Kapadiya, D. B., Dabhi, B. K. and Aparnathi, K. D. (2016). Spices and herbs as a source of natural antioxidants for food. *Int. J. Curr. Microbiol. Appl. Sci.* **5** (7), 280-288.

Karthikeyan, S. (2005). Application of Continuous Microwave Processing and Modified Atmosphere Packaging for Enhancement of Shelf Life of Paneer. Unpublished Ph.D. dissertation. Animal and Fisheries Sci. Univ., India.

Kaur, J. and Bajwa, U. (2003). Effect of pre-treatments of green leafy vegetables on the quality attributes of vegetable impregnated paneer. *J. Food Sci. Technol.* **40** (6), 632-637.

Khatkar, A. B., Ray, A. and Kaur, A. (2017). Studies on shelf life extension of paneer with the addition of plant essential oil and different packaging materials. *Int. J. Curr. Microbiol. Appl. Sci.* . **6** (9), 376-389.

Kim, H. Y., Lee, Y. J., Hong, K. H., Kwon, Y. K., Sim, K. C., Lee, J. Y., Cho, H. Y., Kim, I. S., Han, S. B., Lee, C. W., Shin, I. S. and Cho, J. S. (2001). Isolation of antimicrobial substances from natural products and their preservative effects. *J. Food Sci. Biotechnol.* **10** (1), 59-71.

Kinjal, D., Gokhale, A., Mallik, J., Modha, H. and Patel, H. (2020). Impact of addition of salts on sensory, rheological properties and yield of Paneer. *Int. J. Chem. Stud.* **8** (2), 2049-2058.

Kinjal, D., Patel, H. G. and Gokhale, A. J. (2015). Advancements in manufacture of paneer. National Seminar on Indian Dairy Industry Opportunities and Challenges, 11th Alumni Convention, Gujarat, India. . December 14-17. pp. 170-175. Retrieved from https://www.dairyknowledge.in/sites/default/files/ch16_0.pdf.

Kinsella, J. E. and Whitehead, D. M. (1989). Proteins in whey: chemical, physical, and functional properties. *Adv. Food Nutr. Res.* **33**, 343-438.

Krisch, J., Tserennadmid, R. and Vágvölgyi, C. (2011). Essential oils against yeasts and moulds causing food spoilage. *In:* "Science against Microbial Pathogens: Communicating Current Research and Technological Advances". (A. Mendez Vilas, Eds.) pp. 1135-1142. Spain. Badajoz : Formatex Res. Center, corp.

Kumar, S., Rai., D. C., Niranjan, K. and Bhat, Z. F. (2011). Paneer-an Indian soft cheese variant: a review. *J. Food Sci.Technol.* **51** (5), 821-831.

Kunitz, M. (1946). Crystalline soybean trypsin inhibitor. *J. Gen. Physiol*. **29** (3**),** 291-310.

Lee, K. Y. and Mooney, D. J. (2012). Alginate: properties and biomedical applications. *J. Progress in Polymer Sci.* **37** (1), 106-126.

Liu, Q., Meng, X., Li, Y., , Zhao, C. N., Tang, G. Y. and Li, H. B. (2017). Antibacterial and antifungal activities of spices. *Intl. J. Mol. Sci.* **18** (6), 1283.

Makhal, S. and Kanawjia, S. K. (2005). Effect of calcium chloride and high heat treatment on the yield of direct acidified cottage cheese. *Proc. of National Seminar on Value Added Dairy Products*, National Dairy Research Institute, Karnal .pp 21-22.

Mancini, F., Montanari, L., Peressini, D. and Fantozzi, P. (2002). Influence of alginate concentration and molecular weight on functional properties of mayonnaise. *J. Food Sci. Technol.* **35** (6), 517-525.

Maynes, J. R. and Krochta, J. M. (1994). Properties of edible films from total milk protein. *J. Food Sci.* **59** (4), 909-911.

Mchugh, D. J. (2003). Production, properties and uses of alginates. Retrieved from https://www.fao.org/3/X5822E/x5822e04.htm#TopOfPage. [Accessed 6 April, 2021].

McHugh, T. H. and Krochta, J. M. (1994). Milk-protein-based edible films and coatings. *J. Food Technol.* **48** (1), 97-103.

Menon, N. A. (2000). Studies on the volatiles of cardamom (*Elleteria cardamomum*). *J. Food Sci.Technol.* **37** (4), 406-408.

Mishra, A., Khatri, S., Jha, S.K., and Ansari, S. (2020). Effects of milking methods on milk yield, milk flow rate, and composition in cow. *Int. J. Sci. Res. Pub.* **10** (1), 416-420.

Mistry, C. D., Singh, S. and Sharma, R. S. (1990). Physico-chemical changes during storage of paneer prepared from cow milk by altering the salt-balance of milk. Presented at Int. Dairy Congr. Montreal, Canada. October 8-12 p. 519.

Modi, V. K. and Jain, S. C. (1988). The preservation of paneer by chemical additives. Presented at Food Convocation and Exhibition. CFTRI, Mysore. Feburary 18-23.

Morr, C. V. and Ha, E. Y. W. (1993). Whey protein concentrates and isolates: processing and functional properties A critical reviews. *J. Food Sci. Nutr.* **33** (6), 431-476.

Nabvi, S., Di Lorenze, A., Izadi, M., Sobrozo-Sanchez, E. and Daglia, M. (2015). Antimicrobial effects of cinnamon:from farm to food, cosmetic and pharmaceutical Industries. *J. Nutrients* **7** (9), 7729–7748.

Narayan, R., Kumar, A. and Tejas (2016). Enhancing storage of paneer using low cost hurdle technolgy. *J. Food Dairy Technol.* **4** (3), 49-51.

Nanasombat, S. and Wimuttigosol, P. (2011). Antimicrobial and antioxidant activity of spice essential oils. *J. Food Sci. Biotechnol.* **20** (1), 45-53.

- Packiyasothy, E. and Kyle, S. (2002). Antimicrobial properties of some herb essential oils. *Food Australia* 54 (9):384-387.

Pal, D. and Garg, F. C. (1989). Studies on the utilization of sweet-cream butter milk in manufacturing of paneer. *J. Food Sci Technol.* **26** (5), 259–264.

Pal, D. and Khan, S. U. (2011). Paneer production: a review. *J. Food Sci.Technol.* **48** (6), 645-660.

Pal, M. A. (1998). Microbiologically related biochemical transformation in paneer during storage. *Ind. J. Microbiol.* **38** (1), 21-22.

Pal, M. A. and Kapoor, C. M. (2000). Effect of emulsifying salts on the chemical constitution of processed paneer. *Ind. J. Dairy Biosci.* **11**, 42-46.

Pal, M. A., Yadav, P. L. and Sanyal, M. K. (1993). Effect of paraffining on the physico-chemical, microbiological and sensory characteristics of low fat pneer at low temperature storage. *Indian J. Dairy Sci.* **46** (11), 519-524.

Paranagama, P. A., Abeysekera, K. H. T., Abeywickrama, K. and Nugaliyadde, L. (2003). Fungicidal and antiaflatoxigenic effects of the essential oil of *Cymbopogon citratus* (DC.) Stapf.(lemongrass) against *Aspergillus flavus* isolated from stored rice. *Letters in Applied Microbiol.* **37** (1), 86-90.

Parthasarathy, V. A., Chempakam, B. and Zachariah, T. J. (2008). "Chemistry of Spices.". Cambridge. India.

Patel, S., Prasanth, S., Choudhary, P. L. and Sahu, C. (2007). Techno-economic feasibility of whey based mango-herbal (ginger) beverage. *Ind. J. Dairy Sci.* **60**, 149-155.

Patton, S. (1955). Browning and associated changes in milk and its products: a review. *J. Dairy Sci.* **38** (5), 457-478.

Pinto, S. V., Jana, A. H. and Solanky, M. J. . (2004). Ginger juice based herbal ice cream and its physicochemical and sensory characteristics. *Ind. J. Dairy Sci.* **57** (5), 315-319.

Prajapati, N., Ojha, P. and Karki, T. B. (2015). Antifungal property of essential oil extracted from *Zanthoxylum armatum* (Timur). *J. Nutritional Health & Food Eng.* **3** (1), 266-270.

Prasad, S., Gupta, S. C. and Aggarwal, B. B. (2011). Micronutrients and cancer: add spice to your life. 23–48. *In:* "Herbs, Spices and Medicinal Plants: Processing, Health Benefits and Safety". (M. B. Hossain, N. P. Brunton and D. K. Rai, Eds.). pp. 23-48. Wiley.

Raghavan, S. (2007). "Hand Book of Spices, Seasonings and Flvourings" (2nd ed.). CRC press. Boca Raton.

Rai, S., Goyal, G. K. and Rai, G. K. (2008). Effect of modified atmosphere packaging and storage on the chemical quality of paneer. *J. Dairy Food Home Sci.* **27** (1), 33-37.

Rajarshibhai, B. A. (2012). Evaluation of extracts from selected spices to extend shelf life of paneer,. MSc. Thesis. Ananda Agricultural Univ., India.

Rajorhia, G., Pal, D. and Arora, K. (1984). Quality of paneer marketed in Karnal and Delhi. *Ind. J. Dairy Sci.* **37** (3), 274-276.

Raju, A. and Sasikala, M. S. (2016). Natural antimicrobial edible film for preservation of paneer. *J. Biosci. Biotechnol. Res. Asia*. **13** (2), 1083-1088.

Ramos, O. L., Reinas, I., Silva, S. I., Fernandes, J. C., Cerqueira, M. A., Pereira, R. N. and Malcata, F. X. (2013). Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom. *Food Hydrocolloids*. **30** (1), 110-122.

Roy, S. K. and Singh, S. (1994). Effects of coagulation temperature and hydrocolloids on production and sensory quality of filled paneer. *Ind. J. Dairy Sci.* . **47** (8), 683-687. [Cited in M. A. Pal and S. U. Khan. (2011). Paneer Production: A review. *J. Food Sci. Technol.* **48** (6), 645-660].

Rubio, L., Motilva, M. J. and Romero, M. P. (2013). Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles. *J. Food Sci.Nutr.* **53** (9), 943-953.

Rudolfi, T. A., Schedrina, M. M. and Mindlin, L. O. (1988). Determination of the evaporation rate of essential oils and perfumery compositions using gas chromatography. *Chromatographia*. **25**, 520-531.

Sachdeva, S. (1983). Production packing and preservation of paneer. Ph.D. Thesis. Kurukshetra Univ., India.

Sachdeva, S., Prokopek, D. and Reuter, H. (1991). Technology of paneer from cow milk. *Japan. J. Dairy Food Sci*. **40**:A85-A90.

Sachdeva, S. and Singh, S. (1988a). Incorporation of hydrocolloids to improve the yield, solids recovery and quality of paneer. *Indian J Dairy Sci.* **41** (2), 189-193.

Sachdeva, S. and Singh, S. (1988b). Optimisation of processing parameters in the manufacture of paneer. *J Food Sci Technol.* **25** (3), 142-145. [Cited in M. A. Pal and S. U. Khan. (2011). Paneer production:A review. *J. Food Sci.Technol.* **48** (6), 645-660.

Sachetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, R. and Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *J. Food Chem.* **91**, 621-632.

Sen, D. C. and Rajorhia, G. S. (1996). Efficiency of cardamom as natural preservative for *Sandesh*. *Ind. J. Dairy Sci.* **49** (7), 433-440.

Shan, B., Cai, Y. Z., Brooks, J. D. and Corke, H. (2011). Potential application of spice and herb extracts as natural preservatives in cheese. *J. Med. Food.* **14** (3), 284-290.

Sharma, K. and Verma, B. B. (2006). Herbs and spices: A potential tool for preservation of dairy products. *Indian Food Ind.* **25** (6), 36-38.

Shashikumar, C. and Puranik, D. (2012). Study on the use of lactoferrin for the biopreservation of paneer. *Tropical Agri. Res.* **23**(1)**,** 70-76.

Shrivastava, S. and Goyal, G. K. (2007). Preparation of paneer. *Ind. J. Dairy Sci.* **60** (6), 377-388.

Singh, L., Mohan, M.S., Puttalingamma, V. and Sankaran, R. (1989). Preservation of paneer by sorbic acid. *J. Food Sci.Technol.* **26** (3), 129-132.

Singh, A. D. and Maharaj, R. (2014). Sensory evaluation as a tool in determining acceptability of innovative products developed by undergraduate students in food science and technology at the university of Trinidad and Tobago. *J. Curriculum and Teaching* **3**, 10-27.

Singh, H. and Fox, P. F. (1989). Heat-induced changes in casein [Bull Int. Dairy Fed. No. 238]. pp. 24-30.

Singh, L., Murali, S. and Sankaran, R. (1991). Extension of shelf life of paneer by sorbic acid and irradiation. *J. Food Sci.Technol.* **28** (6), 386-388.

Singh, S. and Kanawjia, S. K. (1988). Development of manufacturing technique for paneer from cow milk . *Ind. J. Dairy Sci.* **41** (3), 322-325.

Souza, E. L., Stamford, T. L. M., Lima, E. O., Trajano, V. N. and Filiho, J. B. (2005). Antimicrobial effectiveness of spices: An approach for use in food conservation systems. *Braz. Arch. Biol. and Technol.* **48,** 92-130.

Sung, B., Prasad, S., Yadav, V. R. and Aggarwal, B. B. (2012). Cancer cell signaling pathways targeted by spice-derived nutraceuticals. *Nutr. Cancer*. **64** (2), 173-197.

Suthar, J. S. (2015). Evaluating the effect of homogenization on the quality of paneer. B.Tech. Disssertation. Anand Agriculture Univ., India.

Tajkarimi, M. M., Ibrahim, S. A. and Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*. **21** (9), 1199-1218.

Thakral, S. (1986). Studies on yeasts in paneer. MSc. Dissertation. Kurukshetra Univ., India.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *J. Food Sci.Technol.* **14** (3), 71-78.

Thippeswamy, L., Venkateshaiah, B. V. and Patil, S. B. (2011). Effect of modified atmospheric packaging on the shelf stability of paneer prepared by adopting hurdle technology. *J. Food Sci. Technol.* **48** (2), 230-235.

Tiwary, M., Naik, S. N. and Tewary, D. K. (2007). Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. *J. Vector Born Disease* **44** (3), 198-204.

Tserennadmid, R., Takó, M., Galgóczy, L., Papp, T., Pesti, M., Vágvölgyi, C. S., Almássy, K. and Krisch, J. (2011). Anti-yeast activities of some essential oils in growth medium, fruit juices and milk. *Int. J. Food Microbiol*. **44** (1), 480-486.

UNIDO and FAO. (2005). Herbs, spices and essential oils - post-harvest operations in developing countries [Report]. Vienna, Austria. Retrieved from https://www.fao.org/3/ad420e/ad420e.pdf. [Accessed 8 March, 2020].

Uribe, S., Ramirez, J. and Pena, A. (1985). Effects of pinene on yeast membrane function. *J. Bactreiology* **161** (3), 1195-1200.

Urtuvia, V., Maturana, N., Acevedo, F., Peña, C. and Díaz-Barrera, A. (2017). Bacterial alginate production: An overview of its biosynthesis and potential industrial production. **33**, 198. [Cited in J. Singh, V. Meshram and M. Gupta. "Bioactive Natural Products in Drug Discovery". Springer Nature Singapore Pte. Ltd. Singapore].

Venugopal, A., Sonal, D. and Rai, S. (2009). Antibacterial effect of herbs and spices extract on *Escherichia coli*. *Electronic J. Biol.* **5** (2), 40-44.

Vignoli, J. A., Bassoli, D. G. and Benassi, M. T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chem*. **124**, 863–868.

Vishweshwaraiah, L. (1987). Studies on dehydration and deep freezing of paneer. *J. Food Sci. Technol.-Mysore* **24** (2), 95-96.

Wijewardhana, U., Gunathilaka, U. G. S. A. and Navaratne, S. (2019). Determination of total phenolic content, radical scavenging activity and total antioxidant capacity of cinnamon bark, black cumin seeds and garlic. *Int. Res. Adv. Eng. Sci.* **4** (2), 55-57.

Yadav, P. L., Rao, K. H., Rao, K. V., Sanyal, M. K. and Singh, R. R. B. (1994). Manufacture of low-fat paneer with enhanced shelf-life at ambient temperature. Presented at Proc. of National Symposium on Meat and Milk Industry: Trends and Developmental Strategies. CCS Haryana Agric.Univ. 25-27 August.

Yilmazer, G., Carrillo, A. R. and Kokini, J. L. (1991). Effect of propylene glycol alginate and xanthan gum on stability of O/W emulsions. *J. Food Sci.* **56** (2), 513-517.

Appendices

Appendix A

ANOVA for sensory analysis for calcium chloride treated paneers

Table A.1 ANOVA table for sensory score of calcium chloride added paneer

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Body and texture	52.733	5	10.547	44.774	.000
	Flavour	62.800	5	12.560	57.284	.000
	Colour	40.150	5	8.030	22.561	.000
	OA	84.550	5	16.910	69.493	.000
Panelist	Body and texture	2.000	9	.222	.943	.498
	Flavour	1.733	9	.193	.878	.551
	colour	3.483	9	.387	1.087	.391
	OA	1.350	9	.150	.616	.777

Table A. 2 Sensory scores of paneer samples treated with calcium chloride

Treatment	Body &Texture	Flavor	Color & appearance	OA
Control	5.3 ± 0.46^{a}	5.7 ± 0.46^{a}	5.5 ± 0.50^{a}	5.2 ± 0.40^{a}
C1	6.3 ± 0.46^{b}	6.8 ± 0.40^{b}	6.9 ± 0.54^{bd}	6.3 ± 0.46^{b}
C2	7.8 ± 0.40^{c}	7.7 ± 0.46^{c}	8 ± 0.45^{c}	8.4 ± 0.46^{c}
C3	7.2 ± 0.40^{c}	7.6 ± 0.49^{c}	7.6 ± 0.49^{c}	7.7 ± 0.46^{d}
C4	6.2 ± 0.40^{b}	5.5 ± 0.5^{a}	6.8 ± 0.87^{b}	6.5 ± 0.50^{b}
C5	5.2 ± 0.60^{a}	5.1 ± 0.3^{a}	6.3 ± 0.46^{b}	5.2 ± 0.40^{a}

Means with different alphabets in the same column are significantly different ($p<0.05$)

Appendix B

ANOVA of calcium treated paneer for compositional parameters

Table B.1 Composition and yield of calcium chloride treated paneer.

Treatment	Moisture	Acidity	FFA	TA	Protein	Fat	Yield
Control	53.160±0.08[a]	0.383±0.02[a]	0.224±0.01[a]	1.86±0.017[a]	18.167±0.047[a]	19.367±0.125[a]	13.38±0.62[a]
C1	53.637±0.12[b]	0.381±0.01[b]	0.226±0.09[a]	1.93±0.012[b]	18.867±0.047[b]	20.733±0.471[b]	14.25±0.41[b]
C2	54.423±0.04[c]	0.335±0.02[b]	0.226±0.08[a]	1.96±0.042[b]	19.567±0.042[c]	21.20±0.516[c]	16.253±0.54[c]
C3	54.253±0.12[d]	0.328±0.05[c]	0.226±0.04[a]	1.98±0.047[b]	19.20±0.82[d]	20.73±0.17[b]	15.833±0.47[d]
C4	54.133±0.17[e]	0.323±0.05[d]	0.225±0.04[a]	2.02±0.016[c]	19.133±0.047[d]	20.70±0.05[b]	15.650±0.041[e]
C5	54.153±0.12[e]	0.307±0.01[e]	0.225±0.01[a]	2.16±0.022[d]	19.167±0.041[d]	20.667±0.42[b]	15.320±0.52[f]

Values represent means± standard deviation. Means with different alphabets in the same column are significantly different ($p<0.05$).

Table B.2 Turkey's post hoc analysis for moisture content

Treatment	N	Subset				
		1	2	3	4	5
Control	3	53.1600				
C1	3		53.6367			
C4	3			54.1333		
C5	3			54.1533		
C3	3				54.2533	
C2	3					54.4233
Sig.		1.000	1.000	.564	1.000	1.000

The error term is Mean Square (Error) = .001

Table B.3 Tukey's post hoc analysis for acidity content

Treatment	N	Subset				
		1	2	3	4	5
C5	3	0.307000				
C4	3		0.322733			
C3	3			0.357633		
C2	3				0.372333	
C1	3				0.374667	
Control	3					0.383333
Sig.		1.000	1.000	1.000	0.809	1.000

The error term is Mean Square (Error) = 5.303E-0065.

Table B.4 Turkey's post hoc analysis for FFA content

Treatment	N	Subset
		1
Control	3	0.22200
C1	3	0.22200
C2	3	0.22200
C5	3	0.22200
C4	3	0.22233
C3	3	0.22267
Sig.		0.967

The error term is Mean Square (Error) = 5.611E-006.

Table B.5 Tukey's post hoc analysis for TA content

Treatment	N	Subset			
		1	2	3	4
Control	3	1.86333			
C1	3		1.92667		
C3	3		1.98000		
C2	3		1.9600		
C4	3			2.02000	
C5	3				2.16000
Sig.		1.000	0.283	1.000	1.000

The error term is Mean Square (Error) = .000

Table B.6 Tukey's post hoc analysis for Protein content

Treatment	N	Subset			
		1	2	3	4
Control	3	18.166667			
C1	3		18.866667		
C4	3			19.133333	
C5	3			19.166667	
C3	3			19.200000	
C2	3				19.566667
Sig.		1.000	1.000	.817	1.000

The error term is Mean Square (Error) = .004.

Table B.7 Tukey's post hoc analysis for Fat content

Treatment	N	Subset		
		1	2	3
Control	3	19.36667		
C5	3		20.66667	
C4	3		20.70000	
C1	3		20.73333	
C3	3		20.73333	
C2	3			21.20000
Sig.		1.000	.987	1.000

The error term is Mean Square (Error) = .017.

Table B.8 Tukey's post hoc analysis for total yield

Treatment	N	Subset					
		1	2	3	4	5	6
Control	3	13.383333					
C1	3		14.250000				
C5	3			15.320000			
C4	3				15.650000		
C3	3					15.833333	
C2	3						16.253333
Sig.		1.000	1.000	1.000	1.000	1.000	1.000

The error term is Mean Square (Error) = .004.

Appendix C

Sensory score for *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated Na alginate coated paneer

Table C.1 Sensory score for *Cinnamomum verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coated paneer.

Treatments	Storage days						
	0	4	8	12	16	20	24
	Color and appearance						
Control	8.10±0.30^{a}	7.60±0.49^{a}	6.0±0.00^{a}	NA	NA	NA	NA
T1	7.90±0.30^{a}	7.90±0.30^{a}	7.4±0.49^{b}	6.80±0.40^{a}	6.50±0.50^{a}	NA	NA
T2	7.90±0.30^{a}	7.90±0.30^{a}	7.4±0.49^{b}	6.80±0.40^{a}	6.50±0.50^{a}	NA	NA
T3	7.90±0.30^{a}	7.90±0.30^{a}	7.6±0.49^{b}	7.30±0.46^{ab}	7.30±0.46^{b}	6.3±0.46^{a}	NA
T4	7.90±0.30^{a}	7.90±0.30^{a}	7.6±0.49^{b}	7.30±0.46^{ab}	7.30±0.46^{b}	6.3±0.46^{a}	NA
T5	7.90±0.30^{a}	7.90±0.30^{a}	7.7±0.46^{b}	7.40±0.49^{ab}	7.40±0.60^{b}	6.5±0.50^{a}	NA
T6	7.90±0.30^{a}	7.90±0.30^{a}	7.8±0.50^{b}	7.80±0.60^{b}	7.80±0.49^{b}	7.4±0.49^{b}	7.0±0.00
T7	7.90±0.30^{a}	7.90±0.30^{a}	7.5±0.60^{b}	7.30±0.46^{ab}	7.30±0.46^{b}	6.3±0.46^{a}	NA
T8	7.90±0.30^{a}	7.90±0.30^{a}	7.6±0.49^{b}	7.40±0.49^{ab}	7.30±0.49^{b}	6.5±0.50^{a}	NA
	Body and texture						
Control	8.40±0.48^{a}	7.90±0.30^{a}	6.3±0.46^{a}	NA	NA	NA	NA
T1	7.90±0.30^{b}	7.80±0.40^{a}	7.4±0.49^{b}	6.70±0.46^{a}	6.00±0.00^{a}	NA	NA
T2	7.90±0.30^{b}	7.90±0.40^{a}	7.4±0.49^{b}	6.70±0.46^{a}	6.00±0.00^{a}	NA	NA
T3	7.90±0.30^{b}	7.80±0.30^{a}	7.5±0.50^{b}	7.20±0.40^{ab}	6.80±0.40^{b}	6.1±0.30^{a}	NA
T4	7.90±0.30^{b}	7.90±0.30^{a}	7.5±0.50^{b}	7.20±0.40^{ab}	6.80±0.40^{b}	6.0±0.00^{a}	NA
T5	7.90±0.30^{b}	7.90±0.30^{a}	7.6±0.49^{b}	7.40±0.49^{b}	6.90±0.00^{b}	6.3±0.46^{ab}	NA
T6	7.90±0.30^{b}	7.90±0.30^{a}	7.8±0.50^{b}	7.60±0.49^{b}	7.00±0.30^{b}	6.8±0.40^{b}	6.5±0.50
T7	7.90±0.30^{b}	7.90±0.30^{a}	7.5±0.40^{b}	7.20±0.40^{ab}	6.80±0.40^{b}	6.1±0.30^{a}	NA
T8	7.90±0.30^{b}	7.90±0.30^{a}	7.6±0.49^{b}	7.40±0.49^{b}	6.90±0.30^{b}	6.3±0.46^{ab}	NA
	Flavor						
Control	7.80±0.40^{a}	6.50±0.50^{a}	5.7±0.46^{a}	NA	NA	NA	NA
T1	7.60±0.48^{a}	7.60±0.49^{b}	7.4±0.49^{b}	6.70±0.64^{a}	6.22±0.46^{a}	NA	NA
T2	7.60±0.48^{a}	7.80±0.49^{b}	7.4±0.49^{b}	6.70±0.64^{a}	6.22±0.46^{a}	NA	NA
T3	7.80±0.40^{a}	7.60±0.40^{b}	7.6±0.49^{b}	7.20±0.40^{ab}	7.00±0.00^{b}	6.3±0.46^{a}	NA
T4	7.60±0.48^{a}	7.60±0.49^{b}	7.6±0.49^{b}	7.20±0.40^{ab}	7.00±0.00^{b}	6.3±0.46^{a}	NA
T5	7.70±0.45^{a}	7.70±0.46^{b}	7.7±0.46^{b}	7.20±0.40^{ab}	7.11±0.50^{bc}	6.4±0.49^{a}	NA
T6	7.80±0.40^{a}	7.80±0.40^{b}	7.8±0.50^{b}	7.70±0.64^{b}	7.44±0.30^{c}	7.1±0.30^{b}	6.8±0.40
T7	7.60±0.48^{a}	7.60±0.49^{b}	7.5±0.60^{b}	7.20±0.40^{ab}	7.00±0.00^{b}	6.3±0.46^{a}	NA
T8	7.80±0.45^{a}	7.70±0.46^{b}	7.6±0.49^{b}	7.20±0.40^{ab}	7.00±0.30^{bc}	6.4±0.49^{a}	NA
	Overall acceptability						
Control	8.30±0.45^{a}	6.60±0.49^{a}	5.8±0.40^{a}	NA	NA	NA	NA
T1	7.60±0.48^{b}	7.60±0.49^{b}	7.4±0.49^{b}	6.80±0.40^{a}	6.00±0.00^{a}	NA	NA
T2	7.60±0.48^{b}	7.80±0.49^{b}	7.4±0.49^{b}	6.80±0.40^{a}	6.00±0.00^{a}	NA	NA
T3	7.80±0.40^{ab}	7.60±0.40^{b}	7.6±0.49^{b}	7.50±0.50^{ab}	7.10±0.30^{b}	6.3±0.46^{a}	NA
T4	7.70±0.45^{ab}	7.70±0.46^{b}	7.6±0.49^{b}	7.50±0.50^{ab}	7.10±0.30^{b}	6.3±0.46^{a}	NA
T5	7.70±0.45^{ab}	7.70±0.46^{b}	7.7±0.46^{b}	7.60±0.49^{b}	7.30±0.64^{bc}	6.5±0.50^{a}	NA
T6	7.80±0.40^{ab}	7.80±0.40^{b}	7.8±0.50^{b}	7.80±0.60^{b}	7.70±0.46^{c}	7.2±0.40^{b}	7±0.45
T7	7.70±0.45^{ab}	7.70±0.46^{b}	7.5±0.60^{b}	7.50±0.50^{ab}	7.10±0.30^{b}	6.3±0.46^{a}	NA
T8	7.70±0.45^{ab}	7.70±0.46^{b}	7.5±0.49^{b}	7.60±0.49^{b}	7.10±0.46^{bc}	6.5±0.50^{a}	NA

Values represent means± standard deviation. Different alphabets (a-c) in superscript represent significant difference between different samples in same day of storage.

Appendix D

ANOVA for moisture content of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coated paneer during storage

Table D.1 Tests of between-subjects effects for dependent variable % moisture

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	88.839[a]	62	1.433	8012.276	.000
Intercept	548816.301	1	548816.301	3068824877.281	.000
Day	74.445	6	12.408	69379.229	.000
Treatment	8.826	8	1.103	6168.874	.000
Day * Treatment	5.568	48	.116	648.641	.000
Error	.023	126	.000		
Total	548905.162	189			
Corrected Total	88.861	188			

Table D.2 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on moisture content of paneer.

Treatment	Storage days						
	0	4	8	12	16	20	24
Control	54.42±0.00[a]	54.16±0.02[a]	53.87±0.01[a]	53.46±0.02a	52.93±0.01[a]	52.35±0.00[a]	51.74±0.01[a]
T1	54.43±0.02[a]	54.33±0.02[b]	54.02±0.01[b]	53.92±0.01[b]	53.67±0.01b	53.38±0.01[b]	53.03±0.01[bc]
T2	54.42±0.01[a]	54.32±0.01[b]	54.02±0.01[b]	53.91±0.01[b]	53.63±0.01c	53.37±0.01[b]	53.05±0.00[b]
T3	54.43±0.02[a]	54.33±0.02[b]	54.03±0.01[b]	53.92±0.01[b]	53.62±0.01c	53.36±0.01[b]	53.03±0.01[bc]
T4	54.44±0.01[a]	54.34±0.01[b]	54.04±0.01[b]	53.93±0.00[b]	53.63±0.00bc	53.38±0.01[b]	53.02±0.01[c]
T5	54.43±0.01[a]	54.33±0.01[b]	54.03±0.01[b]	53.91±0.01[b]	53.66±0.01bc	53.37±0.00[b]	53.02±0.00[c]
T6	54.42±0.01[a]	54.32±0.01[b]	54.02±0.01[b]	53.93±0.00[b]	53.63±0.01bc	53.37±0.01[b]	53.01±0.00[c]
T7	54.42±0.02[a]	54.32±0.02[b]	54.02±0.01[b]	53.92±0.00[b]	53.63±0.01bc	53.38±0.00[b]	53.03±0.00[bc]
T8	54.44±0.01[a]	54.34±0.00[b]	54.03±0.01[b]	53.93±0.01[b]	53.66±0.02bc	53.37±0.01[b]	53.01±0.00[c]

Values represent means± standard deviation. Different alphabets (a-c) in superscript represent significant difference between different samples in same day of storage.

Appendix E

ANOVA for FFA of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coated paneer paneer during storage

Table E.1 Tests of between-subjects effects dependent variable FFA

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.502[a]	62	.266	4146.449	.000
Intercept	41.061	1	41.061	639676.297	.000
Day	7.877	6	1.313	20451.068	.000
Treatment	5.588	8	.698	10881.417	.000
Day * Treatment	3.038	48	.063	985.877	.000
Error	.008	126	6.419E-005		
Total	57.571	189			
Corrected Total	16.510	188			

Table E.2 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on FFA of paneer

Treatment	Days						
	0	4	8	12	16	20	24
Control	0.229±0.003[a]	0.391±0.006[a]	0.588±0.003[a]	0.899±0.007[a]	1.133±0.026[a]	1.473±0.024[a]	1.860±0.016[a]
T1	0.228±0.004[a]	0.236±0.000[b]	0.337±0.002[b]	0.433±0.001[b]	0.57±0.002[b]	0.63±0.001[b]	0.86±0.003[b]
T2	0.226±0.004[a]	0.231±0.002[bc]	0.331±0.003[bc]	0.415±0.001[b]	0.55±0.001[b]	0.61±0.004[bc]	0.79±0.001[c]
T3	0.225±0.004[a]	0.228±0.005[bc]	0.330±0.003[c]	0.386±0.001[bcd]	0.452±0.005[c]	0.579±0.001[d]	0.701±0.006[de]
T4	0.222±0.001[a]	0.229±0.003[bc]	0.327±0.001[c]	0.380±0.006[de]	0.459±0.001[c]	0.586±0.001[cd]	0.716±0.001[d]
T5	0.223±0.002[a]	0.224±0.003[c]	0.281±0.001[d]	0.347±0.023[f]	0.415±0.001[d]	0.476±0.000[e]	0.68±0.004[e]
T6	0.223±0.001[a]	0.223±0.002[c]	0.277±0.001[d]	0.315±0.001[g]	0.373±0.002[e]	0.419±0.002[f]	0.482±0.002[f]
T7	0.229±0.001[a]	0.231±0.002[bc]	0.327±0.001[c]	0.380±0.005[de]	0.461±0.001[c]	0.585±0.004[d]	0.715±0.004[d]
T8	0.226±0.004[a]	0.227±0.003[bc]	0.279±0.001[d]	0.352±0.000[ef]	0.416±0.002[d]	0.474±0.001[e]	0.640±0.007[g]

Values represent means± standard deviation. Different alphabets (a-c) in superscript represent significant difference between different samples in same day of storage.

Appendix F

ANOVA for acidity of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate paneer during storage

Table F.1 Tests of between-subjects effects for dependent variable acidity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	16.414[a]	62	.265	1234.414	.000
Intercept	50.315	1	50.315	234607.127	.000
Day	6.010	6	1.002	4670.871	.000
Treatment	7.534	8	.942	4391.256	.000
Day * Treatment	2.869	48	.060	278.716	.000
Error	.027	126	.000		
Total	66.756	189			
Corrected Total	16.441	188			

Table F.2 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on acidity of paneer

Treatment	Days						
	0	4	8	12	16	20	24
Control	0.303±0.002[a]	0.493±0.005[a]	1.013±0.005[a]	1.160±0.014[a]	1.243±0.021[a]	1.457±0.054[a]	1.827±0.031[a]
T1	0.302±0.000[a]	0.347±0.001[bc]	0.387±0.021[b]	0.433±0.001[bc]	0.482±0.002[b]	0.620±0.008[bc]	0.897±0.012[b]
T2	0.302±0.003[a]	0.345±0.004[bc]	0.366±0.003[bc]	0.437±0.002[c]	0.473±0.002[b]	0.647±0.037[c]	0.903±0.019[b]
T3	0.303±0.001[a]	0.349±0.002[bc]	0.329±0.003[d]	0.406±0.001[d]	0.446±0.003[c]	0.553±0.012[bd]	0.817±0.005[c]
T4	0.304±0.002[a]	0.352±0.002[c]	0.352±0.003[cde]	0.414±0.000[bd]	0.439±0.007[c]	0.557±0.029[bd]	0.790±0.008[c]
T5	0.300±0.001[a]	0.347±0.000[bc]	0.360±0.001[ce]	0.351±0.007[e]	0.395±0.003[d]	0.479±0.001[e]	0.700±0.008[d]
T6	0.300±0.001[a]	0.338±0.002[bd]	0.339±0.001[de]	0.346±0.004[e]	0.351±0.003[e]	0.443±0.002[e]	0.491±0.007[e]
T7	0.308±0.002[a]	0.332±0.006[d]	0.349±0.002[cde]	0.447±0.003[c]	0.439±0.002[c]	0.560±0.014[bd]	0.777±0.009[c]
T8	0.302±0.001[a]	0.348±0.002[bc]	0.340±0.001[de]	0.395±0.003[d]	0.396±0.003[d]	0.457±0.005[e]	0.670±0.016[d]

Values represent means± standard deviation. Different alphabets (a-e) in superscript represent significant difference between different samples in same day of storage.

Appendix G

ANOVA for tyrosine release of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coated paneer

Table G.1 Tests of between-subjects effects dependent variable: Tyrosine release mg/100g

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	90702.628[a]	14	6478.759	45.678	.000
Intercept	176786.775	1	176786.775	1246.409	.000
Day	53487.925	6	8914.654	62.851	.000
Treatment	37214.703	8	4651.838	32.797	.000
Error	24679.619	174	141.837		
Total	292169.023	189			
Corrected Total	115382.248	188			

Table G.2 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on tyrosine release (mg/100g) of paneer samples

Treatment	Days						
	0	4	8	12	16	20	24
Control	10.97±6.3[a]	24.34±3.7[a]	37.84±173[a]	51.52±7.24[a]	91.09±4.11[a]	113.05±7.78[a]	149.97±5.81[a]
T1	10.90±4.12[a]	15.69±3.19[b]	19.42±4.042[b]	27.68±4.84[bd]	37.67±2.85[b]	51.71±4.46[b]	60.97±5.20[b]
T2	10.80±6.11[a]	13.65±4.06[c]	18.41±6.06[c]	24.53±4.07[c]	35.73±3.80[c]	49.16±5.49[b]	58.87±4.65[c]
T3	10.97±5.01[a]	12.30±2.99[d]	17.35±5.01[d]	24.35±5.029[c]	29.82±2.76[d]	39.19±3.63[c]	54.29±5.19[d]
T4	10.83±4.12[a]	12.97±3.00[dc]	16.11±3.05[e]	28.36±3.03[d]	38.16±5.76[b]	43.21±3.91[d]	55.92±4.06[e]
T5	10.94±4.00[a]	11.25±4.04[e]	14.71±5.23[f]	21.96±4.44[e]	24.37±4.49[e]	31.47±5.22[e]	35.17±4.10[f]
T6	10.75±4.12[a]	11.14±6.27[e]	13.26±3.81[g]	16.98±3.10[f]	19.52±5.07[f]	23.81±3.94[f]	28.98±4.87[g]
T7	10.91±5.08[a]	12.96±5.14[d]	17.51±4.13[d]	26.38±3.22[b]	29.68±6.02[d]	37.87±3.02[cg]	52.90±4.29[h]
T8	10.90±6.08[a]	11.40±4.09[c]	15.05±3.09[f]	19.73±3.44[g]	24.51±5.07[e]	35.98±4.39[g]	40.49±5.18[i]

Values represent means± standard deviation. Different alphabets (a-c) in superscript represent significant difference between different samples in same day of storage.

Appendix H

ANOVA for total plate count of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coated paneer

Table H.1 Tests of between-subjects effects on dependent variable: TPC (log cfu/g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	189.591[a]	51	3.717	17261.229	.000
Intercept	2577.765	1	2577.765	11969264.201	.000
Day	166.910	6	27.818	129168.067	.000
Treatment	22.660	8	2.832	13152.039	.000
Day * Treatment	16.339	37	.442	2050.446	.000
Error	.022	104	.000		
Total	3217.928	156			
Corrected Total	189.613	155			

Table H.2 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on total plate count (log cfu/g) content of paneer.

	Days						
Treatment	0	4	8	12	16	20	24
Control	3.81±0.01a	4.67±0.02a	6.64±0.01a	TMTC	TMTC	TMTC	TMTC
T1	3.64±0.00b	3.67±0.02b	3.48±0.02b	4.83±0.00a	4.92±0.00a	5.82±0.01a	TMTC
T2	3.63±0.01b	3.66±0.02bc	3.45±0.01bc	4.74±0.01a	4.81±0.01b	5.71±0.00b	TMTC
T3	3.51±0.00c	3.63±0.01bcd	3.41±0.02c	4.30±0.00b	4.82±0.01b	5.21±0.00c	TMTC
T4	3.53±0.01c	3.63±0.01bcd	3.43±0.01bc	4.38±0.02b	4.87±0.00c	5.44±0.01d	TMTC
T5	3.54±0.01c	3.63±0.01bcd	3.44±0.01bc	4.31±0.02b	4.81±0.00b	5.41±0.00c	TMTC
T6	3.51±0.01c	3.60±0.01d	3.41±0.02c	4.12±0.11c	4.52±0.02d	4.73±0.02e	4.97±0.11
T7	3.52±0.00c	3.62±0.02bcd	3.42±0.03c	4.33±0.02b	4.86±0.01c	5.41±0.00c	TMTC
T8	3.52±0.00c	3.60±0.01cd	3.42±0.01c	4.31±0.01b	4.82±0.00b	5.38±0.00c	TMTC

Values represent means± standard deviation. Different alphabets (a-e) in superscript represent significant difference between different samples in same day of storage.

Appendix I

ANOVA for yeast and mold count of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coated paneer

Table I.1 Tests of Between-Subjects Effects dependent variable yeast and mold (log cfu/g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	125.826[a]	51	2.467	3645.202	.000
Intercept	1306.007	1	1306.007	1929600.953	.000
Day	118.095	6	19.682	29080.457	.000
Treatment	10.243	8	1.280	1891.778	.000
Day * Treatment	6.588	37	.178	263.059	.000
Error	.070	104	.001		
Total	1631.841	156			
Corrected Total	125.896	155			

Table I.2 Effect of *Cinnamomun verum* and *Zanthoxylum armatum* essential oil incorporated Na-alginate coating on yeast and molds count (log cfu/g) content of paneer

Treatment	Days						
	0	4	8	12	16	20	24
Control	2.23±0.016[a]	3.47±0.009[a]	4.20±0.005[a]	VMG			
T1	2.20±0.005[a]	2.20±0.005[b]	2.59±0.009[b]	3.23±0.017[a]	4.32±0.012[a]	4.62±0.024[a]	TMTC
T2	2.21±0.008[a]	2.21±0.008[b]	2.51±0.005[c]	3.15±0.025[ab]	4.24±0.009[b]	4.53±0.024[b]	TMTC
T3	2.20±0.004[a]	2.20±0.004[b]	2.48±0.005[d]	2.95±0.080[c]	4.15±0.000[c]	4.45±0.012[c]	TMTC
T4	2.23±0.016[a]	2.23±0.016[b]	2.51±0.012[c]	3.12±0.032[abd]	4.31±0.014[a]	4.53±0.024[b]	TMTC
T5	2.21±0.012[a]	2.21±0.012[b]	2.42±0.005[e]	3.01±0.041[bcd]	4.13±0.024[c]	4.37±0.024[d]	TMTC
T6	2.20±0.002[a]	2.20±0.002[b]	2.40±0.005[e]	2.78±0.058[e]	3.22±0.024[d]	3.81±0.009[e]	4.10±0.00
T7	2.23±0.016[a]	2.23±0.016[b]	2.51±0.012[c]	3.05±0.037[bcd]	4.32±0.012[a]	4.54±0.017[b]	TMTC
T8	2.21±0.007[a]	2.21±0.007[b]	2.42±0.005[e]	2.99±0.022[dc]	4.12±0.024[c]	4.38±0.024[dc]	TMTC

Values represent means± standard deviation. Different alphabets (a-e) in superscript represent significant difference between different samples in same day of storage

Appendix J

Date:…/……/….

Sensory evaluation score card

Hedonic rating scale

Name:……

Product: Paneer

Please test the samples and check how much you like or dislike and give appropriate scale to show your attitude by giving the point that best describes yours feeling about the samples.

Like extremely	9	Dislike slightly	4
Like very much	8	Dislike moderately	3
Like moderately	7	Dislike very much	2
Like slightly	6	Dislike extremely	1
Neither like nor dislike	5		

Samples	Sensory Attributes			
	Body and texture	Flavor	Color and appearance	Overall Acceptability
Control				
T1				
T2				
T3				
T4				
T5				
T6				
T7				
T8				

Comments:

Signature:……

Appendix K

Calibration curves for the determination of total phenol and tyrosine content

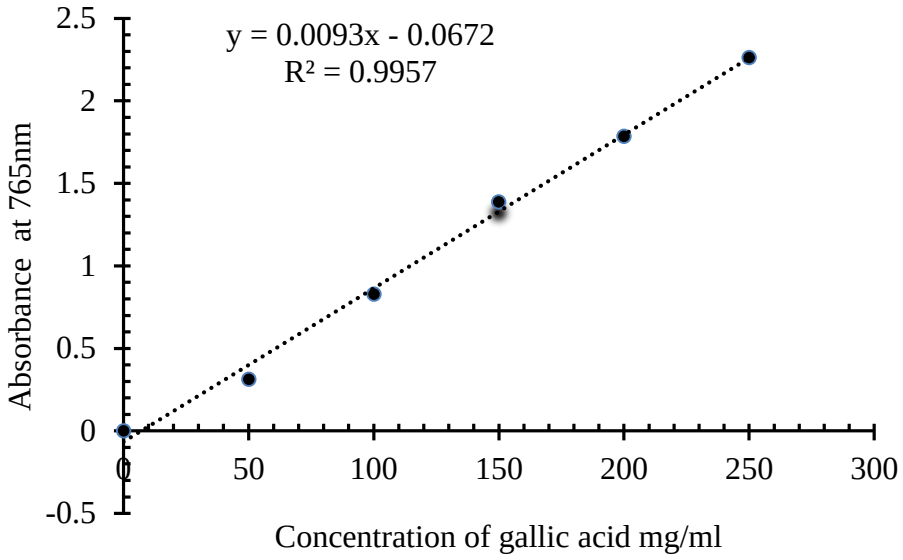


Fig. K.1 Calibration curve for total phenol contents

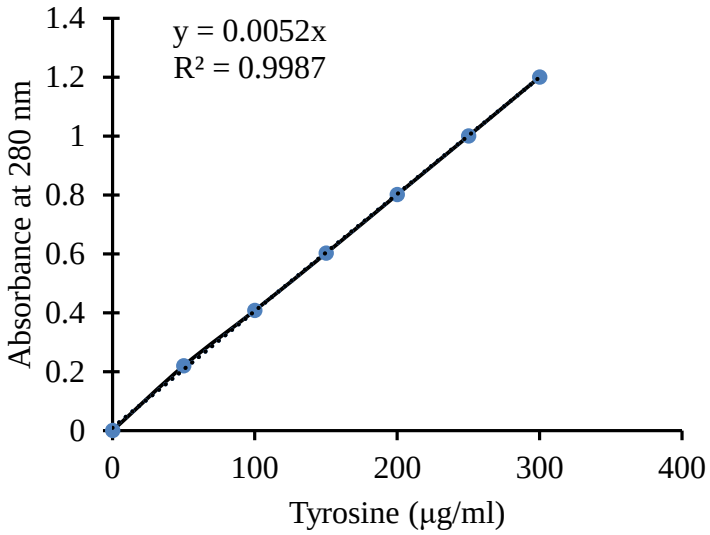


Fig. K.2 Calibration curve for tyrosine release

Appendix L

Absorbance versus concentration curve of radical scavenging activity of essential oils

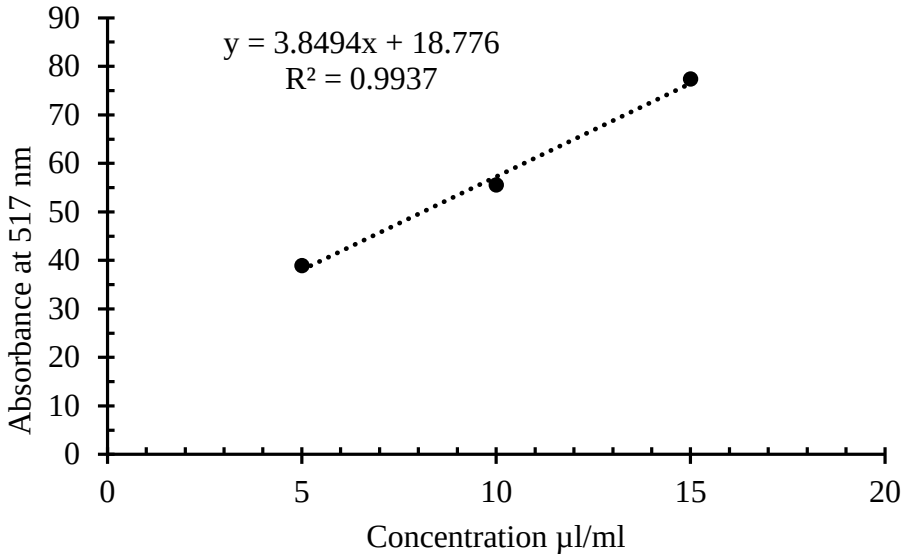


Fig. L.1 Radical savenging activity of *Zanthoxylum armatum* essential oil

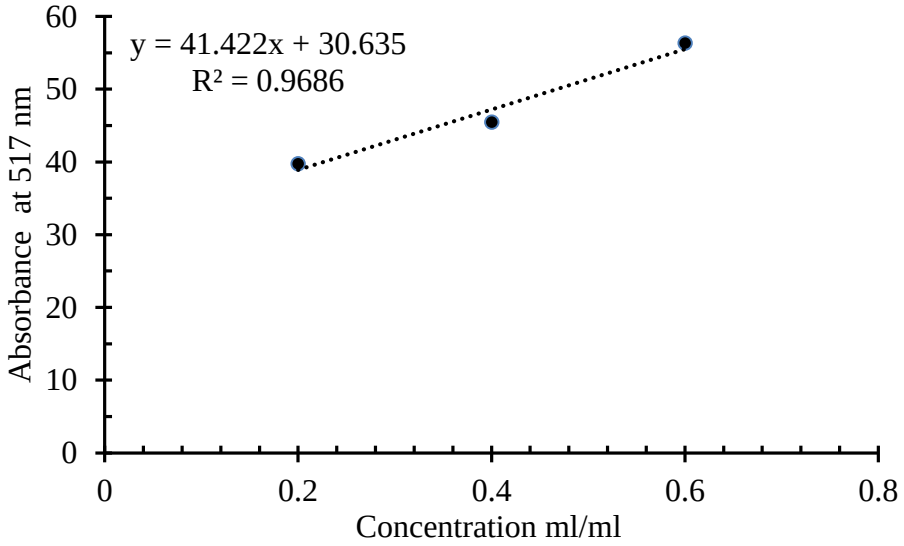


Fig. L.2 Radical scavenging activity of *Cinnamomun verum* essential oil

Appendix M

Table M.1 Cost evaluation essential oil coated paneer

Ingredients	Rate	Quantity	Cost (NRs)
Milk	70/L	2L	140
Citric acid	900/kg	2g	1.8
$CaCl_2$	1440/kg	2g	2.88
Total cost			144.68
Final cost with 20% overhead			173.61
Product prepared			333g
Price per kg			521.35
Zanthoxylum armatum EO	21000/L	1.74ml	38.01
Cinnamomum verum EO	16000/L	0.26ml	4.16
Na- alginate	3600/kg	1.77g	6.372
Total cost of coated paneer			569.89/kg

Appendix N

List of chemicals and equipment

Table N.1 List of major chemical used

Chemicals	Brand	Manufacturer	Purity
Petroleum ether	Fisher	Thermo Fisher Scientific India Pvt. Ltd. 403-404,B-Wing, Mumbai, India	98.08%
Conc. H_2SO4	Fisher	Thermo Fisher Scientific India Pvt. Ltd. 403-404,B-Wing, Mumbai, India	98.00%
Conc. HCL	EMPLURA	Merck Life Science Pvt. Ltd., Godraj One Plrojstyenagar, Mumbai, India	99.00%
Conc. HNO_3	Fisher	Thermo Fisher Scientific India Pvt. Ltd. 403-404,B-Wing, Mumbai, India	98.00%
NaOH	Fisher	Thermo Fisher Scientific India Pvt. Ltd. 403-404,B-Wing, Mumbai, India	97.00%
Catalytic mixture (K_2SO_4+$CuSO_4$)	FOSS	FOSS Analytical A/S, 1 Foss Alle, DK-3400 Hilleroad, UK	97.70%
Boric acid	Fisher	Thermo Fisher Scientific India Pvt. Ltd. 403-404,B-Wing, Mumbai, India	97.00%
DPPH	Fisher	Sigma-Aldrich Inc	97.00%
Folin-ciocalteu reagent	Fisher	Fischer Scientific, India	2N
Gallic acid	Himedia	HiMedia Laboratories Pvt. Ltd.	98 %
Trichliro acetic acid	Himedia	HiMedia Laboratories Pvt. Ltd.	98-102%
Ethyl alcohol	Fisher	CSC Changshu Hongsheng fine chemical co. Ltd	99%
Gycerol	Fisher	Fischer Scientific, India	99%
L- Tyrosine	Himedia	HiMedia Laboratories Pvt. Ltd.	99-101%
Diethyl ether	Qualigens	Qualigens fine chemicals	98%
Sodium carbonate	Fisher	Thermo Fisher scientific, India	99.9%
Potato dextrose agar	Himedia	HiMedia Laboratories Pvt. Ltd.	MH096
Standard plate count agar	Himedia	HiMedia Laboratories Pvt. Ltd.	M091
Voilet red bile agar	Himedia	HiMedia Laboratories Pvt. Ltd.	M049

Appendix O

List of equipment

Table O.1 List of major equipment used

Name of equipment	Model	Specifications
UV-Vis spectrophotometer	Carry 60, Agilent Technologies	190–1100 nm wavelength; 1.5 nm spectral bandwidth
Automatic Kjeldahl N_2 determination set	ATN-300	Range: 0.1mg – 240 mg N (Nitrogen content 0.02%-95%) Recovery:>99.5% Distillation rate:3-7 min/sample
Digital electronic balance	AS-220-R2	Max.220g-Min.10mg
Muffle furnace	LT-15112/B410	Min 30ºC-3000ºC
Refrigerator	GL-B24RPHG	LG
Incubator	ST3 COMF	230V/50Hz/170W R134a

Appendix P

Color plates

Plate P.1 Calcium chloride added paneer ready for sensory evaluation

Plate P.2 Sodium alginate edible coating incorporated with *Zanthoxylum armatum* and *Cinnamomum verum* essential oil mixtures

Plate P.3 Chemical analysis of paneer sample

Plate P.4 Sample ready for microbial analysis

Plate P.5 Performing microbial analysis of paneer samples

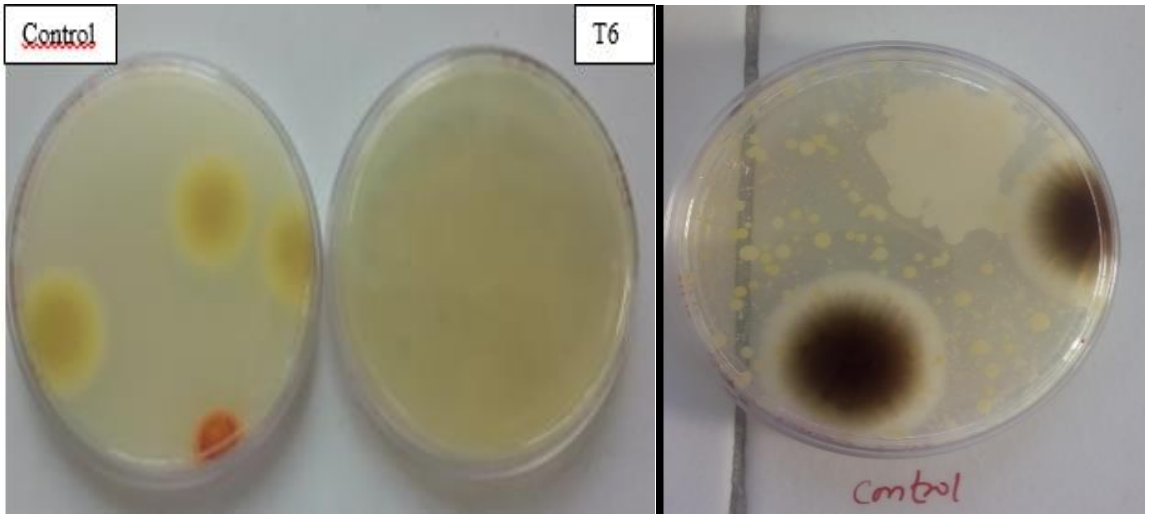


Plate P.6 Visible microbial growth on control sample

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