EFFECT OF HERBAL EXTRACT ON THE SHELF LIFE OF PANEER

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A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

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Approval Letter

This ***dissertation*** entitled ***Effect of Herbal Extract on the Shelf Life of Paneer*** presented by **Bhawana Khadka** has been accepted as the partial fulfillment of the requirement for **B. Tech. degree in Food Technology**

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Abstract

This study was carried out with objectives to extend the shelf life of paneer with addition of herbal extract during the paneer preparations. Paneer samples was prepared by incorporating cloves (*Syzygium aromaticum*) and *pakhanbedh (Bergenia ciliata)* extract mixture each at the rate of 0 to 0.6% of the expected yield of paneer at two different stages of paneer preparation i.e, on milk directly after heat treatment and on curd after 90% drainage of whey and samples were stored at 7±1°C. The changes in sensory characteristics and chemical properties of the treated paneer sample compared to control paneer samples prepared without addition of herbal extracts were studied over the storage periods.

Result showed that the prepared herbal extract exhibited a high antioxidant activity of clove (87.78%) and *pakhanbedh* (89.7%). Total phenolic content was higher in *pakhanbedh* extract than in clove extract. Storage days had significant effect on the sensory and physicochemical properties of paneer. Sensory scores of the control paneer declined rapidly during the storage days and became unacceptable after 5th day of storage. Decrease in sensory scores of the herb extract treated samples showed slower rate in comparison of control samples. The acidity (% lactic acid), FFA (% oleic acid), tyrosine content (mg/100 g) and total plate counts (cfu/g) of herbs treated samples increased at a slower rate in comparison to control samples. The samples of stored paneer obtained in case of herb extract added after heating of milk scored higher. All samples of paneer treated with herbal extract on milk remained acceptable up to 15 days on storage at 7±1°C. Treatment with 0.45% clove and 0.15% *pakhanbedh* extract was found to decrease the sensory and chemical characteristics slower than the other treatments. The yield of paneer was found to be affected by the herb extract addition. % yield increased on the samples treated with herb extract directly on milk and moisture retention was found to increase on addition of herb.

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List of abbreviations

Abbreviations	Full form
BIS	Bureau of Indian Standards
CFU	Colony forming unit
DOE	Design of experiment
FAO	Food and Agricultural Organization
FFA	Free fatty acid
GC-MS	Gas chromatography mass spectrometry
NDDB	National Dairy Development Board
SPC	Standard plate count
TBHQ	Tert-butyl hydroquinone

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PART I

Introduction

1.1 General introduction

Paneer is a South Asian variety of soft cheese obtained by acid coagulation of milk, entrapping all the fat, casein complexed with denatured whey protein and a portion of salts and lactose. It is a non-fermentative, non-renneted, non-melting and un-ripened type of cheese (Khatkar *et al.*, 2017b).

Due to high moisture content as well as nutrients of paneer, it is very prone to microbial spoilage and subsequent biochemical deterioration. The spoilage of paneer is faster at room temperature where the activities of microorganisms are optimum. Several attempts such as use of antimicrobial agents, chemical preservatives, paraffining, deep fat frying, dipping of paneer in treated water, modified atmosphere packaging, low temperature preservation, application of hurdle technology have been made in order to increase the shelf life of paneer (Rajarshibhai, 2012). But at present, consumers are more interested in having foods that are natural or close to natural, minimally processed and free of chemical preservatives (Chauhan *et al.*, 2012). Currently, addition of herb or their extracts and essential oil is widely accepted methods in food preservation and this trend of application can also be applied in paneer to extend its shelf life.

Natural food preservation method refers to application of naturally produced antimicrobial compounds that are obtained from plants, animals and microbes to prevent food spoilages microorganism, proliferation and growth of food borne pathogens in food and foods products. Application of these natural antimicrobial agents is a subject of growing interest for many researchers as a safe replacement for chemical and physical food preservatives which have many side effects and causes health risks to the consumers. Antimicrobial compounds present in foods can also extend shelf-life of unprocessed or processed foods by reducing microbial growth rate or viability (Beuchat and Golden, 1989). 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).

Syzygium aromaticum, commonly known as clove, is a rich sources of antioxidants and antimicrobial compound, and can have an enormous potential for a food preservative. Clove buds is reported to have antibacterial, antifungal, antioxidant, antitumor, anti-inflammatory, insecticidal and flavor imparting characteristics (Ishaq *et al.*, 2019). Similarly, clove represents considerable antioxidant properties. El-Maati *et al.* (2016) studied phenolic extracts of clove for their antioxidant and antimicrobial properties, and concluded that clove extract can be used in food and pharmaceutical products as natural antimicrobial or antioxidant agent.

Bergenia ciliata, commonly known as *pakhanbedh* and a medicinal plant used for the treatment of diarrhea, vomiting, fever, cough, diabetes, cancer, pulmonary disorders and wound healing, is a potential antimicrobial and exhibits a high antioxidant activity (Zafar *et al.*, 2019). Thus, the present study is focused on the utilization of natural herbs cloves and *pakhanbedh* as preservative in paneer.

1.2 Statement of the problem

Paneer is a highly nutritious food containing a good amount of fat and protein. Because of high protein and fat content, and easy availability of the raw material (i.e. milk), paneer can be a good alternative to the meat protein. It is also becoming a great animal protein supplement for vegetarians. Despite being highly nutritious, short shelf life of paneer is a major problem. Paneer, like other indigenous product, is a highly perishable product and suffers from limited shelf life, largely because of its high moisture content (Arora and Gupta, 1980). Its shelf life is reported to be only six days under refrigeration, though its freshness is lost within three days (Bhattacharya *et al.*, 1971). The spoilage of paneer occurs mainly due to the growth of microorganisms, which bring about numerous physico-chemical changes leading to the development of off flavor in the product.

Various methods for increasing shelf life of paneer have been made. Food additives such as sorbic acid, potassium sorbate, solutions of hydrogen peroxide (H_2O_2) and brine, and delvocid have been tried successfully to increase the shelf-life of paneer. Antioxidants like tert-butyl hydroquinone (TBHQ) and butylated hydroxyl anisole (BHA) have also been tried

as possible preserving agents in paneer. There has been increasing concern of the consumers about foods free of chemical preservatives because of their possible toxic effect in human beings. Thus, considering the above facts, the present research is designed to develop herbal paneer using clove and *pakhanbedh* and effect of their addition on paneer was noted.

1.3 Objective of the study

1.3.1 General objective

To study the effect of addition of clove and *pakhanbedh* extract as natural antimicrobial and antioxidant source to enhance shelf life of paneer.

1.3.2 Specific objectives

The specific objectives were as follows:

1. To obtain ethanolic extract of clove and *pakhanbedh* and determine its total phenols and antioxidant activity.
2. To study the effect of herb extract on sensory, chemical and microbiological quality of paneer over storage days compared to control.
3. To study the effect of varying formulations of herb extracts on the sensory, chemical and microbiological quality of paneer.
4. To estimate the shelf life of herb extract treated paneer.

1.4 Significance of the study

This study will facilitate the promotion of use of natural ingredients in paneer to extend its shelf life. This might widen the possibilities for production and distribution of paneer as well as the subsequent utilization of natural herbs. This will also appeal the health sensitive consumers who tend to choose food products with natural ingredients rather than chemically synthesized compounds in their food. Using natural herbs as source of preservative will add value to the compound and would also get a significant market value along with health benefit, we can draw from it. Cloves and *pakhanbedh* are rich in phenols and thus act as antioxidant in our body and apart from this, the antimicrobial property of the herbs work hand in hand to replace or reduce the synthetic preservatives thus assuring food safety. Since cloves and *pakhanbedh* are available locally in Nepal, on one hand their use could be cheap alternatives for preservation of paneer while on the other hand the use of such indigenous herbs would be promoted.

1.5 Limitations

1. Commercially available pre-standardized milk was used and the milk was not standardize at the laboratory.
2. Change in sensory and chemical parameters could not be studied on daily basis.
3. Only two herbs were used for study.
4. Proximate composition of clove and *pakhanbedh* used was not determined.

PART II

Literature review

2.1 History of dairy development in Nepal

Dairy development activities in an organized way in Nepal started from 1952 with the establishment of a Yak cheese factory in Langtang of Rasuwa district under Food and Agriculture Organization (FAO) assistance in 1953. In 1954, a Dairy Development Section was established under the Department of Agriculture (DoA) and also a small-scale milk processing plant was started in Tusal, a village of Kavre district. In 1955, a Dairy Development Commission was formed.

The First Five Year Plan (1956-61) stressed on the need to develop a modern dairy industry. Accordingly, in 1956, a Central Dairy Plant, with an average milk processing capacity of 500 L/h was established in Lainchaur, with the financial assistance from New Zealand and technical assistance from FAO. Around the same time, a second mini milk processing plant was established at Kharipati, in Bhaktapur district. The plant started processing of milk and marketing activities from 1958. History of dairy cooperatives dates back to the First Five Year Plan (1956-61) when the dairy cooperatives were formed in Tusal Village of Kavre district.

In earlier days when there were no organized dairies, demand for milk was fulfilled by raising cows/buffaloes by the people themselves or through the direct supply from the professional milk producers. These producers used to go house by house and deliver the required quantity of milk to the households (FAO, 2010).

2.2 Status of dairy industry

Milk production increased from 13888730 metric tons in the fiscal year 2007/08 to 1911239 metric tons in the fiscal year 2016/17. Among this all, 20% goes to market from formal sector and rest 80% is sold in local markets and used for household consumption. Out of the total milk produced, 70% is obtained from buffaloes and 30% comes from cows. The milk production from buffalo is very much seasonal, whereas cow milk is available all around the year. Currently, the availability of milk is 70 L per head per year and the Ministry of

Agriculture and Livestock Development is committed to increase the milk production to reach 91 L per head per person within 3 years' time (MOALD, 2016/17).

Dairy sector contributes to about 9% of the total Gross Domestic Product and 26.8% of the Agricultural GDP (NDDB, 2074/75).

2.3 Socio-economic impacts

Dairy farming is an integral part of rural livelihood which shows the concept of cooperative approach for gaining common goal of farmers. Dairy cooperatives have made the farmers to unite in a group, which has made them more social. Dairy cooperative is a common venue where farmers meet in the morning and evening daily during milk delivery. So regular meeting has provided them opportunity for mutual harmony and sharing their socio-economic impact. Dairy cooperative makes society organized, harmonized and helpful. Dairy cooperative helps to create awareness in health, sanitation, and education to the farmers. The income from the milk and livestock farming has made them culturally changed such as with good housing, hygienic toilet, bio-plant, television and education. Livestock farming specially dairying is backbone of income for the villagers. Animal and animal by-products keep economic value such as animal sale, milk cash, fertilizer, draught, and biogas and broadly speaking, it has socio-economic importance (Chaudhary and Upadhyaya, 2013).

2.4 Paneer

Paneer is a coagulated milk product obtained by heating and acidulation followed by filtration and pressing. According to prevention of Food Adulteration (PFA) rules (1983), paneer means a product obtained from cow's or buffalo's milk or a combination thereof by precipitation with sour milk, lactic acid, or citric acid. It should not contain more than 70% moisture and the milk –fat contents should not be less than 50.0% of the dry matter.

According to Bureau of Indian Standards (BIS)- 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 there of (milk solids, subjected to the approval by the control committee for food standards suitably processed may also be used) (BIS, 1983).

According to Department of Food Technology and Quality Control (DFTQC), Nepal, paneer is a solid milk product prepared out of pasteurized milk obtained from cow or buffalo or both by precipitation using sour milk, lactic acid or citric acid. It can also be prepared

from milk powder. Milk having rancid off flavors or microbial growth and other colors and food additives are not allowed in paneer (DFTQC, 2018). The allocated standards for paneer as per BIS and DFTQC is given in Table 2.1.

Table2.1 Standards for paneer

Parameters	Requirements	
	As per BIS	As per DFTQC
Moisture % by mass (max)	60.00	70.00
Milk fat % by mass on dry matter basis (min)	50.00	50.00
Titratable acidity % lactic acid, (max)	0.50	-
Standard plate count per g (max)	5×10^5	-
Coliform per g (max)	90	-

BIS (1983); DFTQC (2018)

2.5 Quality characteristics of paneer

A good quality paneer must have a characteristic blend of the flavor of heated milk and acid, i.e. pleasant, mildly acidic and sweet (nutty). Its body and texture must be sufficiently firm to hold its shape during cutting/slicing, yet it must be tender enough not to resist crushing during mastication, i.e. the texture must be compact and smooth. Its color and appearance must be uniform, pleasing white, with a greenish tinge in the case of buffalo milk paneer and light yellow in the case of cow milk paneer (Khan and Pal, 2011; Kumar *et al.*, 2011).

A minimum of 5.5% fat in buffalo milk and 4.5% fat in cow milk is necessary for producing a desirable good quality paneer whereas a lower fat level than the above in milk results in a hard body and coarse texture with increased chewiness. The higher fat content in milk is also not desirable since it produces greasiness, softness and weak body and texture in paneer. The higher fat in milk results in more loss of fat in whey. For manufacture of good quality paneer sweet milk (fresh milk) is the best suitable raw material, developed acidity or sour milk tends to produce sour flavor and bitter taste, which makes it unsuitable for preparation of culinary dishes. Acceptable quality paneer could be produced from slightly acidic and neutralized milk. Buffalo milk admixed with sweet butter milk could be utilized

for making acceptable quality paneer having good frying and cooking characteristics. Good quality of paneer has a typical acidic flavor with slightly sweet taste, firm and cohesive body and closely smooth texture (Singh, 2018).

2.6 Methods of manufacture of paneer

2.6.1 Traditional method

Milk is first heated to boil; coagulation is carried out by adding coagulant with stirring. When whey is clear, it is drained by hanging the curd in a cloth and later by pressing the paneer, which is pressed mechanically into blocks in hoops, by putting weights on them (approx. 2-3 kg per sq. cm) for 15-20 min. Thereafter, the paneer is removed and cut into suitable sizes and immersed in chilled water for 3-4 h to make it firm (Sachdeva and Singh, 1988).

2.6.2 Industrial method

A procedure for the manufacture of paneer at pilot plant level was developed by Bhattacharya *et al.* (1971).

An industrial process for the manufacture of paneer has been developed by the NDDB. The milk is heated to 85°C to obtain a co-precipitate through a plate heat exchanger and pumped to a cheese vat and cooled to 75°C. Hot milk is coagulated by adding 1% citric acid solution with proper mixing. The curd is left to settle for 10 to 15 min without agitation. The whey is then drained and the curd heaps are filled in cheese hoops with a muslin cloth and pressed for 10 to 15 min at a pressure of 3 kg per sq. cm to remove the whey. The final blocks are dipped in pasteurized cold water at 4°C for 3 h for cooling and firming the paneer (Rao *et al.*, 1992).

2.7 Factors affecting quality of paneer

2.7.1 Type of milk

For making good quality paneer, buffalo milk is considered more suitable than cow milk because it contains higher concentration of fat, caseins, and minerals (calcium, phosphorus), which impart a firm and rubbery body to buffalo milk paneer (Farkye, 2017; Sachdeva *et al.*, 1985). In addition to this, the lower voluminosity and solvation of casein micelles in buffalo milk gives buffalo milk paneer a spongy texture (Farkye, 2017). On the other hand, cow milk gives soft, weak and fragile product which is considered to be unsuitable for cooking purpose

(Kumar *et al.*, 2011). Similarly, homogenization of cow milk is recommended to bring about improvement in the yield and organoleptic scores of paneer.

Among all of the milk constituents, fat is found to exert the greatest influence on the quality of paneer. Milk with 5% fat is normally required for making a good quality paneer. However, an acceptable quality paneer was made from milk containing 3.5% fat (Chawla *et al.*, 1985) and from buffalo milk containing 6% fat (Kumar *et al.*, 2008). Kasle (1981) found a significant effect of fat content on the yield as well as chemical and sensory qualities of buffalo milk paneer. Buffalo milk containing 4.5% fat was found to be the best one in terms of overall quality of paneer among 1.5%, 3%, 4.5% and 6% fat containing buffalo milk.

Animal udder infection (mastitis) results in higher pH values for fresh milk whereas lower values show bacterial action. The bacterial action disturbs salt balance i.e., causes progressive removal of calcium and phosphates from caseinate phosphate micelle due to which coagulation is faster simply on heating or on addition of small amount of coagulant which directly affects the quality of the final product (Acharya and Katwal, 2002).

2.7.2 Acidity of milk

Milk having acidity within the range of 0.14-0.16% should be accepted for paneer manufacture. The acidity of milk gradually increases as a result of bacterial action on milk lactose (Acharya and Katwal, 2002). According to De *et al.* (1971), acidic milk having a titratable acidity of 0.2-0.23% yields a product with inferior quality and milk with acidity greater than 0.28% yields paneer with unacceptable flavor that cannot be masked even with added flavors.

2.7.3 pH of coagulation of milk

The variation in pH of coagulation also has a significant impact on the body and texture, total solids recovery and yield of paneer (Farkye, 2017). The moisture content and yield of paneer decreases as we lower the coagulation pH from 5.5 to 5.0 (Sachdeva and Singh, 1988). According to Sachdeva and Singh (1988), sensory quality for paneer prepared from buffalo milk was best when coagulation was at pH 5.3-5.35. Similarly, for cow milk, best sensory attributes were reported when coagulation occurred at pH 5.2-5.25 (Sachdeva *et al.*, 1991).

2.7.4 Coagulant

Conventionally, citric acid is used for coagulating hot milk for paneer making. In addition to this, several other coagulants such as lemon juice, tartaric acid, lactic acid, malic acid, hydrochloric acid, phosphoric acid, acetic acid, fermented milk, sour/cultured whey, yoghurt and lactic cultures have been tried (Khan and Pal, 2011). Dongare and Syed (2018) compared the physicochemical and sensory properties of buffalo milk paneer prepared by different coagulants and found that lactic acid coagulated paneer was better that citric acid, tartaric acid and ascorbic acid coagulated paneer in terms of both physicochemical and sensory attributes. In contrast to this, Karadbhajne and Bhoyarkar (2010) found that ascorbic acid provided the best texture properties as well as chemical and organoleptic properties in buffalo milk paneer among citric acid, ascorbic acid, tartaric acid and lactic acid. Similarly, in cow milk, lactic acid was found to give higher yield, total solids and fat content in paneer but the sensory scores were higher for paneer prepared by using citric acid (Shukla, 2006).

2.7.5 Concentration of coagulant

The concentration of coagulant has a profound effect on the body and texture of paneer. Low acid strength results in soft body and smooth texture, while high acid strength results in hard body. The amount of coagulant required for coagulation of milk depends upon the type of milk, buffering capacity of milk, type of coagulant and the coagulation temperature employed (Khan and Pal, 2011).

The concentration of paneer also has a significant impact on yield of paneer. Kumar *et al.* (2008) reported that paneer made out of buffalo milk coagulated with 0.2% lactic acid brought a significant improvement in the yield, moisture and protein content, total solid recovery, flavor, body and texture and overall acceptability of the product as compared to product prepared by coagulating with 0.6% lactic acid. In another study, the yield of paneer obtained from 1% citric acid was highest (14.2%) than other coagulant concentrations. The sensory evaluation results indicated that the product prepared from citric, tartaric and lactic acids at 1% each could be considered the best product (Karadbhajne and Bhoyarkar, 2010). Similarly, percent yield obtained from 1% citric acid was found to be 14.2%, which is higher than 2% and 3% solution whereas in case of tartaric acid and lactic acid, 2% solution was optimized (Acharya and Katwal, 2002).

2.7.6 Coagulation temperature

The optimum temperature of coagulation differs for different types of milk and their composition, including fat. Coagulation temperature influences moisture retention in paneer (Khan and Pal, 2011). An increase in temperature of coagulation from 60ºC to 90°C decreased the moisture content of paneer from 59% to 49%. Paneer obtained by coagulating milk at 70°C had the best organoleptic quality and had desired frying quality namely integrity/shape retention and softness. Temperatures higher than this resulted in dry and hard paneer while lower temperature yielded product having very moist surface (Sachdeva and Singh, 1988).

2.8 Chemical aspects

2.8.1 Chemical composition

Gross composition of paneer and variation observed in raw material and processing conditions tried out for the preparation have been reported by many workers (Bhattacharya *et al.*, 1971; Sachdeva and Singh, 1988; Syed *et al.*, 1992). The variation in the chemical quality is mainly due to the differences in initial composition of milk, methods of manufacture and milk solid losses in paneer whey (Rao *et al.*, 1992).

Paneer as a product usually consists of almost all the fat, insoluble salts and colloidal material together with part of the moisture which contained lactose, whey proteins, soluble salts, vitamins and other milk components. It contains approximately 53-55% moisture, 23-26% fat, 17-18% protein, 2-2.5% carbohydrate and 1.5-2.0% minerals (Kanawjia *et al.*, 1990). Goel (2000) studied that the total solids, fat, protein, lactose and ash content of laboratory made paneer varied from 57.8 to 56.52, 25.0 to 26.0, 23.08 to 27.02, 2.29 to 2.52 and 1.195 to 1.305% respectively. In market samples the contents varied from 55.29 to 56.28, 19.50 to 26.00, 25.19 to 33.27, 2.32 to 2.61 and 1.495 to 1.605% respectively.

Syed *et al.* (1992) indicated the chemical composition of paneer prepared from different sources of milk is narrated in Table 2.2.

2.8.2 Chemical characteristics

Acidity, free fatty acid content and tyrosine content are the important chemical characteristics of paneer which help to monitor keeping quality of paneer.

Table 2.2 Gross chemical composition of paneer from different sources of milk

Sources of milk	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Yield (%)
Skim milk	62.14	4.00	27.48	1.80	9.85
Cow milk	55.26	24.15	18.43	1.60	14.14
Buffalo milk	53.00	28.22	16.42	1.50	18.24

Syed *et al.* (1992)

2.8.2.1 Acidity

Acidity is an important chemical parameter of paneer and it depends upon the acidity of milk used, type and amount of coagulant used, additives used, additional treatments used, length of storage period, etc. So, acidity of paneer collected from market can vary significantly as a result of a variation in quality of raw material, manufacturing process, market storage conditions and storage period. Gohian (1996) studied the market sample of paneer collected from 18 different producers and showed the mean acidity values ranged from 0.45 to 0.74%. Similarly, on analyzing market paneer samples collected from Agra city of India, average acidity was found to vary between 0.38-0.64% (Goyal *et al.*, 2007).

Shanaziya *et al.* (2018) observed a significant effect of the type of coagulant used on acidity of paneer produced. On manufacturing paneer made out of cow milk by different coagulants, highest acidity was of paneer prepared by using citric acid followed by the ones produced by using lime juice, acetic acid and curd. Similarly, Mistry (1988) observed a lower acidity of cow milk paneer prepared by adding calcium sulphate (acidity values 0.457% and 0.464% for 0.02% and 0.05% calcium sulphate respectively) in comparison to control paneer (acidity value 0.475) while higher acidity was reported for paneer incorporated with disodium hydrogen phosphate (acidity values 0.572% and 0.539% for 0.02% and 0.05% disodium hydrogen phosphate respectively). Likewise, studies have revealed lower acidity of fresh paneer samples treated with nisin (Kantilal, 2010), buffered water (Sachdeva and Singh, 1990a), cardamom (Eresam *et al.*, 2015; Rajarshibhai, 2012), turmeric (Buch *et al.*, 2014), cinnamon (Rajarshibhai, 2012) and ginger (Mhatre, 2018) in comparison to control paneer. In contrast, acidity of paneer treated with acidified brine, acidified water, citric acid,

vinegar, lactic acid, cloves, sorbic acid, formalin, etc. for preservation purpose increased the acidity of fresh paneer samples (Gokhale *et al.*, 2016; Jagannath, 2012; Kamble and Seth, 2017; Patel, 2014; Sachdeva and Singh, 1990a). The effect of black pepper on acidity of fresh paneer is unclear as some workers show increase in acidity (Jagannath, 2012) by black pepper addition while some show a decrease in acidity (Eresam *et al.*, 2015).

During storage, microorganisms act on lactose in paneer and produce lactic acid and various related end products because of which there is an increase in titratable acidity level with progressive storage period (Jagannath, 2012). But the rate of increase in acidity during storage is affected by several factors such as storage temperature (Kumar *et al.*, 2019b), use of preservatives (Khatkar *et al.*, 2017a; Singh *et al.*, 2014) and type of packaging (Shrivastava, 2007). Kumar *et al.* (2019b) attempted to study the effect of storage temperature on acidity of paneer and found the rate of increase in acidity of paneer stored at refrigeration temperature (4±1°C) to be significantly lower than that of paneer stored at ambient temperature (25±1°C). Also, immersing freshly prepared paneer in 0.4% clove essential oil containing chilled water for 2-3 h was found to significantly decrease the rate of increase in acidity of paneer as compared to control paneer (Khatkar *et al.*, 2017a). Similarly, use of nisin (Kantilal, 2010), dipping in brine solution (Sachdeva and Singh, 1990a; Upputuri, 2002), treatment with hydrogen peroxide (Sachdeva and Singh, 1990a; Upputuri, 2002), dipping in banana pseudostem juice (Ray, 2008), addition of sorbic acid (Patel, 2014; Upputuri, 2002), black pepper (Eresam *et al.*, 2015; Jagannath, 2012), cloves (Eresam *et al.*, 2015; Jagannath, 2012), cardamom (Eresam *et al.*, 2015; Jagannath, 2012; Rajarshibhai, 2012), cinnamon (Eresam *et al.*, 2015; Rajarshibhai, 2012) and turmeric (Buch *et al.*, 2014; Singh *et al.*, 2014) has also been found to be effective in reducing the rate of increase in acidity of paneer during storage in comparison to control paneer. In addition to these, Krishnakumar (2001) used the indigenous antimicrobial systems, i.e. activated lactoperoxidase system and lysozyme, and found that these systems stemmed the rise in acidity during storage very effectively.

Similarly, the atmosphere inside packaging material also has a significant impact on the acidity during storage of paneer. Shrivastava (2007) compared the use of different modified atmosphere packaging in paneer and found that paneer packed under vacuum, with 100% CO_2, with 100% N_2 and 50% CO_2/50% N_2 had a minimal rise in acidity values in comparison

to control paneer packed with normal atmosphere. Above all, use of 100% CO_2 was found to be the most effective packaging atmosphere for paneer with least rise in acidity.

2.8.2.2 Free fatty acid (FFA)

The extent of lipolysis in fat rich dairy products is generally determined by estimating FFA. Lipolysis of fats takes place during storage and thus the free fatty acid content gradually increases. But the rate of increase in free fatty acid during storage has been found to be influenced by storage temperature (Kumar *et al.*, 2019b), use of preservatives (Jagannath, 2012; Khatkar *et al.*, 2017a), type of packaging (Shrivastava, 2007), etc. Since buffalo milk paneer is generally higher in fat content, higher FFA content is reported in buffalo milk paneer as compared to cow milk paneer (Ray, 2008).

Several natural preservatives such as cloves, cardamom, cinnamon, black pepper, etc. have been found to be effective in decreasing the rate of increase in free fatty acid content. Khatkar *et al.* (2017a) prepared paneer by using 0.4% clove essential oil incorporated chilling water during chilling for paneer manufacture and observed a profound decrease in rate of increase of free fatty acid in comparison to control paneer. While the FFA content of control paneer increased from an initial 0.175 to 0.541 in 5 days, the FFA content of clove treated sample increased from an initial 0.175 to just 0.409 in 10 days. Similar effect have been demonstrated by using other spices and herbs such as black pepper (Eresam *et al.*, 2015; Jagannath, 2012), cardamom (Eresam *et al.*, 2015; Jagannath, 2012; Rajarshibhai, 2012), cinnamon (Eresam *et al.*, 2015; Rajarshibhai, 2012) and turmeric (Buch *et al.*, 2014). In addition to this, dipping freshly prepared paneer in 0.3% banana pseudostem juice solution was also found to significantly reduce the rate of increase in FFA content (Ray, 2008). Some workers have attempted to work on addition of sorbic acid (Patel, 2014; Upputuri, 2002), nisin (Kantilal, 2010), hydrogen peroxide (Upputuri, 2002) and formalin (Kamble and Seth, 2017) in paneer and have found the results to be equally satisfying regarding the suppression of rise in FFA content.

According to Shrivastava (2007), use of modified atmosphere packaging significantly reduces the rate of increase in FFA content. Vacuum packaging, MAP with 100% CO_2, MAP with 100% N_2 and MAP with 50% CO_2/ 50% N_2 reduce the rate of increase in FFA among which 100% CO_2 was found to be the most effective.

2.8.2.3 Tyrosine content

Measurement of soluble tyrosine is an index of proteolysis. Tyrosine is defined as the extent of partial hydrolysis of milk proteins caused by enzymes present in the milk. Protease enzymes indigenous to milk as well as microbial proteases degrade proteins which lead to release of free amino acids like tyrosine, producing bitter tastes in dairy products (Kumar *et al.*, 2019b). The rate of increase in tyrosine content is significantly reduced by refrigerated storage (Kumar *et al.*, 2019b), use of preservatives (Khatkar *et al.*, 2017a), type of packaging (Shrivastava, 2007), etc.

Kamble and Seth (2017) studied the variation in chemical composition of the paneer samples preserved with formalin during storage. It was observed that addition of formalin in paneer samples significantly suppressed the rise in tyrosine levels in during storage.

Shrivastava (2007) studied the modified atmosphere packaging in paneer at different atmospheres. It was observed that vacuum packaging, MAP with 100% CO_2, MAP with 100% N_2 and MAP with 50% CO_2/ 50% N_2 reduced the rate of increase in tyrosine content among which 100% CO_2 was found to be the most effective.

According to Ray (2008), irrespective of the type of milk used for paneer manufacturing, dipping freshly prepared paneer in 0.3% banana pseudostem juice solution for 2 h can significantly suppress proteolytic activity in paneer and thus decrease the rate of rise in tyrosine content. Similarly, dipping fresh paneer samples in garlic extract has also shown to be effective in suppressing the rise in tyrosine content during storage (Wanjari, 2016).

2.9 Microbiological aspects

2.9.1 Standard plate counts

The microbiological quality of paneer depends mainly upon quality of milk, heat treatment, moisture content in paneer, degree of contamination and storage condition etc. Under sub-standard existing conditions, microorganisms gets entry into the product from various sources such as air, water, utensils, cutting knife and cloth used for filtering as well as from the person handling the product (Aggarwal and Srinivasan, 1980).

During the time of preparation, there is a maximum chance of contamination of paneer due to which the initial microbial load may be high. On comparing the microbial quality of market paneer samples with laboratory made paneer, Goel (2000) found higher microbial

load in market paneer samples which might be obviously due to better hygienic conditions inside laboratory. In addition to this, Gupta (1985) also observed higher microbial load in market paneer samples in comparison to laboratory made paneer samples. Similarly, exposing paneer to an outside atmosphere with higher microbial load resulted in higher initial microbial load in paneer as compared to paneer exposed to lower level of air borne contamination (Goel, 2000).

Storage temperature plays a significant role in microbial proliferation in any perishable food product. Similarly, paneer packed in polythene and stored at 4°C showed significantly fewer standard plate count in paneer samples stored at 30°C because of which the paneer samples stored at 4°C and 30°C had a shelf life of 5 days and 1 day respectively (Shukla, 2006). Kamble (2016) also found that storage of paneer samples at 5-7°C resulted in significantly lower microbial growth as compared to samples stored at 27°C.

Similarly, addition of cardamom (Eresam *et al.*, 2015; Jagannath, 2012; Rajarshibhai, 2012) as well as turmeric (Buch *et al.*, 2014; Singh *et al.*, 2014) has been shown to significantly reduce the rate of growth of microorganisms in paneer. Patel (2014) observed that addition of sodium sorbate during paneer preparation can not only check microbial growth during storage but can also reduce initial microbial load in fresh paneer samples. Similar effect was also demonstrated on addition of nisin in paneer (Kantilal, 2010).

Upputuri (2002) found that application of 0.2% Hydrogen peroxide, 0.1% potassium sorbate and dipping in 5% brine solution can significantly suppress the growth of microorganisms in paneer during storage. In addition to this, dipping freshly prepared paneer in 0.3% banana pseudostem juice solution was also found to significantly reduce the standard plate count of paneer (Ray, 2008). Similarly, Krishnakumar (2001) studied the effect of dipping fresh paneer samples in activated lactoperoxidase system isolated from goat milk and in lysozyme isolated from egg white and found that these treatments reduced the initial microbial load in paneer samples as well as stemmed microbial proliferation.

Similarly, the atmosphere inside packaging material is also found to have a significant impact on total plate count of paneer. While using vacuum packaging was found to reduce the rate of increase in total plate count of paneer during storage, application of MAP with 100% CO_2, MAP with 100% N_2 and MAP with 50% CO_2/ 50% N_2 further decreased the

microbial count in paneer samples during storage. Among them all, use of MAP with 100% CO_2 was reported to be the most effective one which may be because of the bactericidal effect of CO_2 (Shrivastava, 2007). Similarly, application of an edible coating of sodium alginate containing cinnamon essential oil was found to be highly effective in reducing the total plate count in paneer samples (Raju and Sasikala, 2016). Likewise, Punagaiarasi (2015) prepared a whey protein concentrate based edible coating incorporated with different plant essential oils (ginger, garlic and cinnamon) and its application in paneer showed a significant decrease in viable count of paneer up to 2 days of storage at ambient temperature followed by a gradual rise in viable count. Shukla (2006) studied the suitability of different packaging material to extend the shelf life of paneer and found that aluminium foil laminate is better in slowing microbial growth during storage of paneer followed by parchment paper and polythene.

2.9.2 Yeast and mold counts

High moisture content and possessing acidic environment make paneer favorable for the growth of yeast and mold on the surface and hence it is a major limiting factor for shelf life of paneer (Jagannath, 2012). Similarly, chance of contamination during preparation also has a vital role in yeast and mold growth in paneer. Goel (2000) compared laboratory made paneer with market paneer samples and found that the yeast and molds count increased rapidly in market paneer samples as compared to laboratory prepared samples.

Several preservatives possess antifungal properties and thus limit the growth of yeast and mold. In order to reduce the yeast and mold count in paneer during storage, cardamom (Eresam *et al.*, 2015; Jagannath, 2012; Rajarshibhai, 2012), turmeric (Buch *et al.*, 2014; Singh *et al.*, 2014) and dipping in banana pseudostem juice solution (Ray, 2008) have been found to be highly effective.

Upputuri (2002) found that application of 0.2% Hydrogen peroxide, 0.1% potassium sorbate and dipping in 5% brine solution can significantly suppress the growth of yeast and mold in paneer during storage. He also found that application of 0.2% hydrogen peroxide has been found to even significantly reduce yeast and mold count in fresh paneer sample.

Dipping fresh paneer samples in lactoperoxidase thiocyanate solution and in lysozyme solution prior to packaging has been found to show significant reduction in initial yeast and

mold count as well as reduces the rate of growth of yeast and molds in paneer (Krishnakumar, 2001).

Temperature of storage also has a significant role in the growth of yeast and molds in paneer during storage. According to Shukla (2006), storing paneer at 4°C resulted in fewer yeast and mold counts in paneer samples as compared to paneer samples stored at 30°C. Kamble (2016) also found that storage of paneer samples at 5-7°C resulted in significantly lower growth of yeast and mold as compared to samples stored at 27°C.

According to Shrivastava (2007), using vacuum packaging was found to reduce the rate of increase in yeast and mold count of paneer during storage. Similarly, application of MAP with 100% CO_2, MAP with 100% N_2 and MAP with 50% CO_2/ 50% N_2 further decreased the yeast and mold count in paneer samples during storage. Among them all, use of MAP with 100% CO_2 was reported to be the most effective one (Shrivastava, 2007). Similarly, aluminum foil laminate was found to be better in controlling growth of yeast and mold in paneer than parchment paper and polythene during storage of paneer (Shukla, 2006). Application of whey protein concentrate based edible coating incorporated with essential oils of ginger, garlic and cinnamon has been found effective to check yeast and mold growth in paneer (Punagaiarasi, 2015).

2.9.3 Coliforms

A significant reduction of coliform count in paneer samples added with cardamom in comparison to control paneer have been demonstrated (Eresam *et al.*, 2015; Rajarshibhai, 2012). In addition to this, dipping freshly prepared paneer in 0.3% banana pseudostem juice solution was also found to significantly reduce the coliform count of paneer (Ray, 2008). Similarly, dipping fresh paneer samples in lactoperoxidase thiocyanate solution and in lysozyme solution prior to packaging has shown significant reduction in initial coliform count as well as reduces the rate of growth of coliform in paneer (Krishnakumar, 2001).

Temperature of storage also has a significant role in the growth of coliform in paneer during storage. According to Shukla (2006), storing paneer at 4°C resulted in fewer coliform counts in paneer samples as compared to paneer samples stored at 30°C. Kamble (2016) also found that storage of paneer samples at 5-7°C resulted in significantly lower growth of coliform as compared to samples stored at 27°C.

Application of vacuum packaging, MAP with 100% CO_2, MAP with 100% N_2 and MAP with 50% CO_2/ 50% N_2 have been found to reduce the coliform count during storage of paneer (Shrivastava, 2007). Similarly, Shukla (2006) studied the suitability of different packaging material to extend the shelf life of paneer and found that aluminum foil laminate is better in slowing the increase in coliform count during storage of paneer followed by parchment paper and polythene.

2.10 Defects in paneer

Paneer is a nutritious heat acid coagulated indigenous milk product. It is a highly perishable product having very short shelf-life because of its moisture content and high nutrients. The spoilage of paneer occurs mainly due to growth of micro-organisms which bring about various physio-chemical changes leading to the development of off-flavor in the product. Low quality milk, faulty method of production, unhygienic condition, lack of refrigeration facility and proper storage conditions are mainly responsible for defects in paneer (Kumar *et al.*, 2011). Major defects in paneer are mentioned below:

- Flavor defects: Flavor defects includes sour flavor, smoky flavor, rancid flavor and stale flavor.
- Body and texture defects: This defect include hard body and coarse texture.
- Color and appearance defect: This includes moldy surface, surface hardening, dry surface of paneer.
- Foreign matters are seen due to improper straining of the milk and transport of paneer in unhygienic manner (Kumar *et al.*, 2011).

2.11 Shelf life of paneer

Paneer is a highly perishable product (Mishra, 2017). It is rich in nutrients and there is also enough moisture content in it to permit growth of variety of microorganisms. Thus, the shelf life of paneer is quite low. It has been reported that the shelf life of paneer is just 6 days at refrigeration temperature (10ºC) without much deterioration in quality. But the freshness of the product is lost after 3 days; while at room temperature, paneer doesn't keep well for more than one day (Bhattacharya *et al.*, 1971). It has been noticed that the spoilage in paneer occurs mainly due to growth of microorganisms on the surface, with formation of greenish yellow slime on the surface accompanied with off flavor (Mishra, 2017). Since shorter shelf life of paneer is one of the major handicaps for its industrial production, several efforts have

been made to increase its shelf life and the use of additives, modification in manufacturing process, surface treatments and packaging materials have been recommended (Mishra, 2017).

2.12 Measures to improve shelf life of paneer

Various measures tried to improve shelf life of paneer may be broadly categorized as use of antimicrobial agents, application of various treatments and use of hurdle technology. The findings of different workers are summarized in Section 2.12.1 to 2.12.8.

2.12.1 Low temperature storage

Storage temperature is probably the most important factor in maintaining the quality and extending the shelf life of packaged foods. In most cases, an increase in storage temperature degrades the quality and acceptability of packaged foods. Biological reactions tend to increase by a factor of two to three for each 10°C increase in temperature (Singh and Singh, 2005). Since microbiological growth is a major reason for spoilage of paneer, lower temperature storage can contribute significantly in extending its shelf life.

Arora and Gupta (1980) studied the effect of low temperature storage on shelf life of paneer and concluded that the paneer prepared from milk having 4, 5 and 6% fat could be stored for not more than 6 days at 10°C and for at least 120 days at -13°C and – 32°C without much decrease in sensory quality.

Similarly, Kumar (2016) studied the storage stability of paneer nuggets at ambient as well as refrigeration temperature and found the shelf life of paneer nuggets to be much higher at refrigeration temperature. While the shelf life of aerobically packaged and vacuum packaged paneer nuggets was 2 days and 3 days respectively at ambient temperature, the shelf life was as high as 9 days and 18 days for aerobically packaged and vacuum packaged paneer samples respectively at refrigeration temperature.

2.12.2 Microwave treatment and heat sterilization

Microwave has been successfully applied in dairy industries in processing of butter, cheese, yoghurt and ice cream mixes to inactivate number of microbes, enzymes as well as bacteriophages (Karthikeyan, 2005). Several workers have attempted to apply microwave processing in paneer so as to enhance its shelf life and have found the results to be quite satisfactory (Karthikeyan, 2005).

For control paneer, the shelf life was 1 day whereas the shelf life of microwave treated sample was 2 days at 30±1°C. Similarly, the shelf life was 7 days and 21 days for control paneer and microwave treated paneer sample respectively when stored at 7±1°C (Karthikeyan, 2005). Similarly, Barman (2007) found the shelf life of microwave treated paneer at 80% power level and at 90% power level were 44 days and 45 days respectively at 7±1°C storage temperature in comparison to control paneer which had a shelf life of 10 days at the same storage condition.

Heat sterilization of paneer enhances its keeping quality to 4 months at room temperature. Due to sterilization slight browning of paneer occurs which increases during storage. The development of oxidized off flavor after 4 months renders the paneer unacceptable (Kanawjia *et al.*, 1990). Similarly, implication of ohmic treatment at 90°C for 60 s in paneer can have a shelf life up to 21 days at refrigerated storage (7±1°C) (Suman, 2015).

2.12.3 Chemical preservatives

2.12.3.1 Brine

Singh and Kanawjia (1988) claimed that the shelf life of paneer could be extended up to 16 days by dipping in brine (5%) and storing at refrigeration temperature, but the continuous dipping results a very soft, fragile body, and dull yellow appearance of the product at the end of 10 days of storage period.

Paneer dipped in 5% brine solution lasts for nearly 20 days against the control that is spoiled after 6 days of storage at 8-10°C (Narayanan *et al.*, 2016). Similarly, Upputuri (2002) studied the preservation action of brine solution on coconut milk paneer and found that dipping paneer samples in 5% brine solution gave a shelf life of 2 days and 12 days at room temperature and refrigerated storage (4°C) respectively.

Barman (2007) applied brine treatment to extend the shelf life of paneer and found that paneer samples dipped in 17%, 18% and 19% brine solutions had a shelf life of 4 days, 6 days and 8 days respectively on storing at 30±1°C in comparison to control paneer which had a shelf life of 1 day. Similarly, on storing at 7±1°C, control paneer, paneer dipped in 4% brine solution, 5% brine solution and 6% brine solution had a shelf life of 10 days, 20 days, 25 days and 30 days respectively.

2.12.3.2 Sorbic acid, irradiation and benzoic acid

Sorbates are highly effective against yeasts and molds and are commonly applied in cheese for the inhibition of molds. Application of sorbic acid in dairy products include uses in *pedha, burfi, rasgolla, gulab jamun, khoa,* pasteurized milk, cheeses, yoghurt, paneer, etc. (Patel, 2014).

Combination treatment of 0.10% sorbic acid in milk and irradiation of the product at 2.5 kilo Gray (kGy) preserved paneer samples for 30 days at ambient temperature (25–35ºC). The use of benzoic acid (1200 ppm) for the enhancement of shelf life of paneer to 40 days at refrigerated temperature, and to 20 days at 37ºC were also reported (Singh *et al.*, 1989).

Thakral *et al.* (1990) observed that the keeping quality of paneer containing 0.1% potassium sorbate could be extended by 13 days, 3-4 days and 1 day at 7, 22 and 37ºC, respectively. However, the keeping quality was further improved by adding nisin together with the potassium sorbate.

Patel (2014) studied efficacy of sorbic acid in paneer and found out that addition of sodium sorbate (1500 ppm) before initiating the heat treatment gave a shelf life of 15 days for paneer at 7±1°C. Similarly, shelf life of coconut milk paneer was found to be extended up to 2 days and 21 days at room temperature and refrigeration temperature respectively on dipping paneer in 5% brine solution (Upputuri, 2002).

UV treatment of paneer samples for 15 s and 20 s was found to extend the shelf life of paneer up to 15 days and 18 days respectively at 7±1°C storage temperature in comparison to control paneer which had a shelf life of 10 days at the same storage conditions (Barman, 2007).

2.12.3.3 Nisin

Nisin is a member of potent antimicrobial compounds called bacteriocins, which are produced by lactic acid bacteria along with other antimicrobial substances. It has a broad spectrum of antimicrobial activity against gram-positive bacteria and in normal circumstances, it doesn't significantly inhibit yeasts, molds or gram-negative bacteria. It has been applied successfully to extend shelf life of several dairy products such as *khoa*, processed cheese, sterilized milk, paneer, cottage cheese, yoghurt, *lassi*, etc. (Kantilal, 2010).

Kantilal (2010) tried to extend the shelf life of paneer by incorporating nisin in milk at the rate of 12 ppm before initiating the heat treatment of milk and found that application of nisin gave a shelf life of 12 days for paneer at 7±1°C. It was concluded that nisin is not a highly promising preservative for paneer.

2.12.3.4 Hydrogen peroxide

Addition of 0.2% hydrogen peroxide was found to extend the shelf life of coconut milk paneer up to 2 days and 27 days at room temperature and refrigeration temperature respectively (Upputuri, 2002).

Nayak and Bector (1999) reported the results which showed that addition of H_2O_2 to milk significantly decreased the yield of paneer which was very soft, fragile and whiter in appearance. Approximately 20-25% of the H_2O_2 added to milk appeared in paneer which decreased as storage period increased.

2.12.4 Biopreservation techniques

Lactoferrin is one of the bioactive component present in milk and it offers a number of functionalities such as iron absorption, antimicrobial activity, antioxidant activity, antiparasitic, immunomodulatory, antitumor and anti-inflammatory (Kumar, 2009). Similarly, lactoperoxidase system is a naturally occurring system in milk which is found to inhibit many gram negative as well as catalase positive organisms (Krishnakumar, 2001). Likewise, lysozyme is also a protein occurring widely in nature which is more effective against gram positive bacteria (Krishnakumar, 2001).

Kumar (2009) applied lactoferrin isolated from cheese whey to the surface of paneer at 20 ppm concentration prior to storage and found the shelf life of treated samples to be extended up to 7 days at room temperature (30±1°C) and 24 days at refrigerated temperature (4±1°C) while the shelf life of untreated samples was just 2 days at room temperature and 6 days at refrigerated temperature.

Similarly, Krishnakumar (2001) attempted to implement activated lactoperoxidase system isolated from goat milk and lysozyme from egg white for preservation of paneer. In comparison to control paneer whose shelf life was just 8 days at refrigeration temperature, lactoperoxidase system treated samples and lactoperoxidase+lysozyme treated samples extended the shelf life up to 24 days and 28 days respectively at the same storage conditions.

2.12.5 Packaging of paneer

Paneer is highly susceptible to chemical and microbial changes, and its packaging should protect against these changes, maintain quality and effective sales appeal, and add to consumer convenience. It is observed that the technology for production of paneer is well known for over a long time, but its proper packaging needs attention. Various materials have been used for the packaging of paneer, they are; PE sachets, butter paper, parchment paper, wax coated paper, saran coated films, coextruded laminates, heat-induced shrink films (Goyal and Goyal, 2016).

Use of saran-coated packaging films (saran is a polyvinylidene chloride which is a synthetic polymer having low permeability to a wide range of gases and vapors thus making it most valuable for use in food packaging) helped in enhancing the shelf life of paneer to a great extent (Sachdeva and Singh, 1990a).Vacuum packaging of paneer in laminated pouches can help to increase its shelf life to about 30 days at 6 ± 1°C (Narayanan *et al.*, 2016).

Shukla (2006) studied the effect of three types of packaging materials, namely, low density polyethylene (100 gauge), vegetable parchment paper and laminated aluminium foil, on the shelf life of paneer. It was found that laminate provided the longest shelf life among these three packaging materials (15 days at 4°C storage and 2 days at 30°C storage) followed by parchment paper (10 days at 4°C and 1 day at 30°C) and polyethylene (5 days at 4°C and 1 day at 30°C).

Application of whey protein based edible coating incorporated with 1% essential oils (ginger, garlic and cinnamon) was found to extend the shelf life of paneer from 6 days to 12 days at refrigeration temperature 6±1°C (Punnagaiarasi, 2015).

2.12.6 Hurdle technology

Hurdle technology is a technology in which hurdles (preservation parameters) are employed in a suitable combination, and every hurdle is used at an optimum level so that the damage to the overall quality of food is kept to a minimum. The crux of hurdle technology principle is that each hurdle in a food is used at sub-lethal level and all the hurdles together will have a lethal effect just sufficient to preserve the food (Karthikeyan, 2005).

Mishra (2017) tried a combination of five hurdles, namely, heat treatment with steam, reduced pH by soaking in citric acid, drying at 55°C for 4 h to reduce water activity, use of spice extracts (ajwain, black pepper and cloves) and smoke treatment with garlic smoke for extending the shelf life of paneer. On studying the effect of individual hurdles independently, none of the hurdles except smoke could significantly increase shelf life of paneer. But the combination of hurdles significantly increased the shelf life of paneer. The combinations heat treatment+water activity+smoke, pH+water activity+smoke, heat treatment+smoke was found to be the most suitable ones and they gave a shelf life of 37 days, 29 days and 28 days respectively.

Gokhale *et al.* (2016) treated paneer with selected food grade acids viz. vinegar, lactic acid and citric acid followed by partial drying of the treated product under vacuum to reduce its moisture content on the surface and to evaluate its effectiveness in extending shelf life of paneer. Treatment of paneer with lactic acid and vinegar resulted in enhanced shelf-life up to 90 days under refrigerated (7±2°C) storage conditions whereas paneer treated with citric acid was found to be acceptable up to 60 days of storage.

2.12.7 Modified atmosphere packaging (MAP)

MAP is the enclosure of food in a package, inside which the atmosphere is modified with respect to carbon dioxide, oxygen, nitrogen, water vapor and trace gases. Carbon dioxide is an important gas used in MAP and it exhibits bacteriostatic and fungistatic properties. Similarly, nitrogen is considered as an inert and filler gas in order to replace oxygen from the packet. MAP has been applied in a wide variety of dairy products such as milk, yoghurt, cottage cheese, Mozzarella cheese, etc. (Karthikeyan, 2005).

Sweta *et al.* (2008) studied the effect of packaging paneer in high barrier bags (LLD/BA/Nylon-6/BA/LDPE) under different atmospheres and found that MAP had significant influence on moisture, titratable acidity, pH, free fatty acids, and tyrosine content of the paneer samples during storage.

While the shelf life of control paneer packed in polyethylene terephthalate/polyethylene (PET/PE-12/54μm) was found to be 1 day, the shelf life of paneer samples packed under modified atmosphere of 100% CO_2, 100% N_2 and 50% CO_2/50% N_2 was found to be 2 days on storage at 30±1°C. Similarly, on storage at 7±1°C, the shelf life of control paneer was just

7 days whereas the shelf life of paneer packed with a modified atmosphere of 100% CO_2, 100% N_2 and 50% CO_2/50% N_2 was 56 days, 28 days and 42 days respectively (Karthikeyan, 2005).

Shrivastava (2007) applied MAP in paneer and found that use of 100% CO_2, 100% N_2 and 50% CO_2/50% N_2 gave the shelf life of 30 days in comparison to conventional air which gave a shelf life of 10 days only when stored at 3±1°C. Similarly, on deep freeze storage (-15°C), use of modified atmospheres increased the shelf life up to 90 days in comparison to conventional air that only had a shelf life of 45 days.

2.12.8 Use of antioxidants

Kumar and Rai (2009) studied the effect of incorporation of antioxidant butylated hydroxyl anisole (BHA) plus butylated hydroxyl toluene (BHT) in 1:1 ratio and paraffin waxing. For this, four treatments were conducted (i) control (ii) paneer made from milk containing 100 ppm of BHA and 100 ppm of BHT but not dipped in paraffin wax (iii) paneer made from milk containing no antioxidant but dipped in paraffin wax for 5 s (iv) paneer made from milk containing both antioxidants and dipped in paraffin wax. Samples thus prepared were packaged and kept at refrigeration temperature (4±1°C). Samples were drawn and analyzed for different parameters every three days up to 15 days. Addition of antioxidants and paraffin waxing significantly ($P < 0.05$) reduced the microbial load and thiobarbituric acid value. All the samples were quite acceptable up to 9^{th} day of storage, after that control were rated lower for appearance, flavor and overall acceptability. Paneer samples of group 4 remained quite acceptable during the entire storage period.

2.13 Trend towards use of natural ingredients

There has been increasing concern of the consumers about foods free of chemical preservatives because of their possible toxic effect in human beings. Hence, limited use of synthetic additives is in demand (Membre *et al.*, 2001). Consumers are demanding for food with long shelf-life and absence of risk of causing food borne diseases. This has put pressure on the food industry for progressive reduction or elimination of chemical preservative and adoption of natural alternative to achieve concerning microbial safety (Arora and Kaur, 1999).

In recent years many attempts have put emphasis on the search for natural antimicrobial substances that can properly serve the needs of food manufacturers and consumers. Various

herbs and spices have been recognized for their antimicrobial activity and used throughout the past as an alternative approach to preserve foods. Inhibitory activity of herbs and derivatives on the growth of bacteria, fungi and microbial toxins has been well reported (Zaika, 1988). So food technologists and nutritionists are evincing greater interest in the fortification of food and beverages with natural ingredients (Ansari and Kumar, 2012).

2.14 Herbs and spices as preservatives

Botanically, herbs are soft-stemmed plants, the main stem of which dies to the ground level and either does not regrow (annuals), grows again the following year only (biennials) or regrows each year (perennials). Herbs may be used fresh or after dehydration. Herbs, for the most part have a light and very distinct aromatic character although they contain relatively low levels of essential oil (Reineccius, 2006).

A spice is a dried seed, fruit, root, bark or flower of a plant or herb used in small quantities for flavor, color or as a preservative (Kunnumakkara *et al.*, 2009).

Herbs and spices have found many uses in treating number of diseases and their herbal extracts can be used in pharmaceuticals, ayurvedic formulation, confectionery, nutritional foods, ready-to-drink mixes, instant foods, seasonings, dairy products, seasoning blends, etc. Therefore, fortification of herbs in dairy products could provide value added, functional dairy product (Oraon *et al.*, 2017).

Plants and spices are excellent sources of biologically active compounds with potential antimicrobial activity. Essential oils, secondary metabolites produced by plants, have valuable capability of suppressing growth of wide variety of food-spoilage and food-borne microorganisms including bacteria, yeasts and molds. From chemistry point of view, they consist of aromatic and volatile compounds which play an important role in plant defense and possess antimicrobial properties (Hyldgaard *et al.*, 2012). They can be extracted from different parts of plants including flowers, roots, bark, leaves, seeds, peel, fruits, wood, buds and the entire plant (Hammer *et al.*, 1999).

According to Peter and Babu (2004), different parts of herbs (such as roots, buds, flowers, fruits, barks, leaves or seeds) are used. Only little can be said in a general way about their composition. Some of the main active constituents in herbs are as follows:

a. Acids-sour, often antiseptics and cleansing.

b. Alkaloids- bitter, often based on alkaline nitrogenous compounds. They affect the central nervous system and many are toxic and addictive.
c. Anthraquinones- bitter, irritant and laxatives, also act as dyes.
d. Bitters- various compounds, mainly irridoides and sesquiterpenes with a bitter taste that increase and improve digestion.
e. Coumarines- antibacterial, anticoagulants, with a smell of new-mown hay.
f. Flavones- bitter or sweet, often diuretic, antiseptic, antispasmodic and anti-inflammatory. They are typically yellow and present in most plants.
g. Gums and mucilage- bland, sticky or slimy, smoothing and softening.
h. Resins- acrid, astringent, antiseptic, healing. They are often found as oleoresins or oleo-gum resins.
i. Saponins- sweet, stimulant hormonal, often anti-inflammatory or diuretic, soapy in water.
j. Tannins- astringent, often antiseptic, checking bleeding and discharges.
k. Volatile oils- aromatic, antiseptic, Fungicidal, irritant and stimulant.
l. Glycosides- there are main four main kinds, viz.
   i. cardiac: affecting heart contractions
   ii. synogenic: bitter, antispasmodic sedative, affecting respiration and heart rate
   iii. mustard oil: acrid, extremely irritant and
   iv. Sulphur: acrid, stimulant, antibiotic.

Many plant essential oils of herbs are active against various food borne bacteria and molds (Aureli *et al.*, 1992). Herbs and spices have also been well known for their medicinal, preservative and antioxidant properties (Souza *et al.*, 2005), hence they could be used for food preservation as main or adjuvant antimicrobial substances.

2.15 Application of herbs as preservative in functional dairy products

2.15.1 Ghee (clarified butter)

Parmar *et al.* (2013) found that addition of ethanolic extract of *Terminalia arjuna* bark at 7% by weight was highly effective in retarding the auto-oxidation of both cow and buffalo ghee during storage. Ethanolic extract of *arjuna* herb showed significant ability to enhance the antioxidant potential of ghee; the efficacy was more pronounced in case of cow ghee compared to buffalo ghee. The shelf life (accelerated test) of the *arjuna* herbal ghee at

80±1°C was found to be extended to 8 days as compared to 2 days in control ghee sample (devoid of herb).

Sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*) extracts have been the most widely used herbs for prolonging the shelf life of ghee and butter oil (Özcan, 2003). These extracts have antioxidant activity many times stronger than that of synthetic antioxidants (i.e. Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT)) (Estévez *et al.*, 2007).

2.15.2 Yogurt and Labneh (concentrated yoghurt)

Yoghurt containing cinnamon (6.0% cinnamon extract dissolved in 1 L milk) could be successfully stored under refrigeration for up to 28 days; the *Lactobacillus* species count in such yoghurt was 19.46×10^6 cfu/ml when fresh; the *Streptococcus thermophilus* count increased in yoghurt containing cinnamon up to 7 days, when stored under refrigeration (Behrad *et al.*, 2009).

Labneh is basically concentrated yoghurt quite popular in the Middle East. *Labneh* (23.0% TS) containing 0.2 ppm each of thyme, marjoram and sage essential oils has extended shelf life (by 21 days over control) at 5°C. The development of yeast and mold in control sample after 14 days storage at 5°C has been also reported (Otaibi and Demerdash, 2008).

2.15.3 Ice cream

Different forms of ginger i.e., ginger juice at the rate of 4%, ginger shreds at the rate of 4%, sugar syrup treated ginger shreds at the rate of 6% and ginger powder at the rate of 1% were used to prepare 'ginger flavored herbal ice cream' and compared against a control ice cream made from vanilla flavoring. Incorporation of ginger juice or ginger shreds (sugar syrup treated) at rate of 4% by weight of ice cream mix is recommended for obtaining acceptable quality 'ginger flavored ice cream' (Pinto *et al.*, 2009). Trivedi *et al.* (2014) recommended incorporating basil juice at the rate of 6% and freeze-dried basil powder at the rate of 1% by weight of ice cream mix in the preparation of 'basil flavored herbal ice cream'. Basil variety *Ocimum sanctum* was preferred over *O. americanum*, *O. basilicum* and *O. gratissimum*. Incorporation of basil juice led to decrease in fat, protein, carbohydrates, ash and acidity and an increase in pH; melting resistance of ice cream was reduced.

2.15.4 Cheese

A study reported that addition of clove essential oil (minimum inhibitory concentration of 2.0%) showed antibacterial effect against *E. coli* and vancomycin-resistant *Enterococci* in Feta cheese stored at 7°C for 14 days (Sammy, 2011).

2.15.5 Sandesh

Sandesh is a *chhana* (obtained from milk by coagulation with acid) based sweet product very popular in West Bengal, India. Paste of turmeric (*Curcuma longa*), coriander (*Coriandrum sativum*), curry leaf (*Murraya koenigii* L.), spinach (*Spinaciaoleracea*) and aonla (*Emblica officinalis*) were incorporated separately at the 10% level in *sandesh* and were compared with control *sandesh* added with synthetic antioxidants (viz. TBHQ, BHA, BHT). The researchers concluded that herbal *sandesh* could be considered as value-added health food as compared to control *sandesh* containing synthetic antioxidants. The antioxidative effect of herbal *sandesh* decreased in the order: turmeric>curry leaf>aonla>spinach>coriander leaf. The total antioxidative potency of herbal *sandesh* was lower than *sandesh* samples containing TBHQ, but similar to those containing 200 mg/kg BHA and BHT (1:1 w/w).

Incorporation of coriander as herb resulted in increased shelf-life of herbal *sandesh* up to 8 days and 30 days respectively, when stored at 30±1°C and 7±1°C (Bandyopadhyay *et al.*, 2007).

2.15.6 Herb and spices in paneer

Considering the shorter shelf life of paneer, several attempts have already been made to incorporate herbs and spices in paneer. Buch *et al.* (2014) attempted to incorporate turmeric in paneer coagulum at the rate of 0.4% and 0.6% by weight of expected yield of product. The paneer samples containing 0.6% turmeric by weight, remained acceptable up to 12 days as against 7 days for control paneer, when stored at 7±1°C. Turmeric powder when added to milk (0.6% by weight of expected yield of paneer) prior to heat treatment in paneer making, helped in reducing the taste of raw turmeric in resultant product (Buch et al., 2014).

Similarly, Rajarshibhai (2012) studied the effect of selected spices (oleoresins and essential oils) of cardamom, clove and black pepper to extend the shelf life of paneer and has shown an extended shelf life of paneer prepared with 0.01% essential oil of cinnamon, 1:1 combination of essential oil of cinnamon plus cardamom and essential oil of cardamom

alone to 10, 15 and 15 days respectively as compared to 5 days in control paneer at storage temperature of 7°C.

Khatkar *et al.* (2017b) studied on shelf-life extension of paneer with the addition of plant essential oil i.e. cinnamon oil and different packaging materials. There was no major perceivable defect observed in stored samples, except control, but the decrease in flavor score to less than 6.0 during storage limited their shelf life. On decreased flavor basis, control paneer samples showed shelf life of only 10 days with metalized polyester, 8 days with nylon and 5 days with LDPE, while cinnamon treated samples showed shelf life of 18 days with metalized polyester, 14 days with nylon and 9 days with LDPE at 8±1°C.

2.16 Clove *(Syzygium aromaticum)*

Syzygium aromaticum commonly known as clove, is a median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia. For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region (Kamatou *et al.*, 2012).

Spices as clove, oregano, mint, thyme and cinnamon, have been employed for centuries as food preservatives and as medicinal plants mainly due to its antioxidant and antimicrobial activities. Nowadays, many reports confirm the antibacterial, antifungal, antiviral and anticarcinogenic properties of spice plants (Al-Wabel and Fat'hi, 2012; Erturk, 2006; Liu *et al.*, 2017; Zheng *et al.*, 2016). Clove in particular has attracted the attention due to the potent antioxidant and antimicrobial activities standing out among the other spices (Shan *et al.*, 2005).

It is a remarkably versatile molecule incorporated as a functional ingredient in numerous products and has found application in the pharmaceutical, agricultural, fragrance, flavor, cosmetic and various other industries. Its vast range of pharmacological activities has been well-researched and includes antimicrobial, anti-inflammatory, analgesic, anti-oxidant and anticancer activities, amongst others (Kamatou *et al.*, 2012).

2.16.1 Chemical compounds isolated from clove

Eugenol is the main bioactive compound of clove, which is found in concentrations ranging from 9381.70 to 14650.00 mg/100 g of fresh plant material (Kamatou *et al.*, 2012). With regard to the phenolic acids, gallic acid is the compound found in higher concentration

(783.50 mg/100 g fresh weight). However, other gallic acid derivates as hydrolysable tannins are present in higher concentrations (2375.8 mg/100 g) (Cortés-Rojas *et al.*, 2014). Other phenolic acids found in clove are the caffeic, ferulic, ellagic and salicylic acids. Flavonoids as kaempferol, quercetin and its derivates (glycosylated) are also found in clove in lower concentrations.

According to Jirovetz *et al.* (2006) a total of 23 components were identified as constituents of the essential leaf oil of clove comprising about 99% of the total oil composition. The main components were found to be eugenol (76.8%), followed by β-caryophyllene (17.4%), α-humulene (2.1%) and eugenyl acetate (1.2%).

2.16.2 Antimicrobial activity

Ouattara *et al.* (1997) reported that essential oils that contain carvacrol and eugenol have been shown to exhibit the strongest antimicrobial activity.

The antimicrobial activities of clove have been proved against several bacterial and fungal strains. Sofia *et al.* (2007) tested the antimicrobial activity of different Indian spice plants as mint, cinnamon, mustard, ginger, garlic and clove. The only sample that showed complete bactericidal effect against all the food-borne pathogens tested *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus* was the aqueous extract of clove at 3%. At the concentration of 1% clove extract also showed good inhibitory action.

Rana *et al.* (2011) determined the antifungal activity of clove oil in different strains and reported this scale of sensibility *Mucor* sp.>*Microsporum gypseum*>*Fusarium monoliforme* NCIM 1100>*Trichophytum rubrum*>*Aspergillus* sp.>*Fusarium oxysporum* MTCC 284. The chromatographic analyses showed that eugenol was the main compound responsible for the antifungal activity due to lysis of the spores and micelles.

Rajkumar and Berwal (2003) studied the inhibitory effect of clove against *Penicillium chrysogenum*, *Penicilliumverrugosum*, *Aspergillus flavus* and *Aspergillus parasiticus* using spice agar method for using clove to stabilize intermediate moisture meat products. Minimum inhibitory concentration of clove at 80% inhibition level was also calculated. Clove exhibited minimum inhibitory concentration of 0.86, 1.12, 1.08, 1.30 and 0.92% (w/v) against *P. chrysogenum*, *P. verrugosum, Asp. flavus* and *Asp. parasiticus*, respectively. At

5% level of clove all spoilage and toxigenic mold growth was inhibited during 25 days of study period.

Souhair and Nefisa (1980) studied the effects of black pepper, cinnamon, peppermint, cumin, ginger and clove on growth and aflatoxin formation of *Aspergillus flavus* in rice powder corn steep (RC) medium. The effects of the first five spices were judged on the basis of inhibition of aflatoxin formation rather than of mycelial growth. Clove completely inhibited both mycelial growth and aflatoxin formation at a concentration above 0.1%.

2.16.3 Antioxidant activity

Shan *et al.* (2005) identified the main phenolic compounds in 26 spices and quantified them by high performance liquid chromatography, followed by the in vitro antioxidant activity analysis by the ABTS method. Results showed high correlation between the polyphenols content and the antioxidant activity. Clove (buds) was the spice presenting higher antioxidant activity and polyphenol content. The major types of phenolic compounds found were phenolic acids (gallic acid), flavanol glucosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins. It was highlighted the huge potential of clove as radical scavenger and as a commercial source of polyphenols.

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. Gulcin *et al.* (2012) demonstrated higher free radical scavenging activity of cloves in comparison to several artificial antioxidants such as BHA, BHT, α-tocopherol and Trolox. It was found that 45 µg/ml of clove oil was found to show 83.6% inhibition during DPPH assay. DPPH free radical scavenging activity of clove oil also increased with an increasing concentration.

Ethanol and aqueous extracts of clove and lavender at concentrations of 20, 40 and 60 µg/ml showed inhibitions up to 95% when tested as metal quelants, superoxide radical capture and scavenging of the DPPH radical. The powerful antioxidant activity of both extracts may be attributed to the strong hydrogen donating ability, metal chelating ability and scavenging of free radicals, hydrogen peroxide and superoxide (Cortés-Rojas *et al.*, 2014).

2.17 *Pakhanbedh* (*Bergenia ciliata*)

B. ciliata Sternb belongs to the family Saxifragaceae which consist of 30 genera and 580 species. *B. ciliata* commonly known as hairy *Bergenia* is a perennial herb found between the height of 800–3000 m throughout the temperate Himalayas from Afghanistan to Southeast Tibet (Ruby *et al.*, 2012). In Bhutan it is found in Deothang, Phuntsoling, Mongar and Ha districts. In India it is reported from Lushai hills, West Bengal, Arunachal Pradesh, Meghalaya, Himalayas (Kumaon), Kyongnosla, Karponanag, Gangtok in Sikkim, district Almora Uttarakhand (Hasan *et al.*, 2013).

In Nepal it occurs in Makanwanpur district, Karepalanchwok district and Dolakha district. In Pakistan it is distributed in northern parts mainly FATA region of Khyber Pukhtunkhwa province, Poonch valley, Swat, Abbottabad, Galliyat and Chitral. It was long since this plant has been used as medicines for the treatment of different human ailments. In Himalaya region many rural communities use *B. ciliata* for the treatment of various diseases. For century's rhizome of *B. ciliata* has been used for curing pulmonary infections, leucorrhea, piles and for dissolving bladder and kidney stones (Ahmad *et al.*, 2018).

2.17.1 Chemical components isolated from *pakhanbedh*

Phytochemical screening of *B. ciliata* showed the presence of terpenoids, tannins, flavonoids, saponins, steroids (Uddin *et al.*, 2012)**.** Gyawali and Kim (2012) reported 48 volatile organic compounds in *B. ciliata*. These 48 compounds were divided into 11 categories which are phenol (19%), alcohol (19%), volatile organic compound (VOCs) (16%), terpenoids (14%), fatty acids (8%), sterol (5%), glycosides (5%), carboxylic acids (5%), flavonoids (3%), cinnamic acid (3%) and nitro compounds (3%).

Phenols are the most important constituents of *B. ciliata*. Different phenolic compounds like bergenin,tannicacid, gallic acid, catechin, [10]-3-O-galloylcatechin and [10]-3-O-galloylepicatechin are present in *B. ciliata* (Dwivedi *et al*., 2012).

2.17.2 Antimicrobial activity of *pakhanbedh*

Several workers have attempted to study the antimicrobial potential of *Berginia ciliata* on a variety of microorganisms. Through GC-MS analysis, it has been identified that phytochemicals in *Berginia ciliata* rhizomes such as 1-Monolinoleoylglyceroltrimethylsilylether/ Monolinolein TMS, Ethyl iso-allocholate and β-

Caryophyllene possess antimicrobial activity and Gallic acid possess antifungal activity (Verma *et al.*, 2019).

Rajbhandari *et al.* (2007) tested the antibacterial activity of root and leaves extract viz ethanol, hexane, ethyl acetate, chloroform, butanol and aqueous (5 mg/ml) aliquots of *Bergenia ciliata.* The root extract was found to inhibit the growth of gram-positive bacteria as compared to gram negative strain. Similarly, Chauhan *et al.* (2012) observed that the rhizome extract of *B. ciliata* had much higher antibacterial activity than its leaf extract owing to the presence of important phytocomponents in rhizome than in the leaves.

Ethanolic extract of *B. ciliata* with 50 mg/ml concentration showed inhibition against *B. subtilis, Klebsiella pneumonia, S. aureus, E. coli, Salmonella typhi, Shigella dysentriae, Saccharomyces cerevisiae* (Khan *et al.*, 2012). Similarly, ethanolic root extracts of *Bergenia ciliata* at the concentration of 200 µg/disc showed maximum inhibition against bacterial strains of *S. typhimurium, E. coli* (Verma *et al.*, 2019).

2.17.3 Antioxidant activity of *pakhanbedh*

Berginia ciliata consists of several phytochemicals such as bergenin, gallic acid, carotene, beta sitasterol, cetene and hexadecanoic acid which possess antioxidant potentials (Verma *et al.*, 2019).

Several works have been performed to explore the antioxidant activity of *pakhanbedh*. Zafar *et al.* (2019) demonstrated that crude extract of *pakhanbedh* showed 87.37% free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Similarly, Rajkumar *et al.* (2010) also studied the antioxidant activity of *pakhanbedh* and found out 100 µg extract showed the % inhibition of 97% on DPPH radicals.

B. ciliata rhizome extracts were found to possess antioxidant activity, including reducing power, free radical scavenging activity and lipid peroxidation inhibition potential (Bhandari *et al.*, 2008). Adhikari (2016) tested 15 medicinal herbs found in Nepal for antioxidant and antimicrobial property. *Bergenia ciliata* was considered to be the best antioxidant among the sampled plant samples. Many reports have shown *Bergenia ciliata* as a potential antioxidant but its use on food preservation has not been much tested. Thus, this study focuses on the utilization of *pakhanbedh* as a preservative in paneer.

PART III

Materials and methods

This chapter covers details on the manufacturing of paneer and rate of addition of spice extracts in paneer. It also covers details regarding storage and analysis schedule of paneer. It also encompasses details of the methods used for monitoring chemical changes, procedure followed for the microbiological analyses and sensory evaluation of paneer during storage. Finally, statistical design used for analysis of data is given.

3.1 Collection of raw materials

3.1.1 Collection of milk

For preparation of paneer, commercially available milk {3% fat and 8% solid not fats (SNF)} manufactured by NMC dairy milk was taken.

3.1.2 Collection of cloves and *pakhanbedh*

Cloves and *pakhanbedh* were collected from local market of Dharan.

3.1.3 Packaging material

Polypropylene bags were used for packaging of samples during storage study.

3.2 Materials used

The materials required were used from the labs of Central Campus of Technology. The glass wares, chemicals and instruments used in the research purpose are listed in Appendix H.

3.3 Experimental Design

Extracts of two herbs, namely cloves and *pakhanbedh* were added at different concentrations to paneer and their effect on physicochemical as well as sensory properties during storage were studied. For cloves, several studies have found addition of cloves extract upto 0.6% concentration in paneer to be acceptable (Eresam *et al.*, 2015; Jagannath, 2012). Thus, an upper threshold value of 0.6% was set for cloves based on these studies.

Similarly, for *pakhanbedh*, no any studies were found to be performed and thus preliminary trial was performed by preparing paneer samples with different concentrations of *pakhanbedh* extracts, namely, 0.3, 0.6 and 0.9% of yield of paneer. Among different

samples, the one containing up to 0.6% extract was found to be acceptable and 0.9% containing sample exhibited high astringent flavor of *pakhanbedh* and thus 0.6% was set as the upper threshold concentration of *pakhanbedh* for incorporating in paneer.

A total of six different formulations were selected for the two components i.e. clove and *pakhanbedh* extract each with a lower level of 0% and upper level of 0.6% of yield of paneer which are shown in Table 3.1.

Table 3.1 Different formulations of herb extracts added to paneer

Samples	Formulation
Control	0% cloves + 0% *pakhanbedh*
A, F	0% cloves + 0.6% *pakhanbedh*
B, G	0.15% cloves + 0.45% *pakhanbedh*
C, H	0.3% cloves + 0.3% *pakhanbedh*
D, I	0.45% cloves + 0.15% *pakhanbedh*
E, J	0.6% cloves + 0% *pakhanbedh*

Control represents the paneer sample where no herbs extracts were added. Samples A, B, C, D, E represent the paneer samples where herbs extracts are directly treated on milk and samples F, G, H, I, J represent the samples where herb extract are treated on curd after coagulation and drainage of whey.

3.3.1 Preparation of herb extract

Herb extract was prepared according to the method followed by Baljeet *et al.* (2015). Cloves and *pakhanbedh* were cleaned, dried at 50°C for 3-4 h and grinded to powder form. The flow sheet for extract preparation is shown in Fig. 3.1.

3.3.2 Analysis of spice extract

3.3.2.1 Determination of extraction yield and spice extract concentration

In order to determine the extraction yield of spices, the extract was filtered through a filter paper while the remaining residue was dried properly so as to remove all of the solvent used. Then the amount of spice extracted along with the solvent was determined by subtracting the weight of dry residue remaining after filtration with the amount of spice sample initially taken for extraction.

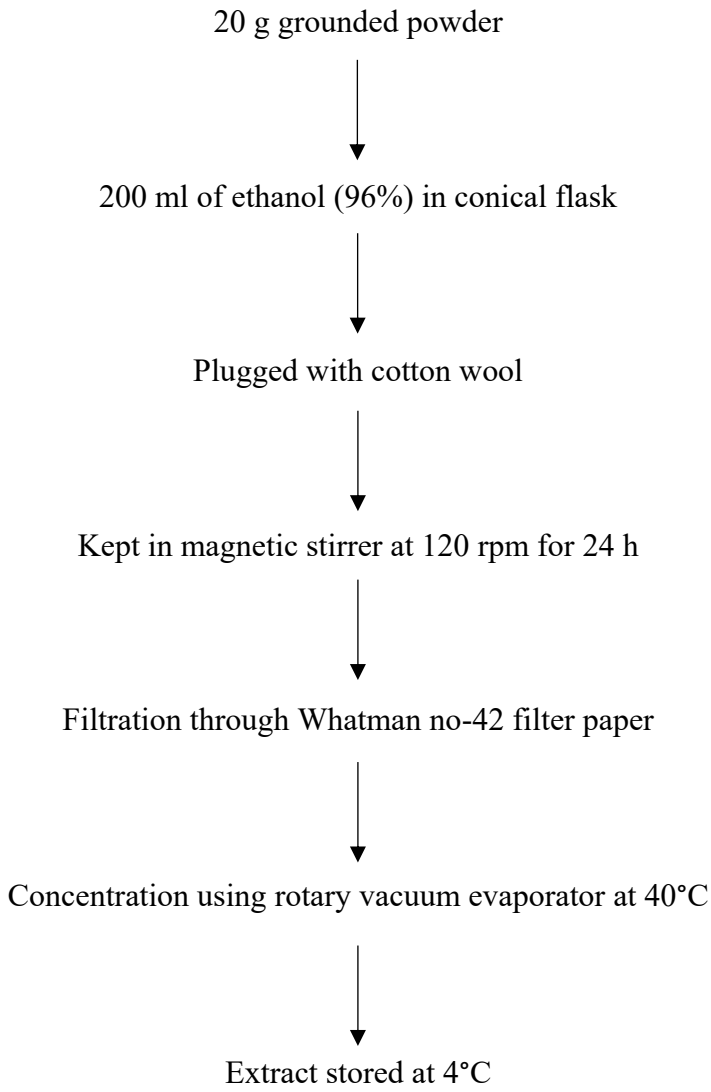

Fig. 3.1 Preparation of herb extract

Baljeet *et al.* (2015)

For concentration of herb extracts, rotary vacuum evaporator was used. During concentration, it was assumed that the solvent is only evaporated while the portion of herb in the extract remains unchanged. Thus, the concentration of herb extract concentrate was determined.

Amount of herb extracted in solvent = Wt. of dried herb taken for extraction – wt. of dried residue after extraction

$$\text{Concentration of concentrated herb extract} = \frac{\text{amount of herb extracted in solvent}}{\text{final weight of concentrated extract}}$$

3.3.2.2 Determination of total phenolic content

Total phenolic content of the clove and *pakhanbedh* extract was determined according to Genwali *et al.* (2013). For the standard curve, various concentrations of gallic acid solutions in methanol (10, 25, 50 and 75 µg/ml) were prepared. In a 20 ml test tube, 1 ml gallic acid of each concentration was added and to that 5 ml of Folin-Ciocalteau reagent (10%) and 4 ml of 7% Na_2CO_3 were added to get a total volume of 10 ml. The blue colored mixture was shaken well and incubated for 30 min at 40°C in a water bath. Then the absorbance was measured at 760 nm against blank.

Various concentrations of the extracts (25, 50, 100 and 200 µg/ml) were prepared. Following the procedure described for standard, absorbance for each concentration of extract was recorded. Total phenolics content of the extracts was expressed as mg gallic acid equivalents (GAE) per g of sample in dry weight (mg/100 g).

3.3.2.3 Determination of antioxidant activity by DPPH assay

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by (Blois, 1958). 0.1 mM stock solution of DPPH was prepared. The solution was further diluted to adjust the absorbance value of approximately 0.9 at the wavelength of λ=517 nm. The absorbance of DPPH radical solution (A_0, control) was measured by adding 2.5 ml working DPPH solution to 0.5 ml of 80% methanol.

Three different concentrations of methanolic solutions of each extracts were prepared by the serial dilution of the stock solution (10 mg/ml) of the respective extract. To each 0.5 ml extract solution (A_s Sample), 2.5 ml of 0.1 mM DPPH solution was added. These samples were shaken well and kept in dark for 30 min at room temperature. The absorbance was measured at 517 nm against the control solution. The radical scavenging activity was expressed as the radical scavenging percentage using the following equation:

Inhibition % = 100 $(A_0-A_s)/A_s$, where:
A_0 = average absorbance of the control (DPPH) solution
A_s = average absorbance of the sample

3.3.3 Proximate analysis of milk

Fat in milk was determined according to the Gerber method as per NDDB (2001). Protein in milk was determined by formal titration method and SNF in milk was determined as per

(NDDB, 2001). Moisture content in milk was determined by hot air oven method as per Ranganna (1986).

3.3.4 Preparation of paneer

Paneer was prepared in the laboratory using method described by De (1983) with slight modifications and the procedure is shown in Fig. 3.2.

Filtration of milk

↓

Heating to 95°C for 5 min

↓

Cooling to 70°C

↓

Addition of Coagulant– Citric acid (1 %) at 70°C

↓

Holding 5 min

↓

Drainage of whey

↓

Pressing

↓

Removal of paneer from block

↓

Chilling (4°C)

↓

Removal of paneer cubes from chilling water

↓

Allow to drain water (15 min)

↓

Packaging

↓

Refrigerated storage (5-7 °C)

Fig. 3.2 Preparation of paneer

De (1983)

Herb extract treatment of paneer

The different combinations of herb extract were either added directly to milk before coagulation or to the curd after coagulation. In the first type of treatment, milk was first heated to 95°C for 5 min and when cooled to a temperature of 80°C, the calculated herb extract combination was added to the milk and stirred well to distribute the extract uniformly throughout the milk. Then as the temperature dropped down to 70°C, coagulant was added and paneer was prepared by procedure as shown in Fig. 3.2.

Similarly, for second type of treatment, curd was first obtained in muslin cloth after coagulation. After almost 90% of whey was drained off, calculated amounts of herb extract combination were added to the curd. Then with the help of a spatula, the curd was mixed with the extract so as to distribute the extract as uniformly as possible. The herb extract treated curd was then allowed to drain more of the whey followed by pressing and chilling.

3.3.5 Analysis of paneer for proximate composition

3.3.5.1 Determination of Moisture

Moisture content in paneer was determined according to BIS (1983) procedure specified for paneer under IS: 10484.

About 3 g of paneer sample was weighed in a previously dried and tarred aluminum dish, mixed thoroughly with about 5 ml of water and placed on a boiling water bath for 25-30 min. The dish was then transferred to oven maintained at 102±1°C. After 4 h the dish was immediately transferred to desiccator. After cooling for about 30 min the dish was weighed. The process of heating, cooling and weighing was repeated until the loss of weight between two successive weighing was less than 1 mg.

Calculation of moisture content:

$$\text{Moisture (\% w/w)} = \frac{W_1 - W_2}{W_2 - W} \times 100$$

Where,

W = 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

3.3.5.2 Determination of Fat

Fat content in paneer was determined by Van Gulik method as given by NDDB (2001). 3 g grated paneer was taken in Van Gulik beaker placed on a rubber stopper and it was fixed firmly to Van Gulik butyrometer. About 15 ml sulfuric acid (sp. gr. 1.53-1.60) was added to the butyrometer and placed in water bath at 65°C followed by shaking. After the paneer dissolved in sulfuric acid, 1 ml amyl alcohol was added to the butyrometer and the liquid level in butyrometer was brought to about 35% mark by adding more sulfuric acid. The butyrometer was closed with small rubber stopper on the top and the contents were mixed thoroughly by inverting the butyrometer several times. The butyrometer was centrifuged in Gerber centrifuge for 5 min at around 1100-1200 rpm and placed in water bath at 65°C for 5 min. The reading was taken directly from the fat column of butyrometer.

3.3.5.3 Determination of protein

Protein content was determined by Kjeldahl method as described by Ranganna (1986) the conversion factor used is 6.38. Briefly, 5 g paneer sample was transferred quantitatively on Kjeldahl digestion flask followed by addition of catalyst mixture (1 g potassium sulfate and 0.2 g copper sulfate) and 25 ml concentrated sulfuric acid. Digestion was carried out in heating mantle for 3-4 h. After digestion completed, digest was transferred to a 100 ml volumetric flask and volume makeup was done with distilled water. Then 10 ml of this digested solution was transferred to previously rinsed distillation assembly followed by 20 ml of 50% sodium hydroxide solution. 10 ml of 4% boric acid was placed at the tip of the condenser so that the tip is immersed in boric acid solution in order to trap the ammonia produced. Then distillation was begun by heating the steam generator flask. After about 60-70 ml distillate was collected in the flask placed at the condenser tip, the tip was rinsed with 5 ml distilled water and the distillate was finally titrated against 0.02 N hydrochloric acid solution. The volume of hydrochloric acid consumed during titration was noted as V ml. Similarly, distillation followed by titration was conducted for blank digest as well by using 1 g sucrose instead of paneer. The volume of hydrochloric acid consumed during titration was noted as B ml. The total protein content was determined as

$$\text{Total protein \% in paneer} = \frac{14.007 \times (\text{V-B}) \times \text{N} \times 100 \times 100}{1000 \times 10 \times \text{W}} \times 6.38$$

Where,

V= volume of hydrochloric acid consumed by sample

B= volume of hydrochloric acid consumed by blank

N= normality of hydrochloric acid

W= weight of sample taken

3.3.5.4 Determination of lactose

Lactose content in paneer was estimated by difference. Supposing that lactose is the only carbohydrate present in milk, lactose content was determined by subtracting the sum of all other chemical constituents (moisture, fat, protein and ash) from 100%.

3.3.5.5 Determination of Ash

The ash content of the paneer sample was estimated by the method of Ranganna (1986). About 8-10 g grinded paneer sample was taken in a clean and dry silica crucible and burnt over bunsen flame to volatize as much organic matter as possible. Once smoke stopped to come out of the sample, the crucible was placed inside muffle furnace maintained at a temperature of around 500°C. Ashing was continued for 3-4 h and if some traces of black residues was noticed, 1-2 ml of conc. nitric acid was added to it and again put in muffle furnace until the ash turned completely white. Finally, the crucible was cooled in a dessicator and weighed.

$$\text{Total ash content in paneer (\% dry basis)} = \frac{\text{Ash (g)} \times 100 \times 100}{\text{Sample (g)} \times \text{Dry matter (\%)}}$$

3.3.6 Analysis of paneer for chemical characteristics during storage

The chemical changes taking place in the paneer are analyzed by using different methods as described in Section 3.3.6.1 to 3.3.6.4.

3.3.6.1 Determination of Acidity

The acidity of the paneer sample was estimated according to BIS (1983) procedure specified for paneer under IS: 10484.

About 2 g of the sample was weighed and ground with 3 ml of boiling water using pastel and mortar. The contents were transferred into a dish using 17 ml of boiling distilled water. However, the contents of dish were cooled to room temperature and 10 ml of 0.1 N NaOH and 1 ml phenolphthalein were added. The sample was titrated against 0.1 N HCl till disappearance of pink color.

Calculation of acidity:

$$\text{Acidity (\% lactic acid by weight)} = \frac{10\text{-V}}{\text{M}} \times 100\%$$

Where,

V = Volume in ml of standard acid used in titration

M = Mass in g of sample taken for the test.

3.3.6.2 Determination of Free Fatty Acid

The fat breakdown in paneer was determined by estimating free fatty acid (FFA) as % oleic acid by adopting procedure given by Thomas *et al.* (1954).

Extraction of free fatty acids was carried out from 5 g of sample once with a mixture of 4 ml ethanol, 7 ml diethyl ether and 10 ml petroleum ether (40-60°C) and then thrice with a mixture of 7 ml diethyl ether and 10 ml petroleum ether. The extracts were pooled in a conical flask and titrated with 0.02 N NaOH using 1 ml phenolphthalein as indicator (0.5% w/v).

Calculation of free fatty acids:

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

Where,

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

W = Weight in g of the sample taken

3.3.6.3 Determination of Tyrosine Content

Proteolytic changes in terms of tyrosine content in stored paneer samples were estimated by the procedure suggested by Hull (1947) with slight modification.

A known quantity of grated paneer sample (5 g) was accurately weighed and thoroughly mixed with 10 ml of warm (45ºC) distilled water. 5 ml of the suspension was pipetted into a clean and dry 50 ml conical flask. Then 10 ml of 0.72 N trichloroacetic acid (TCA) and 1 ml of distilled water were added. The mixture was then thoroughly shaken and allowed to stand for 30 min followed by filtration, using filter paper Whatman No. 42. 5 ml of this filtrate were pipetted into a dry 50 ml conical flask. To this were added 10 ml mixture of sodium carbonate and sodium polyphosphate solution (this solution was prepared by dissolving 75

g of sodium carbonate, and 10 g of sodium polyphosphate in 500 ml of distilled water). The mixture was then gently shaken and 3 ml of diluted Folin- reagent (1 part of Folin-Ciocalteau's reagent and 2 parts of distilled water v/v) were added with continuous shaking. A reagent blank was also prepared. The readings were taken after the lapse of 5 min at 650 nm in a spectrophotometer.

Preparation of standard curve of L-tyrosine

100 mg of L (-) tyrosine were dissolved in glass double distilled water and the volume was made up to 100 ml. One ml of this stock solution (Solution - I) contained 1 mg of tyrosine. Then 10 ml of Solution-I was diluted to 100 ml by using glass-distilled water. One ml of this solution (Solution-II) contained 0.1 mg of tyrosine. Now 20 ml of Solution-II was further diluted to 100 ml. 1 ml of this solution (Solution-III) contained 0.02 mg of tyrosine.

A known volume (corresponding to 0.02 mg to 0.42 mg of tyrosine) of Solutions-II and / or III was transferred into a series of 50 ml clean and dry conical flasks. The final volume in each flask was made to 6 ml using glass double distilled water. Then 10 ml of 0.72 N trichloroacetic acid (TCA) were added to each flask. 5 ml aliquots of the above mixture were taken out from every flask for color development and the readings were recorded as described earlier. A linear curve was obtained by plotting absorbance against concentration of tyrosine.

3.3.6.4 Determination of total plate count

Microbial count in paneer sample was determined by following method given by National Dairy Development Board (2001). Plate count agar used for microbial enumeration was prepared as per manufacturer's guidelines in distilled water and sterilized in an autoclave. Paneer test sample was prepared by putting 10 g smashed paneer samples in 90 ml of diluent (phosphate buffer adjusted to pH 7.5±0.1) and properly dispersed by using a shaker. Then series of dilutions were prepared from this primary dilution as per required. 1 ml of each of these dilutions was first placed in separate sterile petri plates. Then 12-15 ml of lukewarm plate count agar media was poured into each plate. The prepared plates were mixed properly and allowed to solidify by leaving to stand on a cool horizontal surface. The plates were incubated at a temperature of 30±1°C for 48 h in inverted position. The colonies were then counted by using colony counting equipment.

3.3.7 Sensory evaluation of paneer

Control paneer and herb extract incorporated paneer samples were subjected to sensory evaluation by 5 semi-trained panelists. 9 point hedonic rating test was used and a sensory evaluation card as shown in Appendix H was provided to the panelists. Each paneer sample was brought to room temperature and cut into cubes prior to evaluation. Each of the sample were analyzed for color and appearance, body and texture, flavor and overall acceptance on the 0^{th}, 5^{th}, 10^{th} and 15^{th} day of storage.

3.3.8 Statistical analysis

Analysis was carried out in triplicates and sensory analysis was done in 5 replications. Statistical calculations were performed in Microsoft Office Excel 2016. All the data obtained in this experiment was analyzed for significance test at 5% level of significance by two-way ANOVA using IBM SPSS statistics 20.

PART IV

Results and discussion

The results of the present study are presented and discussed, in this chapter.

4.1 Chemical composition of milk

The proximate composition of milk is shown in Table 4.1.

Table 4.1 Composition of milk used for paneer preparation.

Constituents	% (wb)
Moisture	88.260±0.290
Fat	2.933±0.058
SNF	7.988±0.020
Protein	2.892±0.087
Acidity (as % lactic acid)	0.144±0.009

4.2 Yield of Spice Extract

The yield of clove extract was found to be 39.29% and *pakhanbedh* extract was found to be 28.19%. Similar observation was reported for yield of clove extract by Ishaq *et al.* (2019) and by Chauhan *et al.* (2016) for *pakhanbedh*.

4.3 Estimation of total phenolic content of clove and *pakhanbedh* extract

The total phenolic content of the ethanolic extracts were calculated by using calibration curve (Fig. F.1 in Appendix F) obtained from the Gallic acid solutions ranging from 10 µg/ml to 75 µg/ml. Based on the equation obtained, total phenolic content was calculated and expressed in mg GAE/g of dried extract. The phenolic content is shown in Table 4.2.

The above data obtained for total phenolic content of the clove and *pakhanbedh* extract correlate with the data obtained by Turgay and Esen (2015) and Kanth *et al.* (2019).

4.4 Estimation of total radical scavenging activity

The DPPH assay measures the ability of the extract to donate hydrogen to the DPPH radical, resulting in bleaching of the DPPH solution. It is a method for determination of antioxidant activity. Both clove and *pakhanbedh* extract showed a high antioxidant activity expressed in

Table 4.2 Total phenolics of cloves and *pakhanbedh* extract

Concentration of extract (µg extract/ml)	Total phenolic content (mg GAE/g of dried extract)	
	Pakhanbedh	Cloves
25	1549.6123±2.54	692.043±5.44
50	1723.811±2.72	584.32±2.72
100	1251.2068±4.72	418.41±2.56
200	1327.532±2.72	388.57±4.72

the form of % of inhibition. The high amount to phenolic content contributed to the high antioxidant activity of the extracts. According to Genwali *et al.* (2013) the phenolic compounds are responsible for DPPH free radical scavenging of the extracts. The result is presented in Fig. 4.1.

All concentrations of both cloves and *pakhanbedh* extract showed high antioxidant activity. *Pakhanbedh* extract showed maximum inhibition of 89.7% and cloves extract showed maximum inhibition of 87.78%. Similar observations were made by Muhson and Mashkor (2015) for clove fruit extract and Zafar *et al.* (2019) for *pakhanbedh* extract.

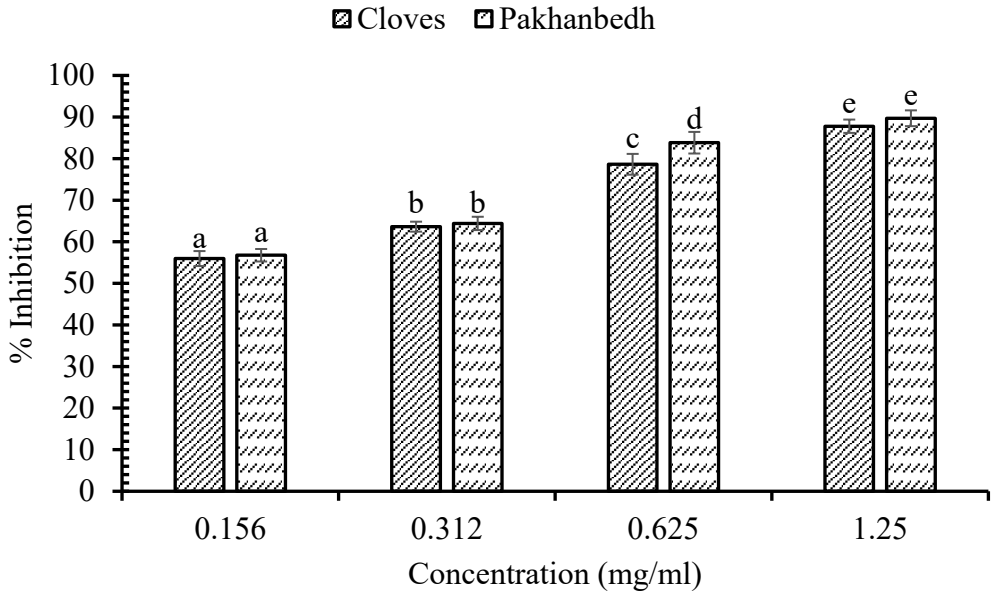

Fig. 4.1 DPPH radical scavenging activity% inhibition of clove and *pakhanbedh* extract

*Values with different superscripts are significantly different

Although not significantly different (P>0.05), *pakhanbedh* extract demonstrated relatively higher % inhibition as compared to cloves extract at 0.156, 0.312 and 1.25 mg/ml concentraction of extract. In contrast, *pakhabbedh* extract showed significantly higher (P<0.05) % inhibition at 0.625 mg/ml concentration of herb extract.

According to the results, clove and *pakhanbedh* extract showed higher phenolic compounds and higher antioxidant activity. It can be concluded that, both can be considered as an excellent source of antioxidant compounds.

4.5 Proximate composition of paneer

The proximate composition of prepared paneer is presented in Table 4.3.

Table 4.3 Proximate composition of paneer

Chemical parameter	Percentage (% wb)
Moisture	56.05±0.478
Fat	18.17±0.057
Protein	21.53±0.020
Ash	2.07±0.0577
Lactose	2.18±0.466
Acidity	0.423±0.006

*Values represent means± standard deviation.

Goel (2000) and Syed *et al.* (1992) also reported similar values as obtained in this study.

4.6 Yield of paneer

The % yield of paneer varied from 13.81% to 16.05 % with an average of 15.01 %. The result agrees with the observations of Kumar *et al.* (2019a). The yield of paneer samples is shown in Fig. 4.2.

Among the different samples, samples A-E represent the paneer samples where herbs extracts are directly treated on milk and samples F-J represent the samples where herb extract are treated on curd after coagulation and drainage of whey. Maximum samples showed higher yield than the control samples. This may be due to higher moisture retention by the herbal extract treated paneer samples. Moisture content of the control paneer was 56.64% whereas the moisture content of herb treated samples ranged from 55.10-58.85%, showing

samples where herbs extracts were treated directly on milk had higher moisture retention as well as higher yield. Natural crude polyphenols such as green tea, grape and cranberry extracts have been found to demonstrate strong protein-polyphenol interactions with calcium caseinate (Han *et al.*, 2019) and such interactions bring changes in functional properties of protein such as water absorption and binding (Ozdala *et al.*, 2013; Yildirim-Elikoglu and Erdem, 2017). This might be the reason for higher moisture retention in herb extract incorporated paneer. Badola *et al.* (2018) found similar trend in increase in moisture content of paneer samples when treated with cardamom and black pepper. Increase in moisture retention on adding banana pseudo stem juice as preservative in paneer was also observed by Ray (2008).

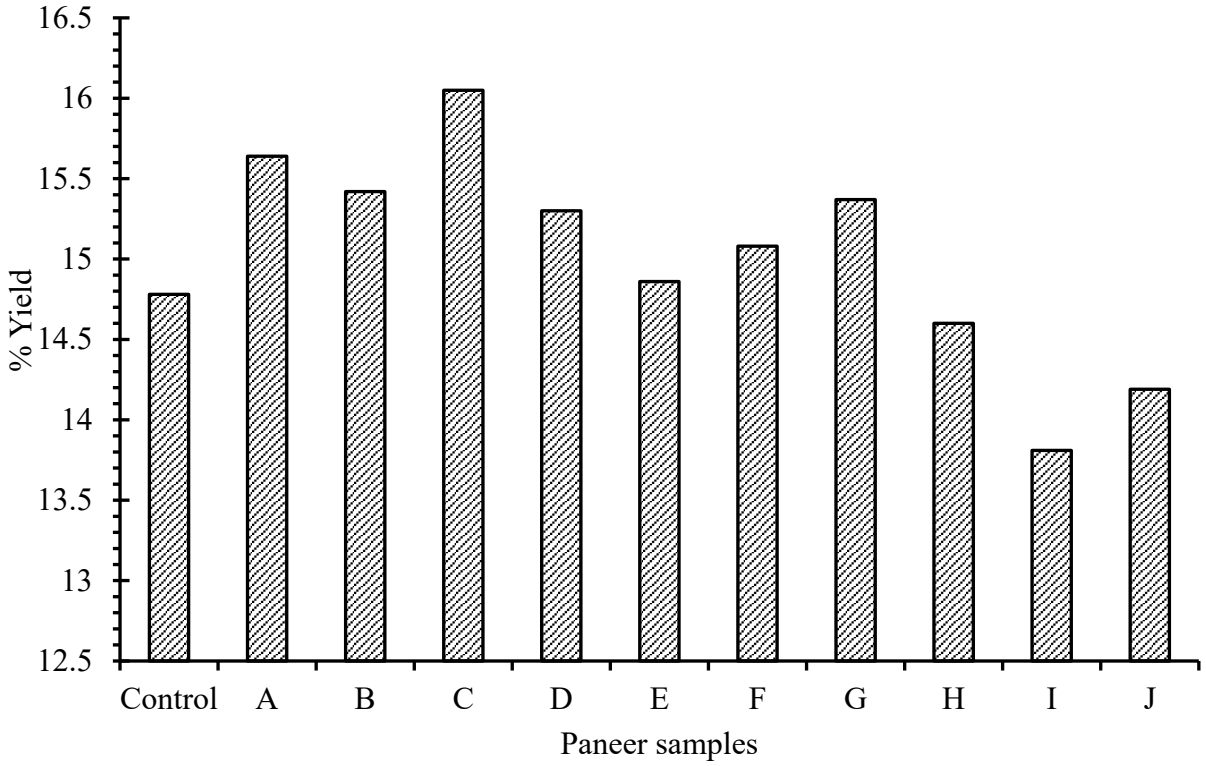

Fig. 4.2 % Yield of paneer samples

*Samples A-E represent the paneer samples where herbs extracts are directly treated on milk and samples F-J represent the samples where herb extract are treated on curd after coagulation and drainage of whey. A, F: 0.6% PE+0% CE, B, G: 0.45% PE+0.15% CE, C, H: 0.3% PE+0.3% CE, D, I: 0.15% PE+0.45% CE and E, J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

4.7 Effect of addition of clove and *pakhanbedh* extract and the stage of addition of the extract on the sensory attributes of paneer during storage

The sensory quality of product decides acceptance or rejection of the product for consumption. These qualities include flavor, body and texture, color and appearance and overall acceptability. The paneer samples were evaluated for the sensory quality. The results of sensory parameters of the paneer samples without addition of herbal preservatives as well as addition of herbal preservatives at two different stage of processing are discussed in subsection 4.7.1 to 4.7.4.

4.7.1 Color and appearance

The effect of storage on color and appearance of paneer samples on treatment with different proportions of clove and *pakhanbedh* extract mixture added directly on milk after heat treatment and in curd after coagulation are presented in Appendix A and B and trend is depicted in the Fig. 4.3 and Fig. 4.4 respectively.

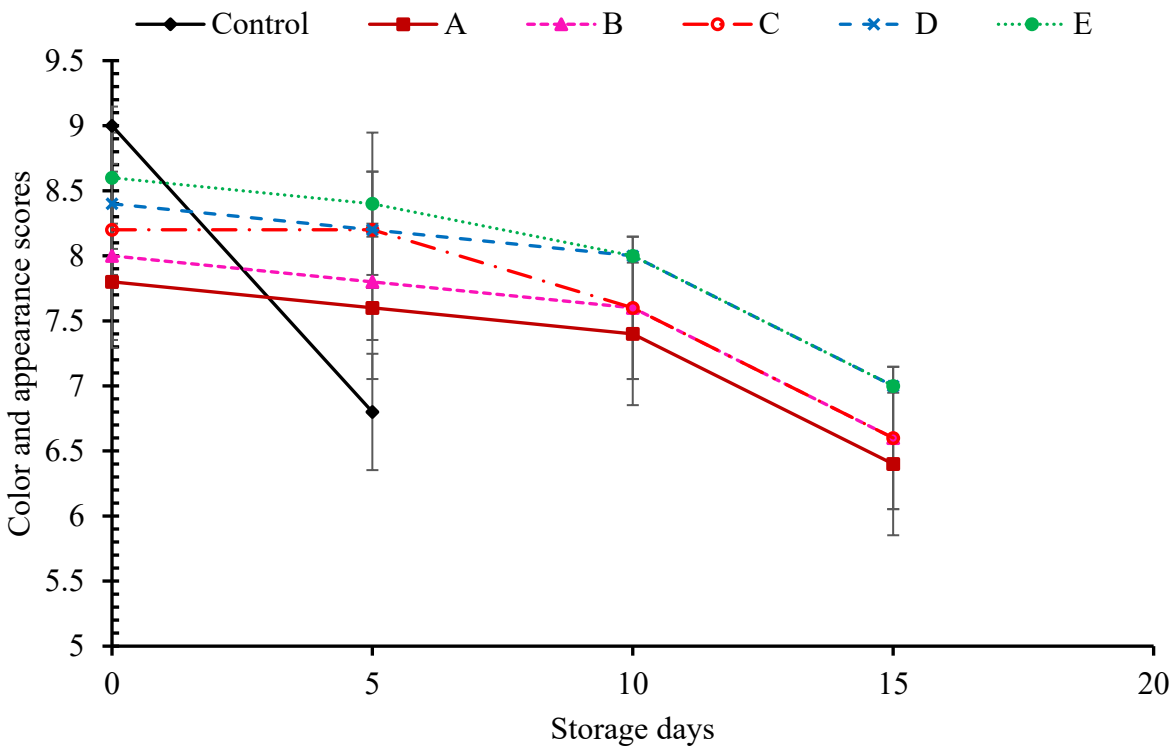

Fig. 4.3 Effect of herbal extract on color and appearance of paneer (added on milk)

*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

Fig. 4.3 and 4.4 indicated that a decreasing trend on appearance and color scores of stored paneer samples were seen with the increase in storage period. The storage period and addition of clove and *pakhanbedh* extract significantly (P<0.05) affected the color and appearance scores of paneer samples. The scores of control sample decreased significantly from an initial 9 to 6.8 in 5th day of storage. Whereas the decrease in the color and appearance scores of the herbs extract treated samples on milk were not significant (P<0.05) till 10th day of storage and the scores decreased significantly on the 15th day of storage.

The score for color and appearance of samples of paneer with herbal extract declined at almost with same trend during the storage period. However, amongst all the treated samples of paneer sample D and E showed minimum rate of decrease in score of color and appearance. The color and appearance score of all the samples of paneer treated with herbal preservatives remained within acceptable level throughout the storage period.

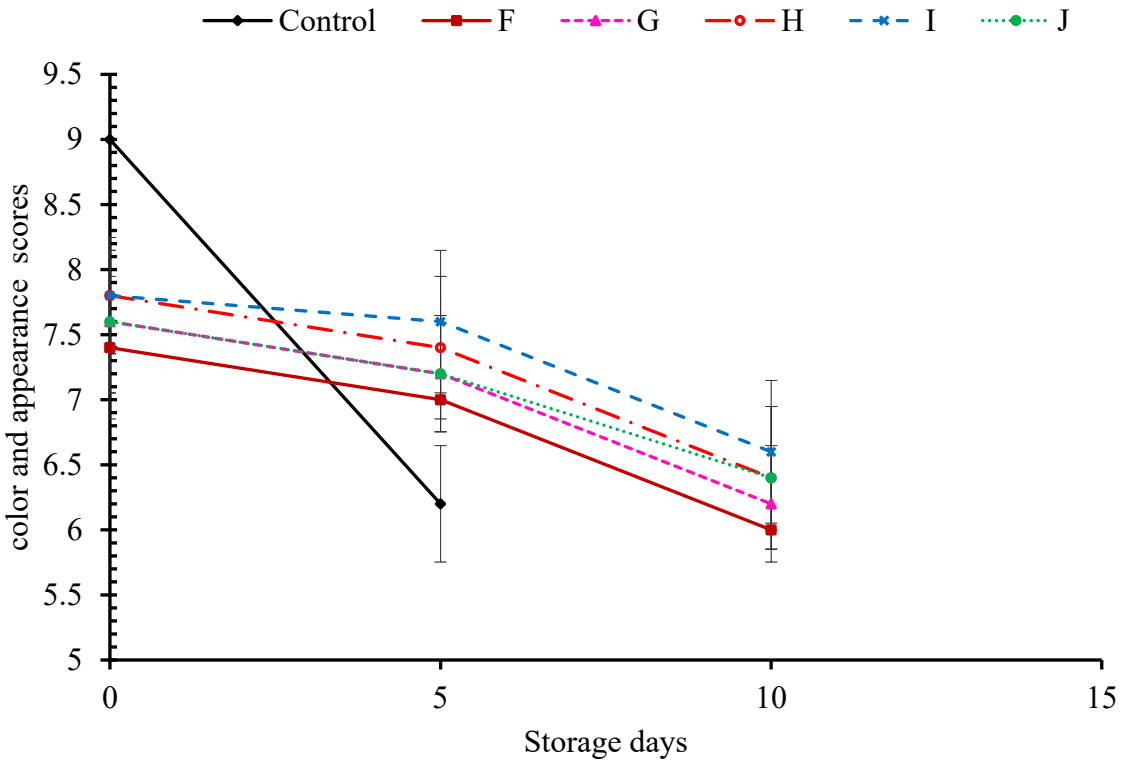

Fig. 4.4 Effect of herbal extract on color and appearance of paneer (added on curd)

*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

In case of paneer samples where herbal preservatives were added after coagulation on curd the initial color scores were lower than that of the samples where herbal preservatives were added directly on milk. This is due to the uneven mixing of the extract in the paneer samples. There was decreasing trend on the color and appearance scores of paneer samples significantly ($P<0.05$) on 10^{th} day of storage. The decrease in appearance score of herbs extract treated paneer sample was comparatively with slower extent as compared to control sample that could be mostly due to antimicrobial action of components of clove and *pakhanbedh* extract that inhibit other biochemical reaction resultant in deterioration of product. Similar trend in decrease of the color and appearance score of paneer samples on treatment with cinnamon was observed by (Khatkar *et al.*, 2017b).

4.7.2 Flavor

The data obtained for change in flavor scores of paneer samples during storage are presented in Appendix A and B and the trend is depicted in Fig. 4.5 and Fig. 4.6.

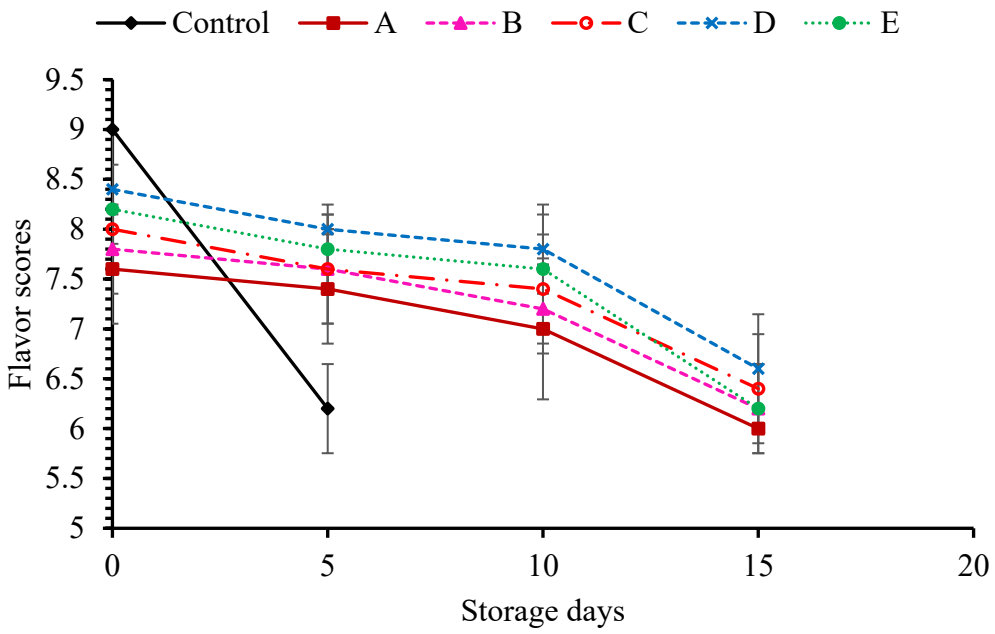

Fig. 4.5 Effect of herbal extract on the flavor scores of paneer samples (added on milk)
*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

Storage days and addition of herbal extract showed significant ($P<0.05$) effect in the flavor scores of all paneer samples. The flavor score of control paneer decreased significantly

from an initial 9 to 6.2 at 5th day of storage and the control paneer became unacceptable after 5th day of storage due to visible mold growth. The samples of paneer treated with herbal extract showed a lower decrease in flavor scores than that of the control samples. Similar observations were made in other studies (Buch *et al.*, 2014; Eresam *et al.*, 2015; Khatkar *et al.*, 2017b).

The paneer samples where herbal extracts were added directly on milk showed decline in flavor scores sharply after 10th day of storage and became unacceptable after 15th day of storage. Among all the herbs extract treated samples, sample D showed the minimum decrease in flavor scores.

Samples treated with individual herbal extract showed maximum decrease in the flavor scores in comparison of the mixture treated samples. This may be due to the combined inhibitory effect of the clove and *pakhanbedh* on microbial growth. Similar trend in the flavor scores were seen in the samples where herbal extracts were added after coagulation in curd. In this stage of addition, the flavor scores decreased initially as compared to addition on directly on milk due to the raw and astringent flavor of the clove and *pakhanbedh* extract.

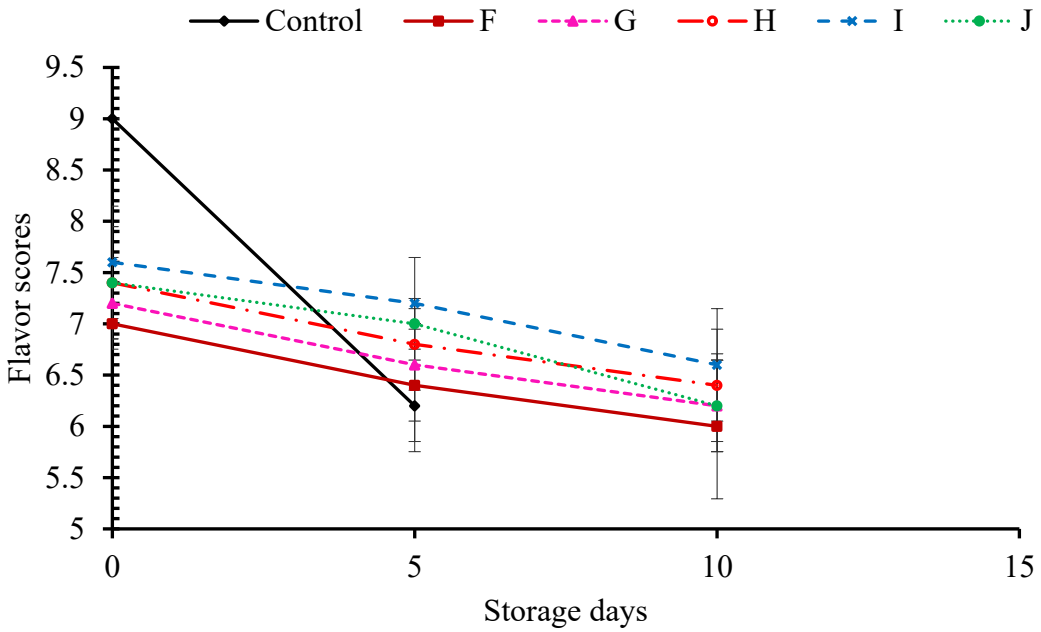

Fig. 4.6 Effect of herbal extract on the flavor scores of paneer samples (added on curd)
*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

Among the curd treated samples the flavor scores of the paneer samples F and G decreased significantly on 5th day of storage and other samples showed significant decrease on flavor scores on day 10th. Among the samples, sample I showed minimum decrease in flavor scores. Off flavors and slime formation was observed after 10th day and 15th day of storage in samples treated with herb extracts in milk before coagulation and in samples treated with herb extracts in curd after coagulation respectively.

4.7.3 Body and Texture

The data obtained for change in Body and Texture scores of paneer samples during storage are represented in Appendix A and B and the trend is depicted in Fig. 4.7 and Fig. 4.8.

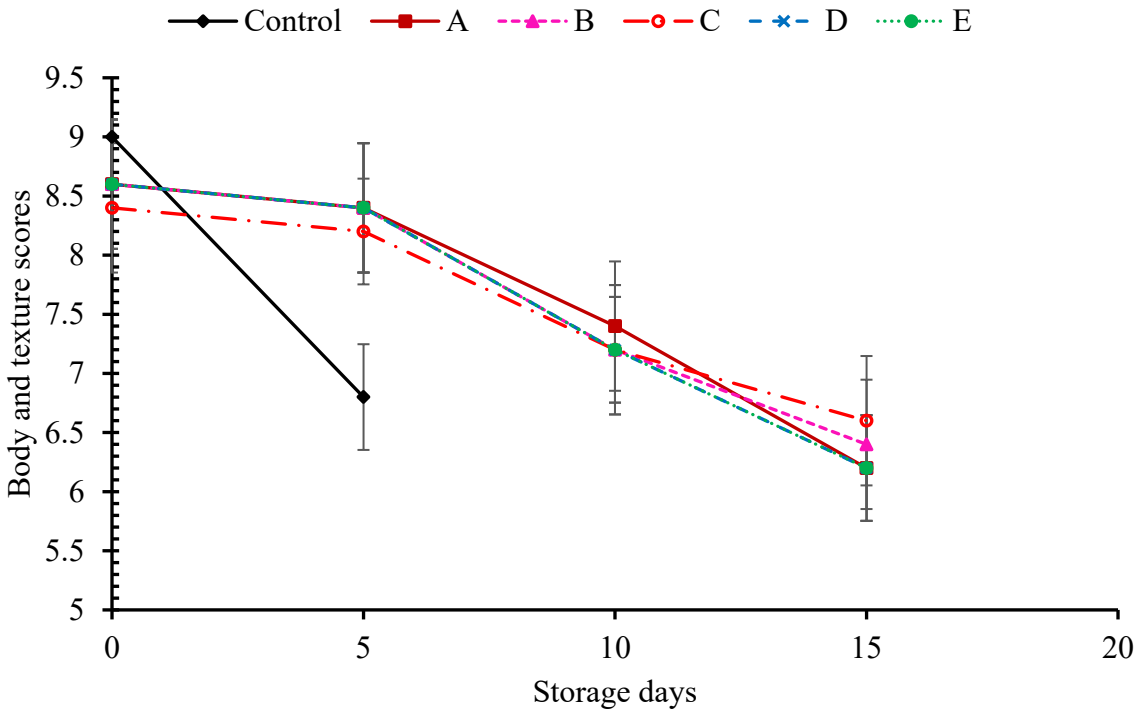

Fig. 4.7 Effect of herbal extract on the body and texture scores of paneer samples (added on milk)

*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

The results indicated storage days had significant ($P<0.05$) effect on the body and texture of paneer samples. Addition of herbal extract had significant ($P<0.05$) on the body and texture of paneer samples. The body and texture scores of samples where herbal extracts

were added directly on milk were slightly lower than control due to high moisture retention. In case of fresh curd treated samples, the scores were lower as compared to the control and milk treated samples due to the disturbance in the curd while mixing of herbal extract. Also, the addition of all the herbal extracts found to reduce score of the body and texture of all sample of paneer. This effect may be due to interference of particles of herb extracts in development of body and texture of the paneer.

The control samples of paneer showed decreasing trend in score of body and texture on 5th day of storage and later it become unacceptable due to yeast and mold growth. 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.

In milk treated samples the body and texture scores showed acceptable level till 15th days of storage. Sample C and B showed minimum decrease in the body and texture scores. Among the curd treated paneer samples scores remained acceptable till 10th days of storage, sample I showing the minimum decrease and slime was observed on all the samples afterwards.

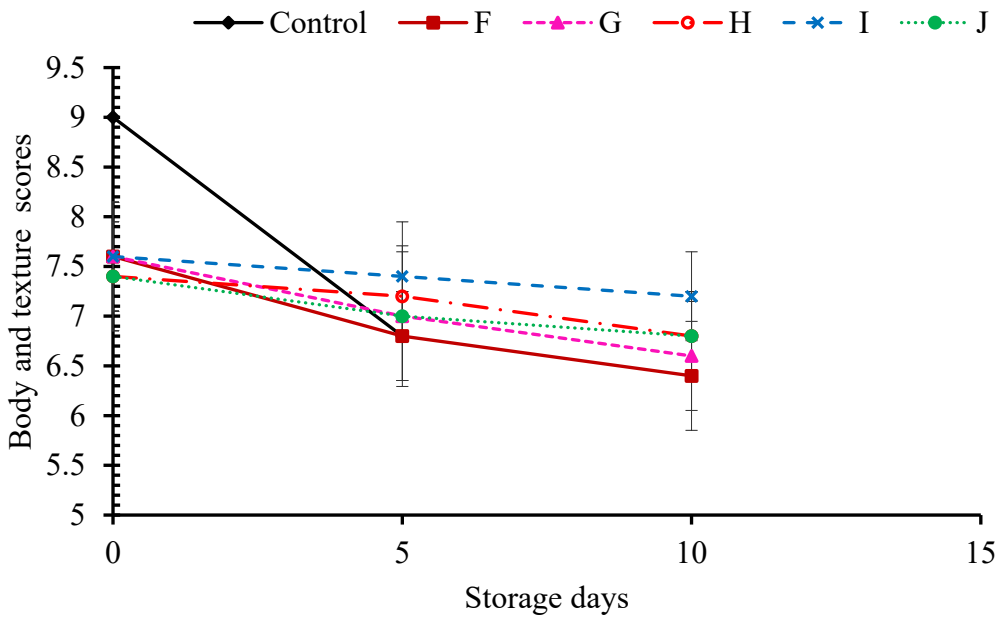

Fig. 4.8 Effect of herbal extract on the body and texture scores of paneer samples (added on curd)

*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

4.7.4 Overall acceptability

The data obtained for change in Overall acceptability scores of paneer samples during storage are represented in Appendix A and B and the trend is depicted in Fig. 4.9 and Fig. 4.10.

The result indicated storage period and addition of herbs extract significantly ($P<0.05$) affected the overall acceptability of the samples. The overall acceptability of control and herb extract treated paneer samples added on both stages showed a decreasing trend on storage.

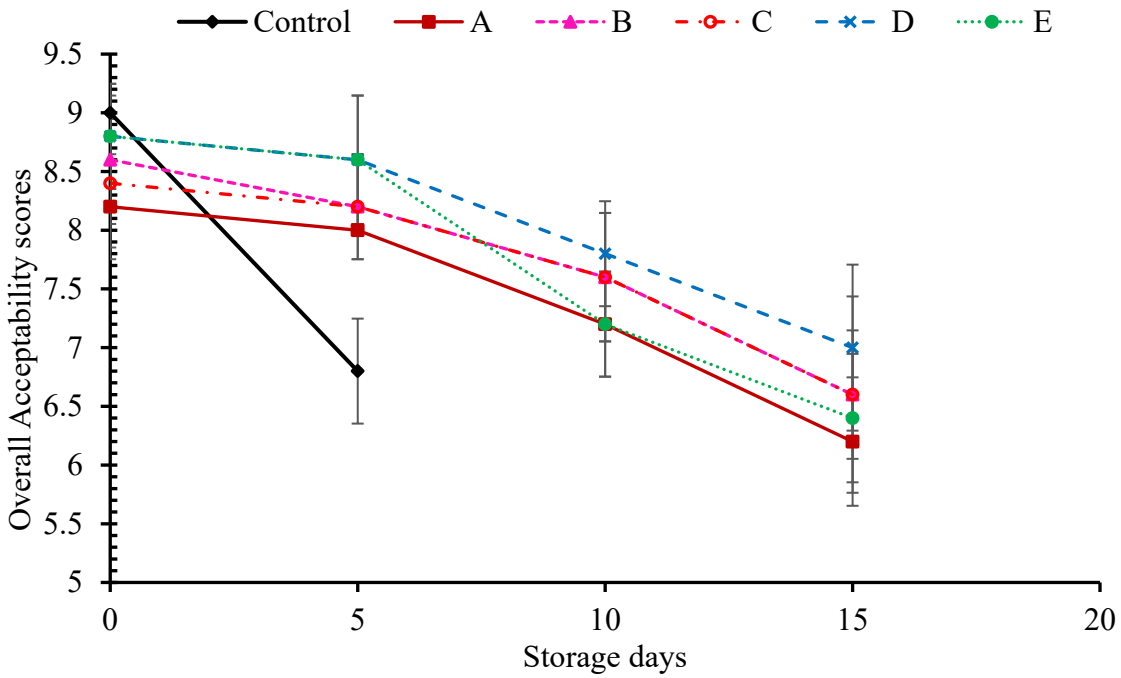

Fig. 4.9 Effect of herbal extract on overall acceptability of paneer samples (added on milk)
*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

The scores for control sample decreased rapidly from an initial 9 to 6.8 at 5th day of storage and later the control sample became unacceptable due to surface mold growth. For samples added with herb extracts on milk, overall acceptability scores didn't change significantly upto 5th day of storage. Whereas the scores decreased rapidly after 10th day of storage but the scores were within acceptable limits till the 15th day of storage with sample D showing the minimum decrease. All the samples became unacceptable afterwards due to

surface slime formation and mold growth. The overall acceptability of the stored paneer depends upon several factors like degree of proteolysis, lipolysis, flavor changes and microbial activity. The overall acceptability was found to be better for the samples in which the proteolysis, lipolysis, flavor changes and microbial growth had been lesser i.e. sample D> sample C >sample B > sample E > sample A respectively.

In curd treated samples the overall acceptability score was initially lower as compared to control and milk treated samples due to the effect of herbal extract addition in other sensory scores. Later, on storage, all the samples showed a decreasing trend in the overall acceptability and after 10th day with sample I showing minimum decrease in score and all samples became unacceptable due to the slime formation and mold growth.

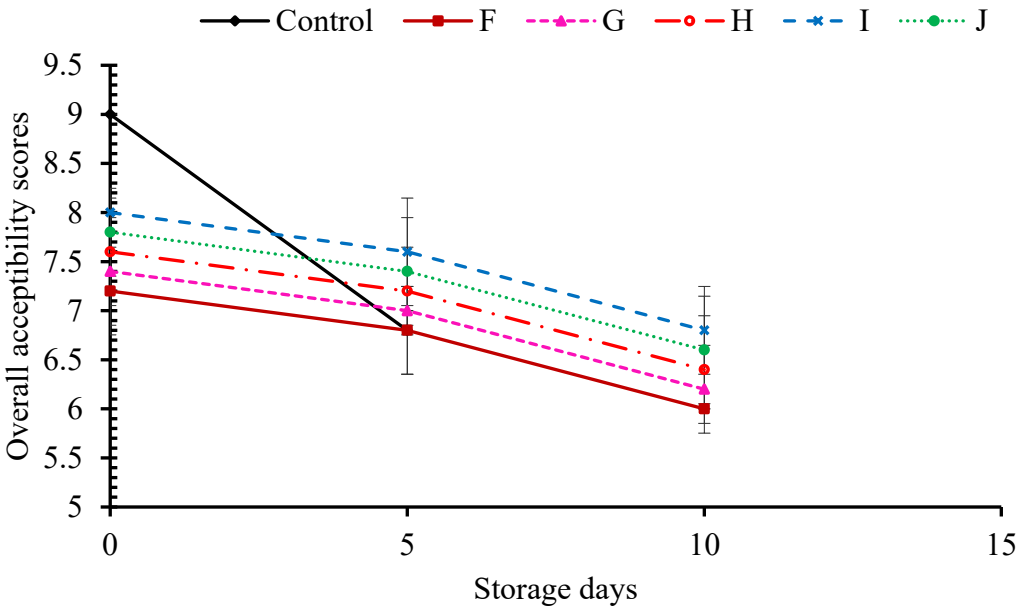

Fig. 4.10 Effect of herbal extract on overall acceptability of paneer samples (added on curd) *F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

According to the result all the combinations of herbal extract tended to maintain the sensory scores of the paneer samples at both stage of addition in comparison to the control sample which showed acceptability only up to 5th day. The mixture of clove and *pakhanbedh* extracts showed more control over the sensory changes than those of the individual extracts used.

4.8 Effect of clove and *pakhanbedh* extract concentration and stage of addition of the extract on the chemical characteristics of paneer during storage

4.8.1 Acidity

The data obtained for change in acidity of paneer samples during storage are represented in Appendix C and D and the trend is depicted in Fig. 4.11 and Fig. 4.12.

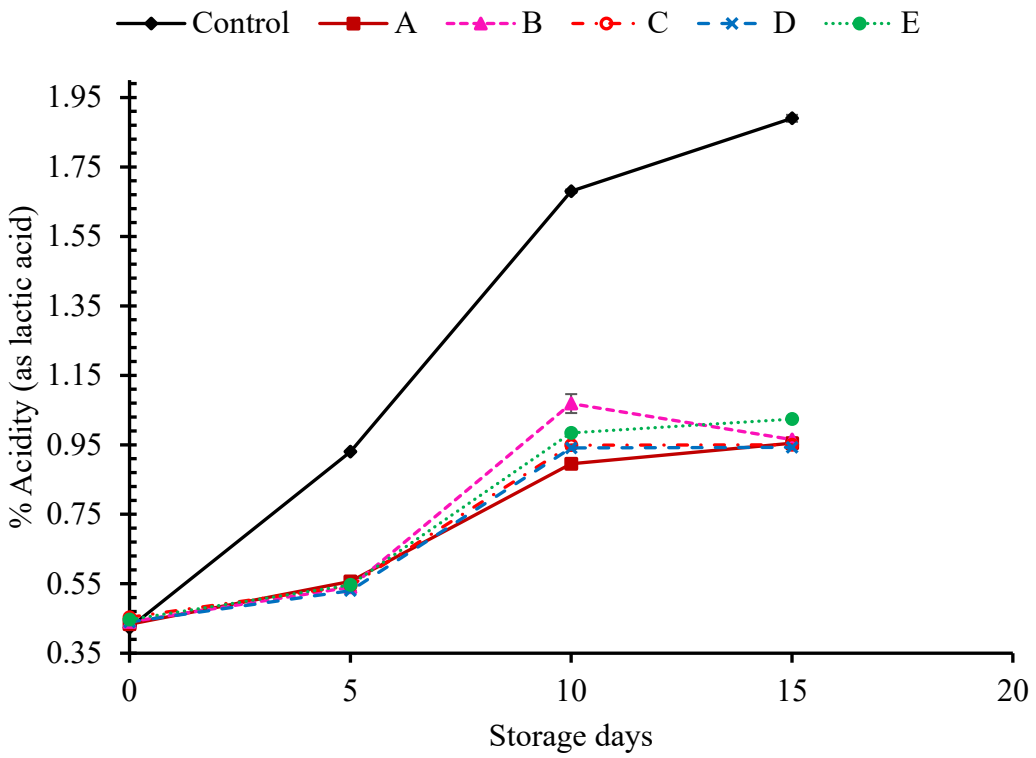

Fig. 4.11 Effect of herbal extract on acidity of paneer samples (added on milk)

*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

The titratable acidity is one of the indications of the microbiological activity in paneer. It is also a determinant of sensory quality of the product during storage. Storage days and addition of herbal extracts significantly ($P<0.05$) affected the acidity of paneer samples.

The average acidity (as % lactic acid) of the fresh control samples of paneer was 0.426% (as lactic acid).The acidity of paneer was reported to vary from 0.20 to 1.17 % (Sachdeva and Singh, 1990b). The initial average acidity of all herb extract treated paneer samples

ranged from an initial 0.43-0.46% (Lactic acid). The acidity of herbs treated samples were slightly higher than that of control sample which may be due to the acidic nature of cloves. Similar results related to the effect of clove acidic nature on paneer acidity was found by (Eresam *et al.*, 2015).

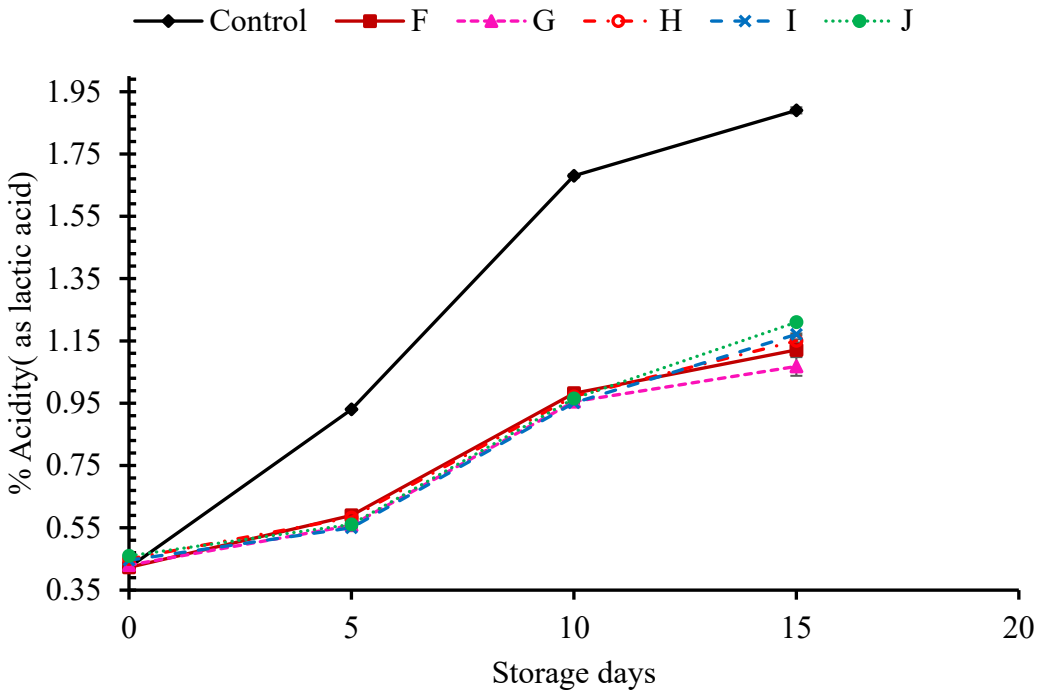

Fig. 4.12 Effect of herbal extract on acidity of paneer samples (added on curd)
*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

The acidity of herb extract treated samples was found to increase significantly on storage but at a significantly lower rate in comparison to control paneer. Similar trends in change in acidity of paneer samples during storage were noted by Gohian (1996) and Gokhale *et al.* (2016). In contrast to these observations, acidity of sample B in which herb extract was added to milk before coagulation decreased after the 10th day of storage. According to Sachdeva and Singh (1990b), the cycle of increase and decrease in acidity value of paneer during storage is due to production and subsequent utilization of some of the acidic and or basic compounds as a result of microbial activity. Such cyclic trend has been observed in acidity value of the paneer during storage in this study.

For paneer samples in which herb extracts was added to milk before coagulation, sample D showed the minimum rise in the acidity value till 15th day of storage. After 15th day of

storage all the samples showed slime formation and visible mold growth in the surface and were unfit for consumption.

Paneer samples in which herbs extracts was added to curd after coagulation were found to be acceptable only up to 10^{th} day of storage. Within this storage period, minimum rise in acidity was observed in sample I. On 15^{th} day of storage, acidity of samples, in which herb extract was added to curd, was found to be higher than the samples in which herb extract was added to milk before coagulation. It may be due to higher microbial load in samples where herb extract was added to curd after coagulation than in samples where herb extract was added to milk before coagulation which is shown in Appendix C and D. In addition to this, as mentioned earlier, milk proteins have high affinity towards polyphenols leading to protein-polyphenol interactions and this interaction in affected by temperature and pH (Yildirim-Elikoglu and Erdem, 2017). Although no literature has clearly mentioned the effect of pH and temperature on interaction of cloves and *pakhanbedh* extracts with milk proteins, addition of herb extracts directly to milk before coagulation might have resulted in higher retention of herb extracts in paneer which eventually showed better antimicrobial activity and thus lesser rate of increase in acidity as compared to paneer where herb extracts were added to curd. Similarly, addition of herb extracts in milk resulted in more uniform distribution of extracts in paneer samples in comparison to the samples where extracts were added to curd, due to which the herbs might have been more effective in the former paneer samples.

4.8.2 Free fatty acid

The data obtained for change in free fatty acid of paneer samples during storage are represented in Appendix C and D and the trend is depicted in Fig. 4.13and Fig. 4.14.

Although the initial FFA values for control as well as herb extract treated samples was similar just after preparation, the values for control paneer rose at a significantly ($P<0.05$) higher rate than in herb extract incorporated samples. It may be due to antimicrobial (which reduced the microbial load in paneer) and antioxidant property of herb extract. The FFA of all samples was significantly affected by the storage days ($P<0.05$). Similar observations were reported by Eresam *et al.* (2015) and Rajarshibhai (2012) in which addition of cloves oil caused an increase in FFA of paneer at a slower rate as compared to control paneer. No works related to use of *pakhanbedh* in food products for preservative purpose was found.

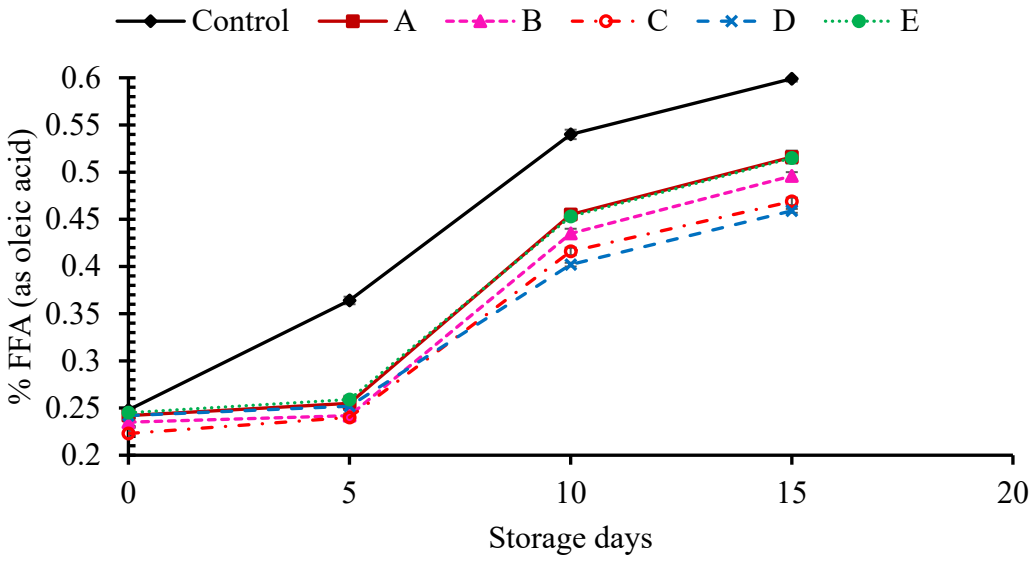

Fig. 4.13 Effect of herb extract in FFA content of paneer samples (added in milk)

*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

Concentration of herb extract was found to affect the FFA content significantly ($P<0.05$) over the storage period. For paneer samples in which herb extract was added to milk before coagulation, very slow rise in FFA up to 5th day of storage was observed, followed by a rapid increase on the 10th day and finally a slower rate up to the 15th day. This initial slow rise in FFA in comparison to control sample is due to reduction in lipid oxidation by the antioxidant and antimicrobial activity of the herb extracts. The least increase in FFA was observed for sample D till the 15th day of storage. On the 15th day, FFA content of paneer samples where herbs were added to milk was found to be lower than the samples where herbs were added to curd. This might be because of higher herb extract retention due to better protein-polyphenol interactions and more homogenous mixing of the herbs in the former than the latter.

Trend similar to the samples in which herb extract was added to milk was also found for samples in which herb extract was added to curd and least increase in FFA was also observed for sample I till 10th day of storage. All the samples were found to unsuitable for consumption after 10th day of storage.

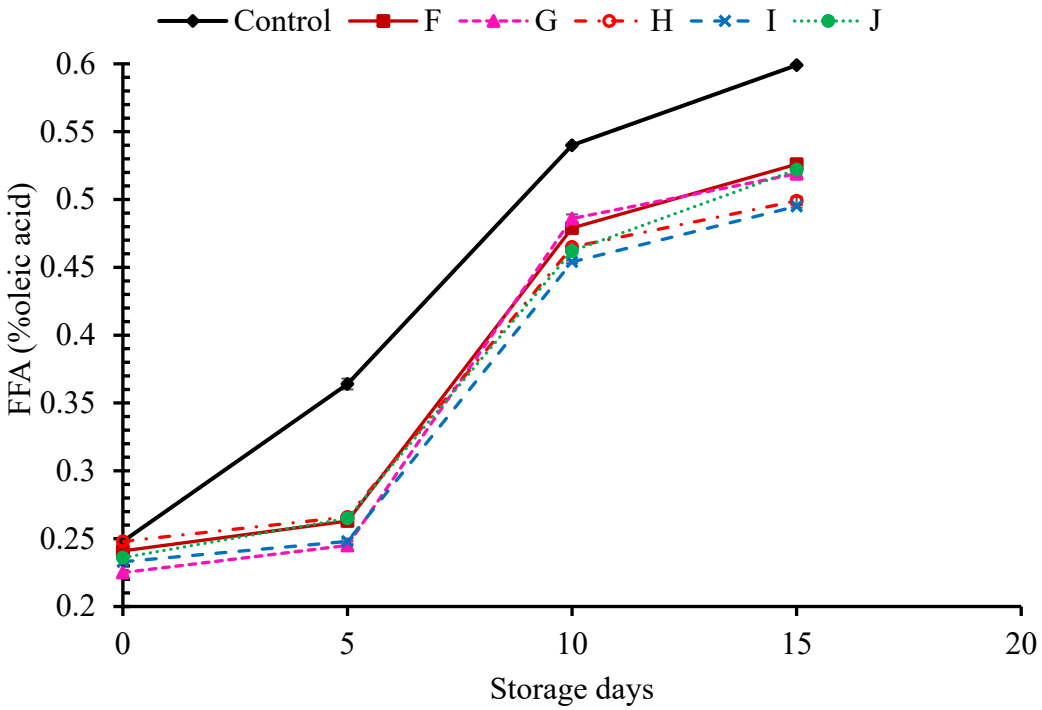

Fig. 4.14 Effect of herb extract in FFA content of paneer samples (added in curd)

*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

4.8.3 Tyrosine content

The data obtained for change in tyrosine content of paneer samples during storage are represented in Appendix C and D and the trend is depicted in Fig. 4.15 and Fig. 4.16.

Tyrosine content is an indication of the extent of proteolysis in paneer. Although the initial values for control as well as herb extract treated samples is similar, the tyrosine content of control paneer was found to increase significantly at a higher rate during the storage period. It indicates slower rate of proteolysis in herb extract treated samples for both stages of addition due to antimicrobial property of the herbs.

For paneer samples in which herb extract was added to milk before coagulation, different concentrations of herb extract had a significant effect ($P<0.05$) on FFA of paneer during the entire storage period. Least increase in tyrosine content was observed from an initial 10 mg/100 g to 27.57 mg/100 g for sample D while the highest increase was observed from an initial 10.043 mg/100 g to 42.568 mg/100 g for sample E. Similar observations for increase in tyrosine content on storage was obtained by Khatkar *et al.* (2017b).

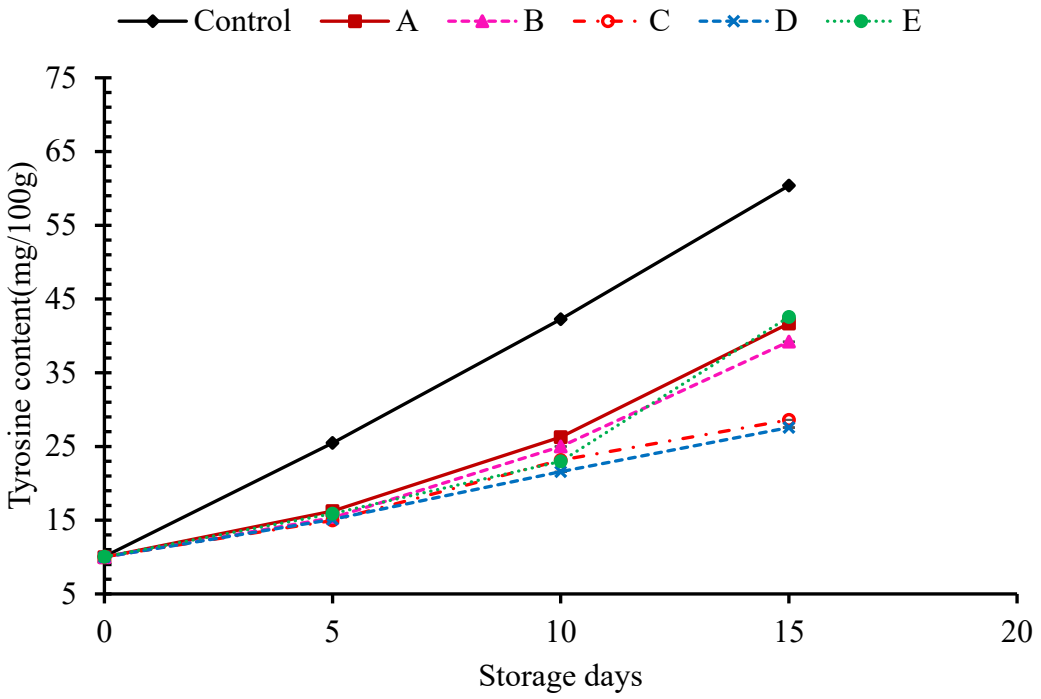

Fig. 4.15 Effect of herb extract in tyrosine content of paneer samples (added in milk)
*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

Trend, similar to that of the samples in which herb extracts was added to milk before coagulation, was observed in tyrosine content of paneer samples in which herb extracts was added to curd after coagulation. Throughout the entire storage period, sample D exhibited minimum increase in tyrosine content which indicate least proteolytic activity in the sample D. After each storage period, the tyrosine content of samples in which herb extract was added to curd after coagulation was found to be higher than samples in which herb extract was added to milk before coagulation. It indicates better preservative action of herbs when added to milk than to curd. Although no literature has been mentioned, more of the herb extract might have interacted with milk proteins when added to milk prior to coagulation due to the effect of pH and temperature leading to better retention of the extracts in paneer while only smaller quantity of herb might have retained in paneer when added directly to curd. No data have been found on the effect of stage of addition on the effectiveness herbal extract in paneer. However, similar observations have been made by Rajkumar *et al.* (2010) on use of nisin as preservative in paneer where it was found that addition of nisin was more effective in extending shelf life when added directly in milk prior to heat treatment.

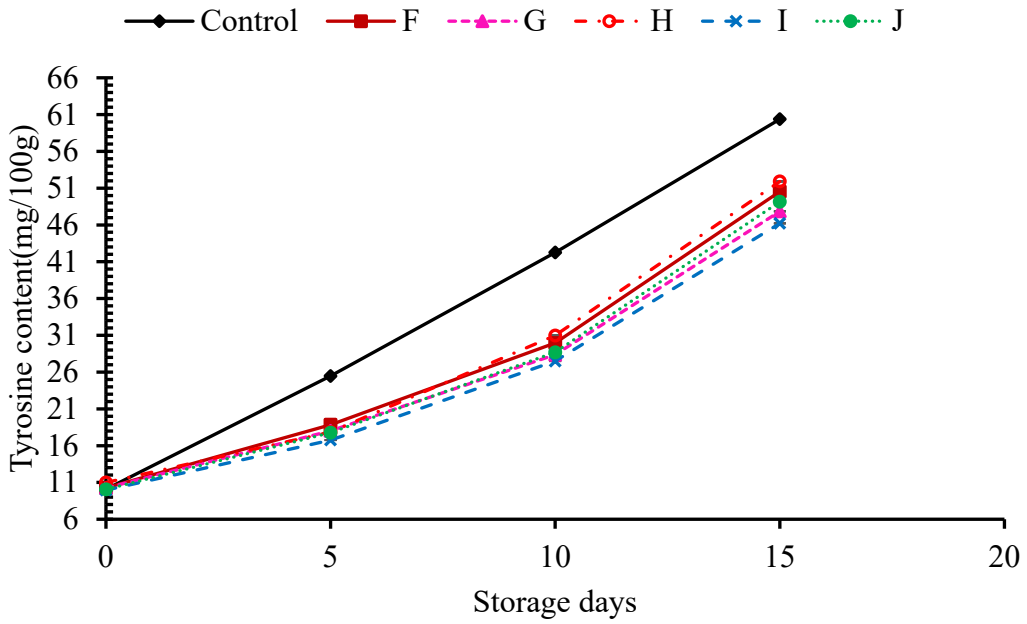

Fig. 4.16 Effect of herb extract in tyrosine content of paneer samples (added in curd)
*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

4.8.4 Total plate count

The data obtained for change in total plate count of paneer samples during storage are represented in Appendix C and D and the trend is depicted in Fig. 4.17 and Fig. 4.18.

The paneer contain moisture, which favor the growth of microorganism. The major spoilage of paneer is only due to the growth of microbes. Hence, the SPC of paneer samples were studied. The study shows that the storage days has significant ($P<0.05$) effect on the standard plate count of the paneer samples of both control and herbal extract treated samples at both stages.

The microbial counts of the control sample increased rapidly from an initial 1.5×10^3 cfu/g to 1.25×10^4 cfu/g on 5^{th} day of storage. Later control sample became unacceptable due to visible mold growth. Whereas, samples treated with herbs extract directly on milk showed slower increase in microbial count in comparison to control sample till 5^{th} day of storage. This may be due to the antimicrobial activity of the clove and *pakhanbedh* extract. 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. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death of the microbes.

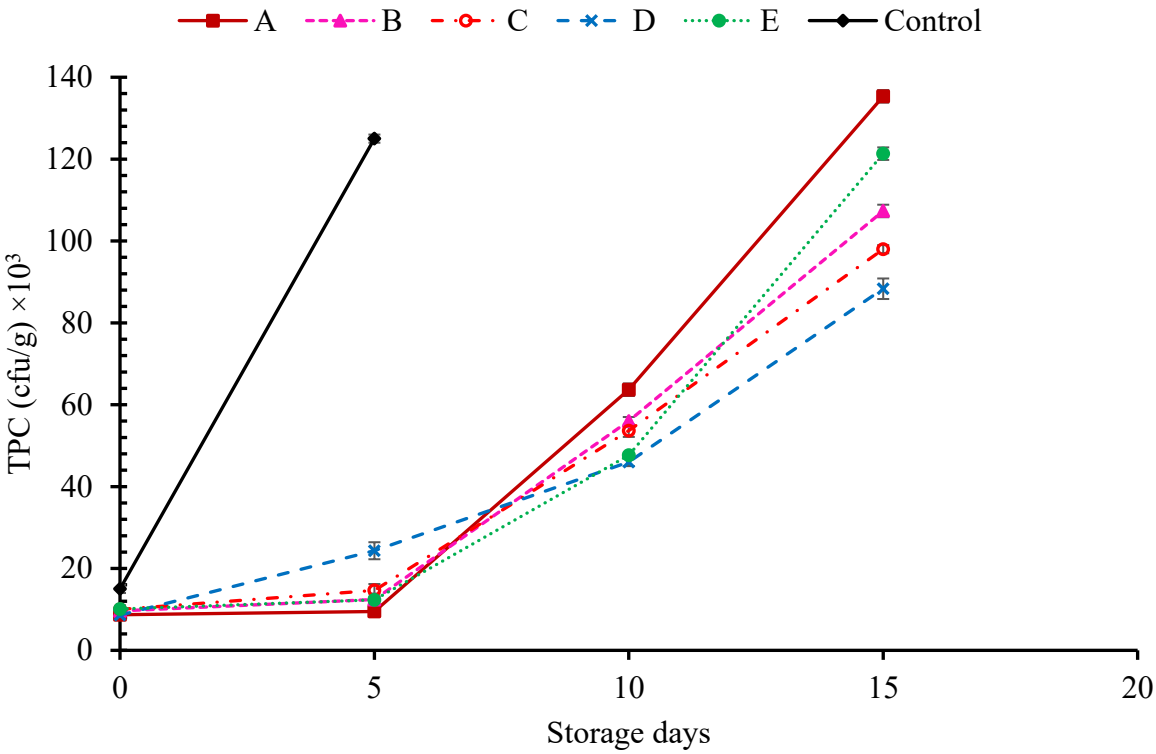

Fig.4.17 Effect of herbal extract on SPC of paneer samples (added on milk)

*A: 0.6% PE+0% CE, B: 0.45% PE+0.15% CE, C: 0.3% PE+0.3% CE, D: 0.15% PE+0.45% CE and E: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

The result showed that the concentration of herbal extract had significant ($P<0.05$) effect on the total plate counts of the paneer samples during storage days. Among the herbal extract treated samples, sample C and D showed minimum increase in the microbial counts. Combination of clove and *pakhanbedh* extract mixture as preservative proved to be effective in controlling the microbial growth in comparison to the individual herbs used. Microbial count of paneer until 15th day of storage remained within the acceptable limit and after 15th day of storage slime development and mold growth was visible on the samples stored at 7±1°C. Similar trend of increase in microbial count in stored sample paneer was noted by (Shanaziya *et al.*, 2018). Gupta (1985) studied the total bacterial load in paneer and it was found that the standard plate counts of fresh sample of market paneer ranged from 2.5×10^3 to 3.5×10^5 cfu/g.

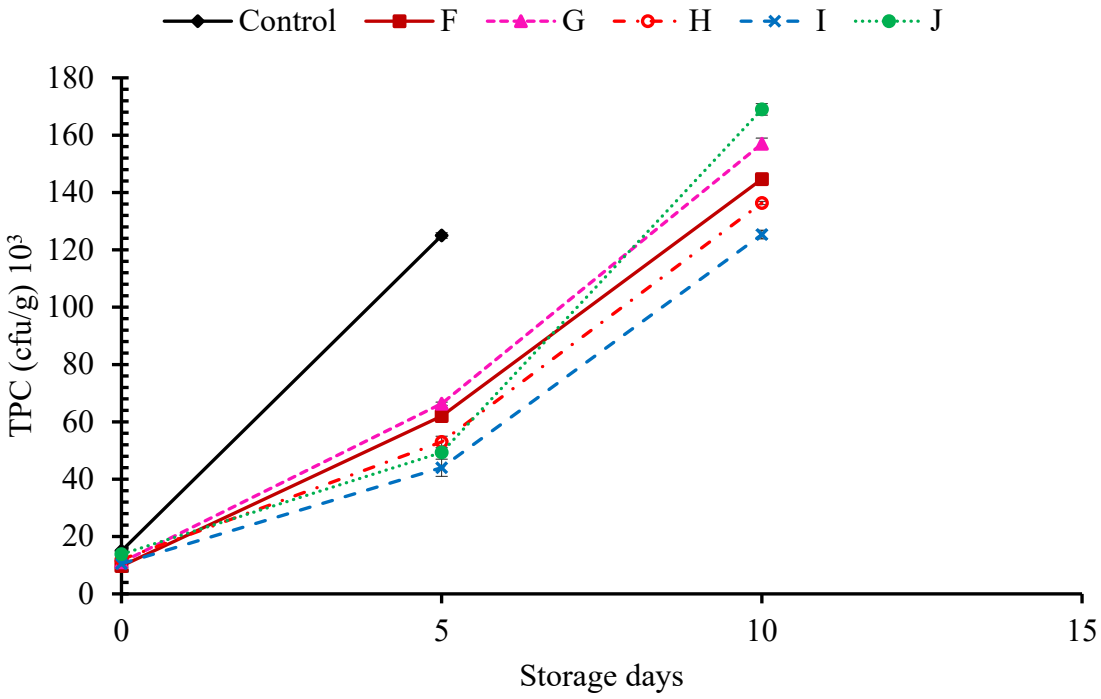

Fig. 4.18 Effect of herbal extract on TPC of paneer samples (added on curd)

*F: 0.6% PE+0% CE, G: 0.45% PE+0.15% CE, H: 0.3% PE+0.3% CE, I: 0.15% PE+0.45% CE and J: 0% PE+0.6% CE. PE represent *pakhanbedh* extract and CE represent clove extract.

In the paneer samples where herbs extract is treated in curd after coagulation the initial microbial load was found higher than that of the milk treated samples. Though same proportions of herbal extract were added to the curd treated samples of paneer for evaluating the preservative action, all samples of paneer deteriorated after 10^{th} day of storage and were not fit for consumption. The added herbal extract showed the preservative action till 10^{th} day of storage. Among the curd treated samples, sample I showed the minimum decrease in the microbial count till 10^{th} days of storage.

The faster deterioration in the curd treated paneer samples may be due to the possible aerial contamination of the paneer samples during mixing treatment. The mixing treatment resulted in the greater surface area available for contamination. It also provided opportunity for incorporation of microorganism into the interior of the product. Due to which the incorporated herb extract could not exert its maximum protective effect during the storage in comparison to the milk treated paneer samples. Uneven mixing of the herb extract in paneer when added directly to curd might also be the reason for less effectiveness of the

extracts against microorganisms. Similarly, there have been evidences regarding protein-polyphenol interactions in milk proteins (Han *et al.*, 2019) and the extent of this interaction is found to be affected by pH, temperature, nature of polyphenols and the nature of proteins (Yildirim-Elikoglu and Erdem, 2017). Although no literature has mentioned the optimum conditions for maximum protein-polyphenol interaction for milk proteins, the prevailing set of conditions might have resulted in higher retention of extracts in paneer when added to milk rather than in paneer where herbs were added directly to curd. Similar observations were observed by Gálvez *et al.* (2007) in nisin, which is a antimicrobial agent used as preservative in food items. According to the study, the effectiveness of nisin to control microbial growth depends upon the initial microbial load of the food samples.

It was observed that the shelf life of control paneer was 5 days under storage at 7±1°C. The shelf life of paneer samples treated with clove and *pakhanbedh* extract at different concentrations showed effective action on increasing the shelf life on paneer up to 10 days in samples where the extracts are treated in curd after coagulation and drainage of whey and the samples where herbs extracts were added on milk after heating at 80°C shelf life was extended to 15 days and later the samples started losing the sensory scores. Mold growth, slime development started in the sample leading the samples to spoilage.

Results proving the effectiveness of addition of the herbs extract at different stages of paneer preparation have not been found but the findings of this study correlate with that of Buch *et al.* (2014) who observed that addition of turmeric in paneer as preservative showed most effective action when added to milk along with heat treatment.

Nunez and Aquino (2012) studied the effect of use of cloves oil combined with heat treatment on microbial inactivation and concluded that any increase in temperature is linked to increased sensitivity of microorganisms towards cloves oil. Similar observations were reported by Hurst (1981) and Maisnier-Patin *et al.* (1995) in case of nisin, wherein better action of nisin is reported in reducing the growth of bacteria when it was used in combination with heat treatment. According to Maisnier-Patin *et al.* (1995), nisin enhances the effect of moderate heat and had a greater effect when used along with heat. This explains the reason behind longer shelf life of paneer samples in which herb extracts were added to milk just after heat treatment than that of paneer samples in which herb extracts were added to curd after coagulation whose temperature was much lower during herb extract addition.

4.9 Cost of herbal paneer

The cost of the herbal paneer was calculated by considering the cost of raw materials, transportation cost and processing cost used in paneer with 10% overhead cost of the final product. The cost of paneer was calculated to be NRs. 572.82/kg. The cost calculations are given in Appendix G.

Part V

Conclusions and recommendations

5.1 Conclusions

As per the objectives, methodologies as mentioned was followed and based on the result and discussions of research followed, following conclusions can be made:

- Yield of ethanolic extract of clove and *pakhanbedh* was found to be 39.29% and 28.19%.
- Both cloves and *pakhanbedh* extract showed a high phenolic content and high free radical scavenging activity.
- The addition of herbal extract and storage days significantly affected the sensory scores of the paneer samples.
- Addition of herbal extract and storage days significantly affected the acidity, FFA, tyrosine content and the microbial counts of the paneer samples.
- Stage of addition of herbal extract in paneer also affected the effectiveness of the extract on the sensory and physico-chemical characteristics of paneer samples. Addition of herb extract to milk is better than adding in curd during paneer preparation which may be because of better retention of herbs due to protein-polyphenol interactions, uniform mixing and lesser chances of microbial contamination during paneer preparation in the former.
- Different formulations of the herbal extract also significantly affected the shelf life of the paneer samples.
- Addition of herbal extract caused moisture retention on the paneer samples and the yield of paneer samples increased i.e. control 14.78% to 16.05% highest yield was obtained on sample containing 0.3% clove+0.3% *pakhanbedh* extract added on milk.
- Shelf life of herb extract treated paneer samples were higher than that of the control paneer sample up to 15 days on milk treated samples and up to 10 days on curd treated samples.
- Among the herb extract treatments, paneer samples treated with 0.45% cloves and 0.15% *pakhanbedh* was found to cause minimum change in the sensory scores and in the chemical characteristics during the storage period in both stages of addition.

- All the formulations of herb extract were proved to have preservative action on the paneer samples and had a longer shelf life in comparison to the control sample.
- Cost of herb extract treated sample was estimated to NRs. 572.82/kg.

5.2 Recommendations

The present work is a preliminary study of preservative action of selected herbs extracts in paneer. Due to time constraints, limited work has been done in this research. From this study, following points can be recommended:

- Antimicrobial potential of the herbal extract can be studied.
- Other common herbs and spices can be tested for their potential to act as preservatives in paneer.
- Cloves and *pakhanbedh* extract can be used as preservative in other food item.
- Preservation of paneer samples can be by varying on the packaging material and storage temperature.

Part VI

Summary

Paneer, an important indigenous dairy product has very short shelf life. This hinders its storage and acceptance for trade by organized sector. One of the measures to enhance the shelf life of paneer is use of preservative. Spices and herbs are well known for their medicinal, preservative and antioxidant properties. Among different natural herbs and spices this study was focused on utilizing cloves, which is a powerful antimicrobial and antioxidant proved by various studies as a preservative in paneer. Also, *pakhanbedh* a medicinal herb found in Himalayan region of Nepal which has a high potential to act as preservative due to its high antimicrobial and antioxidant activity has been used to study its preservative effect. Individual treatment as well as mixture of cloves and *pakhanbedh* extract was used as preservative. The effect on stage of addition of the herbs extract on the physical as well as chemical parameters of paneer samples was studied.

To study the effect of cloves and *pakhanbedh* on the storage stability of paneer samples, cloves and *pakhanbedh* were collected from local market of Dharan and ethanolic extracts were prepared. The extract was then analyzed for total phenols and total radical scavenging capacity. Both cloves and *pakhanbedh* extract showed a high phenolic content and exhibited a high free radical scavenging activity. Paneer samples with different combinations ranging from 0 to 0.6% of each extract was incorporated at two different stages of paneer preparation was done and the respective samples were analyzed for effect of storage days on the control samples as well as herbs treated samples.

The sensory scores of control paneer declined rapidly from beginning of the storage. The sensory scores of control paneer samples decreased rapidly on 5^{th} day of storage and later became unacceptable due to visible mold growth. Samples where herbs extract was added directly on milk showed slower decrease on the sensory scores and were acceptable until 15^{th} days of storage. Later, samples became unacceptable due to slime formation and mold growth. Samples where herb extract was treated after coagulation on curd, initial sensory scores were slightly lower than that of the paneer samples where herbs extract was treated on milk. Curd treated samples also showed a slower rate of decrease in the sensory scores. All samples remained acceptable till 10^{th} day of storage. Among the different combination

of herbs extracts 0.45% cloves +0.15% *pakhanbedh* mixture showed a slower decline on the sensory scores in comparison to the other combinations.

Addition of herbs extract and storage days significantly affected the chemical characteristics of paneer samples during storage. Control paneer samples showed a sharp increase in acidity, FFA, tyrosine content and total plate count throughout the storage period. The herbs treated samples added at both stages of paneer preparation showed slower growth rate on the acidity, FFA, tyrosine content and standard plate count content in comparison to control. The paneer samples where herb extracts were treated directly on milk showed longer shelf life than the paneer samples which contain herbs extract added on curd i.e. 10^{th} day and 15^{th} day respectively.

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Appendices

Appendix A

Table A.1 Sensory scores of paneer samples treated with herbs extract on milk

Spice formulations	Storage days			
	0	5	10	15
	Color and appearance			
Control	9 ± 0.00^{pa}	6.8 ± 0.45^{qa}	NA	NA
A	7.8 ± 0.45^{pb}	7.6 ± 0.55^{pab}	7.4 ± 0.55^{pa}	6.4 ± 0.55^{qa}
B	8 ± 0.71^{pb}	7.8 ± 0.45^{pb}	7.6 ± 0.55^{pqa}	6.6 ± 0.55^{qa}
C	8.2 ± 0.45^{pab}	8.2 ± 0.45^{pb}	7.6 ± 0.55^{pa}	6.6 ± 0.55^{qa}
D	8.4 ± 0.55^{pab}	8.2 ± 0.45^{pb}	8 ± 0.00^{pa}	7 ± 0.00^{qa}
E	8.6 ± 0.55^{pab}	8.4 ± 0.55^{pb}	8 ± 0.00^{pa}	7 ± 0.00^{qa}
	Body and texture			
Control	9 ± 0.00^{pa}	6.8 ± 0.45^{qa}	NA	NA
A	8.6 ± 0.55^{pa}	8.4 ± 0.55^{pb}	7.4 ± 0.55^{qa}	6.2 ± 0.45^{ra}
B	8.6 ± 0.55^{pa}	8.4 ± 0.55^{pb}	7.2 ± 0.45^{qa}	6.4 ± 0.55^{qa}
C	8.4 ± 0.55^{pa}	8.2 ± 0.45^{pb}	7.2 ± 0.45^{qa}	6.6 ± 0.55^{qa}
D	8.6 ± 0.55^{pa}	8.4 ± 0.55^{pb}	7.2 ± 0.55^{qa}	6.2 ± 0.45^{ra}
E	8.6 ± 0.55^{pa}	8.4 ± 0.55^{pb}	7.2 ± 0.55^{qa}	6.2 ± 0.45^{ra}
	Flavor			
Control	9 ± 0.00^{pa}	6.2 ± 0.45^{qa}	NA	NA
A	7.6 ± 0.55^{pc}	7.4 ± 0.55^{pb}	7 ± 0.71^{pa}	6.2 ± 0.45^{qa}
B	7.8 ± 0.45^{pbc}	7.6 ± 0.55^{pb}	7.2 ± 0.45^{pa}	6.2 ± 0.45^{qa}
C	8 ± 0.00^{pbc}	7.6 ± 0.55^{pb}	7.4 ± 0.55^{pa}	6.4 ± 0.55^{qa}
D	8.4 ± 0.55^{pab}	8 ± 0.00^{pb}	7.8 ± 0.45^{pa}	6.6 ± 0.55^{qa}
E	8.2 ± 0.45^{pbc}	7.8 ± 0.45^{pb}	7.6 ± 0.55^{pa}	6.2 ± 0.45^{qa}
	Overall acceptability			
Control	9 ± 0.00^{pa}	6.8 ± 0.45^{qa}	NA	NA
A	8.2 ± 0.45^{pa}	8 ± 0.00^{pb}	7.2 ± 0.45^{qa}	6.2 ± 0.55^{ra}
B	8.6 ± 0.55^{pa}	8.2 ± 0.45^{pqb}	7.6 ± 0.55^{qa}	6.6 ± 0.55^{ra}
C	8.4 ± 0.55^{pa}	8.2 ± 0.45^{pb}	7.6 ± 0.55^{pqa}	6.6 ± 0.84^{qa}
D	8.8 ± 0.45^{pa}	8.6 ± 0.55^{pqb}	7.8 ± 0.45^{qra}	7 ± 0.71^{ra}
E	8.8 ± 0.45^{pa}	8.6 ± 0.55^{pb}	7.2 ± 0.45^{qa}	6.4 ± 0.45^{ra}

Values represent means± standard deviation. Different alphabets in superscript represent significant difference. Alphabets (a-b) represent difference in values between different samples in same day of storage. Alphabets (p-r) represent difference among samples with increase in number of days.

Appendix B

Table B.1 Sensory scores of paneer samples treated with herbs extract on curd

Spice formulations	Storage days			
	0	5	10	15
		Color and appearance		
Control	9.00 ± 0.000^{pa}	6.20 ± 0.447^{qa}	NA	NA
F	7.40 ± 0.548^{pb}	7.00 ± 0.000^{pab}	6.00 ± 0.000^{qa}	NA
G	7.60 ± 0.548^{pb}	7.20 ± 0.447^{pb}	6.20 ± 0.447^{qa}	NA
H	7.80 ± 0.447^{pb}	7.40 ± 0.548^{pb}	6.40 ± 0.548^{qa}	NA
I	7.80 ± 0.447^{pb}	7.60 ± 0.548^{pb}	6.60 ± 0.548^{qa}	NA
J	7.60 ± 0.548^{pb}	7.20 ± 0.447^{pqb}	6.40 ± 0.548^{qa}	NA
		Body and texture		
Control	9.00 ± 0.000^{pa}	6.80 ± 0.447^{qa}	NA	NA
F	7.60 ± 0.548^{pb}	6.80 ± 0.447^{pqa}	6.40 ± 0.548^{qa}	NA
G	7.60 ± 0.548^{pb}	7.00 ± 0.707^{pqa}	6.60 ± 0.548^{qa}	NA
H	7.40 ± 0.548^{pb}	7.20 ± 0.447^{pa}	6.80 ± 0.447^{pa}	NA
I	7.60 ± 0.548^{pb}	7.40 ± 0.548^{pa}	7.20 ± 0.447^{pa}	NA
J	7.40 ± 0.548^{pb}	7.00 ± 0.000^{pa}	6.80 ± 0.447^{pa}	NA
		Flavor		
Control	9.00 ± 0.000^{pa}	6.20 ± 0.447^{qa}	NA	NA
F	7.00 ± 0.000^{pb}	6.40 ± 0.548^{pqab}	6.00 ± 0.707^{qa}	NA
G	7.20 ± 0.447^{pb}	6.60 ± 0.548^{pqab}	6.20 ± 0.447^{qa}	NA
H	7.40 ± 0.548^{pb}	6.80 ± 0.447^{pqab}	6.40 ± 0.548^{qa}	NA
I	7.60 ± 0.548^{pb}	7.20 ± 0.447^{pqb}	6.60 ± 0.548^{qa}	NA
J	7.40 ± 0.548^{pb}	7.00 ± 0.000^{pab}	6.20 ± 0.447^{qa}	NA
		Overall acceptability		
Control	9.00 ± 0.000^{pa}	6.80 ± 0.447^{qa}	NA	NA
F	7.20 ± 0.447^{pb}	6.80 ± 0.447^{pa}	6.00 ± 0.000^{qa}	NA
G	7.40 ± 0.548^{pbc}	7.00 ± 0.000^{pa}	6.20 ± 0.447^{qab}	NA
H	7.60 ± 0.548^{pbc}	7.20 ± 0.447^{pqa}	6.40 ± 0.548^{qab}	NA
I	8.00 ± 0.000^{pc}	7.60 ± 0.548^{pa}	6.80 ± 0.447^{qb}	NA
J	7.80 ± 0.447^{pbc}	7.40 ± 0.548^{pqa}	6.60 ± 0.548^{qab}	NA

Values represent means± standard deviation. Different alphabets in superscript represent significant difference. Alphabets (a-c) represent difference in values between different samples in same day of storage. Alphabets (p-q) represent difference among samples with increase in number of days.

Appendix C

Table C.1 Values of chemical parameter for herbs added in milk during storage

Spice formulations	Storage days			
	0	5	10	15
	Acidity (% lactic acid)			
Control	0.423 ± 0.006^{pa}	0.930 ± 0.000^{qa}	1.680 ± 0.005^{ra}	1.890 ± 0.010^{sa}
A	0.433 ± 0.006^{pab}	0.556 ± 0.006^{qd}	0.895 ± 0.005^{rb}	0.954 ± 0.005^{sbc}
B	0.440 ± 0.000^{pabc}	0.540 ± 0.000^{qbc}	1.070 ± 0.027^{rd}	0.965 ± 0.005^{sc}
C	0.453 ± 0.006^{pc}	0.544 ± 0.0051^{qc}	0.949 ± 0.002^{rc}	0.950 ± 0.000^{rbc}
D	0.440 ± 0.01^{pabc}	0.530 ± 0.000^{qb}	0.941 ± 0.002^{rc}	0.942 ± 0.004^{rb}
E	0.46 ± 0.006^{pbc}	0.546 ± 0.006^{qcd}	0.984 ± 0.005^{re}	1.020 ± 0.005^{sd}
	FFA (% oleic acid)			
Control	0.248 ± 0.0030^{pa}	0.364 ± 0.0040^{qa}	0.540 ± 0.0050^{ra}	0.599 ± 0.0030^{sa}
A	0.242 ± 0.0095^{pa}	0.255 ± 0.0070^{pb}	0.455 ± 0.000^{qb}	0.517 ± 0.0068^{rb}
B	0.235 ± 0.0090^{pab}	0.242 ± 0.0020^{pcd}	0.435 ± 0.0050^{qc}	0.496 ± 0.0040^{rc}
C	0.223 ± 0.0030^{pb}	0.240 ± 0.0040^{qd}	0.416 ± 0.0040^{rd}	0.469 ± 0.0040^{sd}
D	0.242 ± 0.0040^{pa}	0.252 ± 0.0030^{qbc}	0.402 ± 0.0020^{re}	0.459 ± 0.0021^{sd}
E	0.245 ± 0.0025^{pa}	0.259 ± 0.0040^{qb}	0.453 ± 0.0030^{rb}	0.515 ± 0.0050^{sb}
	Tyrosine content (mg/100g)			
Control	10.076 ± 0.045^{pa}	25.469 ± 0.004^{qa}	42.248 ± 0.004^{ra}	60.375 ± 0.005^{sa}
A	9.983 ± 0.025^{pa}	16.231 ± 0.0401^{qb}	26.261 ± 0.020^{rb}	41.682 ± 0.040^{sb}
B	9.986 ± 0.012^{pa}	15.325 ± 0.030^{qc}	24.993 ± 0.055^{rc}	39.211 ± 0.020^{sc}
C	10.054 ± 0.015^{pa}	14.968 ± 0.002^{qd}	23.152 ± 0.040^{rd}	28.606 ± 0.006^{sd}
D	10.005 ± 0.050^{pa}	15.111 ± 0.010^{qe}	21.572 ± 0.027^{re}	27.567 ± 0.003^{se}
E	10.043 ± 0.037^{pa}	15.891 ± 0.030^{qf}	22.972 ± 0.040^{rf}	42.568 ± 0.045^{sf}
	Total plate count (cfu/g) × 10^3			
Control	15.00 ± 1.000^{pa}	125.00 ± 1.00^{qa}	VMG	VMG
A	8.700 ± 0.200^{pb}	9.500 ± 0.100^{pb}	63.667 ± 1.53^{qa}	135.33 ± 1.53^{ra}
B	9.633 ± 0.153^{pb}	12.400 ± 0.100^{qbc}	56.000 ± 1.00^{rb}	107.33 ± 1.53^{sb}
C	10.00 ± 1.000^{pb}	14.667 ± 1.53^{qc}	53.667 ± 1.53^{rb}	98.00 ± 1.00^{sc}
D	8.600 ± 0.100^{pb}	24.333 ± 2.08^{qc}	46.000 ± 1.00^{rc}	83.33 ± 2.51^{sd}
E	10.100 ± 0.100^{pb}	12.333 ± 1.53^{pbc}	47.667 ± 0.57^{qc}	121.33 ± 1.53^{re}

Values represent means±standard deviation. Different alphabets in superscript represent significant difference. Alphabets (a-f) represent difference in values between different samples in same day of storage. Alphabets (p-s) represent difference among samples with increase in number of days**.**

Appendix D

Table D.1 Values of chemical parameter for herbs added on curd during storage

Spice formulation	Storage days			
	0	5	10	15
	Acidity (% lactic acid)			
Control	0.423 ± 0.006^{pa}	0.930 ± 0.000^{qa}	1.680 ± 0.005^{ra}	1.890 ± 0.010^{sa}
F	0.423 ± 0.006^{pa}	0.590 ± 0.005^{qb}	0.982 ± 0.002^{rb}	1.121 ± 0.003^{sd}
G	0.430 ± 0.010^{pa}	0.558 ± 0.003^{qcd}	0.955 ± 0.005^{rc}	1.068 ± 0.030^{se}
H	0.450 ± 0.000^{pb}	0.583 ± 0.006^{qb}	0.972 ± 0.002^{rd}	1.150 ± 0.005^{scd}
I	0.446 ± 0.006^{pb}	0.550 ± 0.000^{qd}	0.951 ± 0.001^{rc}	1.171 ± 0.003^{sc}
J	0.460 ± 0.000^{pb}	0.561 ± 0.003^{qc}	0.965 ± 0.005^{rd}	1.210 ± 0.005^{sb}
	FFA (% oleic acid)			
Control	0.248 ± 0.003^{pa}	0.364 ± 0.0040^{qa}	0.540 ± 0.0050^{ra}	0.599 ± 0.0030^{sa}
F	0.241 ± 0.003^{pb}	0.263 ± 0.002^{qb}	0.479 ± 0.002^{rb}	0.526 ± 0.003^{sb}
G	0.225 ± 0.002^{pd}	0.245 ± 0.003^{qc}	0.486 ± 0.003^{rd}	0.519 ± 0.002^{sc}
H	0.248 ± 0.003^{pa}	0.265 ± 0.001^{qb}	0.465 ± 0.002^{rc}	0.499 ± 0.003^{sd}
I	0.234 ± 0.001^{pc}	0.248 ± 0.002^{qc}	0.454 ± 0.001^{re}	0.495 ± 0.003^{sd}
J	0.236 ± 0.002^{pbc}	0.265 ± 0.002^{qb}	0.462 ± 0.001^{rc}	0.522 ± 0.003^{sbc}
	Tyrosine content (mg/100g)			
Control	10.076 ± 0.0456^{pa}	25.469 ± 0.004^{qa}	42.248 ± 0.0040^{ra}	60.375 ± 0.0050^{sa}
F	10.260 ± 0.030^{pc}	18.836 ± 0.003^{qb}	29.95 ± 0.020^{rb}	50.532 ± 0.0030^{sb}
G	10.252 ± 0.030^{pc}	18.012 ± 0.033^{qc}	28.365 ± 0.005^{rc}	47.843 ± 0.0060^{sc}
H	10.990 ± 0.030^{pd}	17.855 ± 0.005^{qd}	30.973 ± 0.0030^{rd}	51.916 ± 0.0035^{sd}
I	9.954 ± 0.053^{pb}	16.761 ± 0.005^{qe}	27.495 ± 0.0050^{re}	46.210 ± 0.020^{se}
J	10.055 ± 0.015^{pa}	17.765 ± 0.005^{qf}	28.693 ± 0.035^{rf}	49.156 ± 0.0030^{sf}
	Total plate count (cfu/g) × 10^3			
Control	15.00 ± 1.000^{pa}	125.00 ± 1.00^{qa}	VMG	
F	9.70 ± 0.200^{pc}	62.00 ± 1.00^{qb}	144.66 ± 1.53^{ra}	VMG
G	11.00 ± 0.200^{pbc}	66.33 ± 0.577^{qb}	157.00 ± 2.00^{rb}	VMG
H	11.96 ± 0.152^{pb}	53.00 ± 2.00^{qc}	136.33 ± 0.577^{rc}	VMG
I	10.50 ± 0.400^{pc}	44.00 ± 3.00^{qd}	125.33 ± 1.53^{rd}	VMG
J	13.83 ± 0.251^{pa}	49.33 ± 0.577^{qc}	169.00 ± 2.00^{re}	VMG

Values represent means±standard deviation. Different alphabets in superscript represent significant difference. Alphabets (a-f) represent difference in values between different samples in same day of storage. Alphabets (p-s) represent difference among samples with increase in number of days.

Appendix E

Table E.1 Moisture content and Yield of Paneer

Samples	Moisture content (% wb)	Yield (% w/w)
Control	56.64±0.51	14.79
A	58.85±0.21	15.66
B	58.015±0.05	15.42
C	57.36±0.50	16.05
D	56.73±0.38	15.31
E	57.55±0.63	14.86
F	57.95±0.07	15.08
G	56.85±1.62	15.37
H	55.15±0.21	14.61
I	55.105±0.15	13.82
J	56.14±0.19	14.20

Values represent means± standard deviation for moisture content.

Appendix F

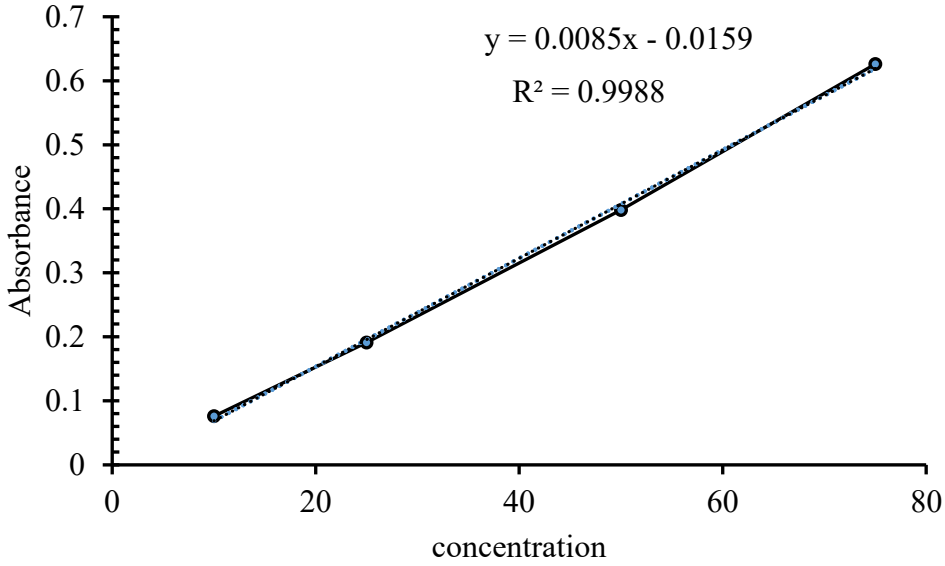

Fig. F.1 Calibration curve for total phenolic content

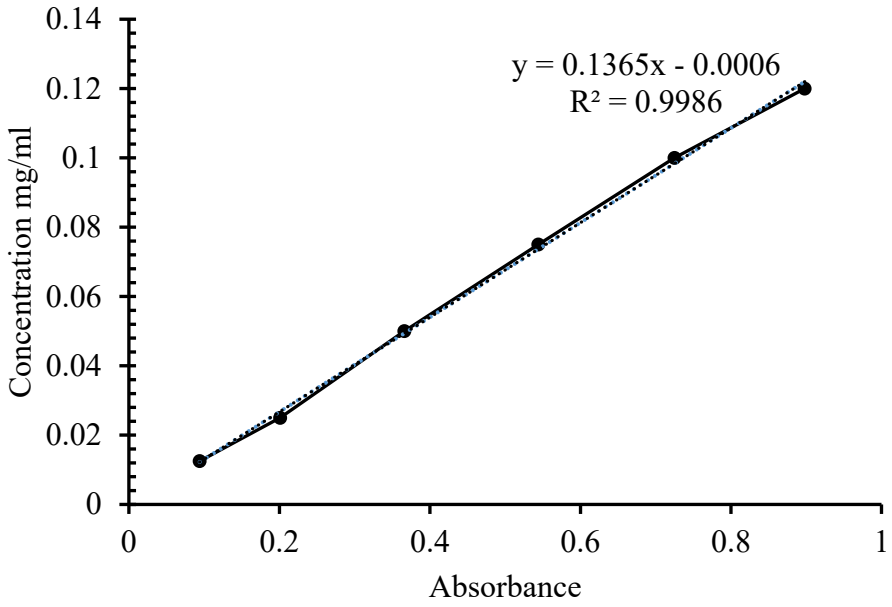

Fig. F.2 Calibration curve for tyrosine content determination for day 1

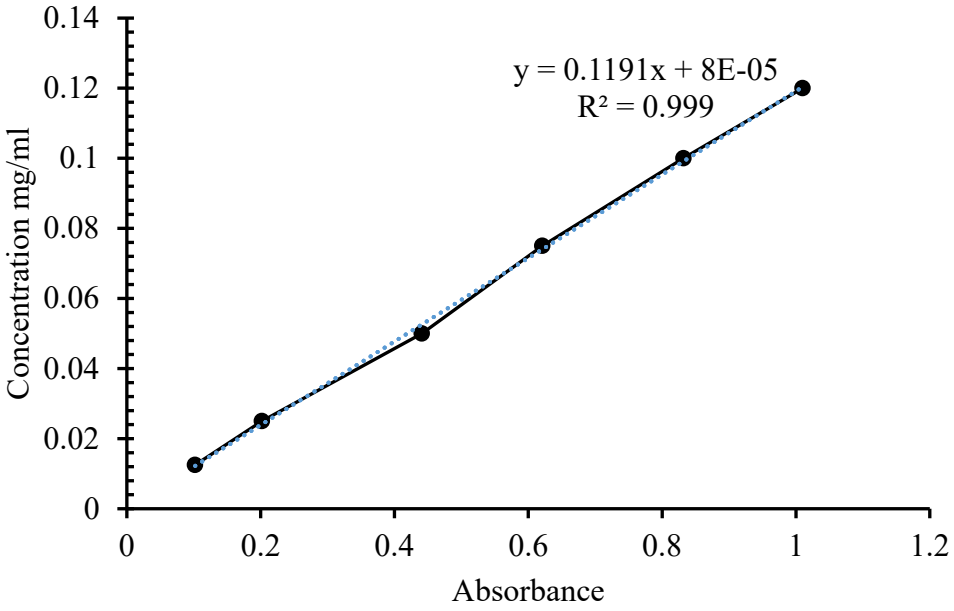

Fig. F.3 Calibration curve for tyrosine content determination for day 5

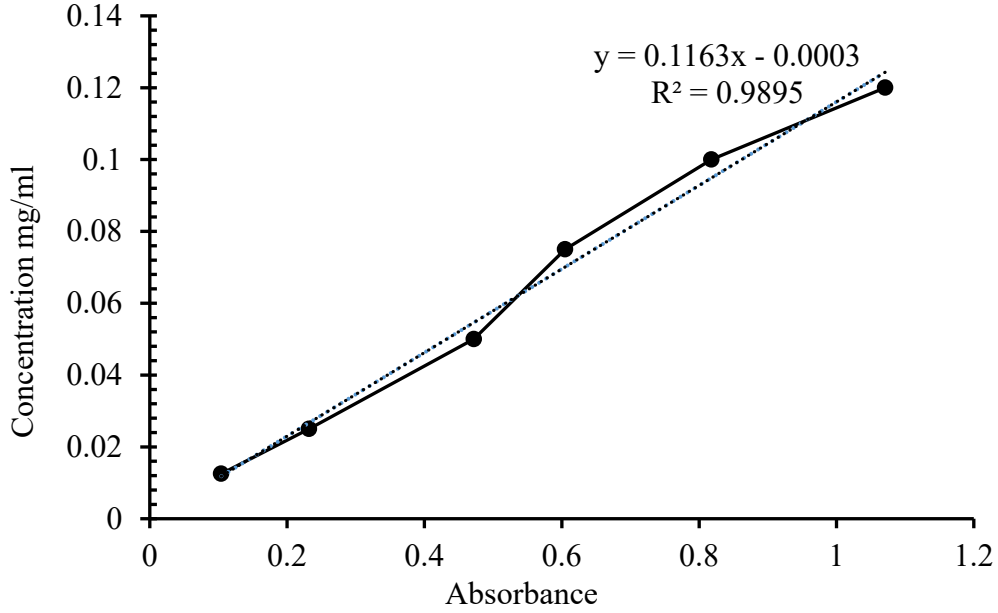

Fig. F.4 Calibration curve for tyrosine content determination for day 10

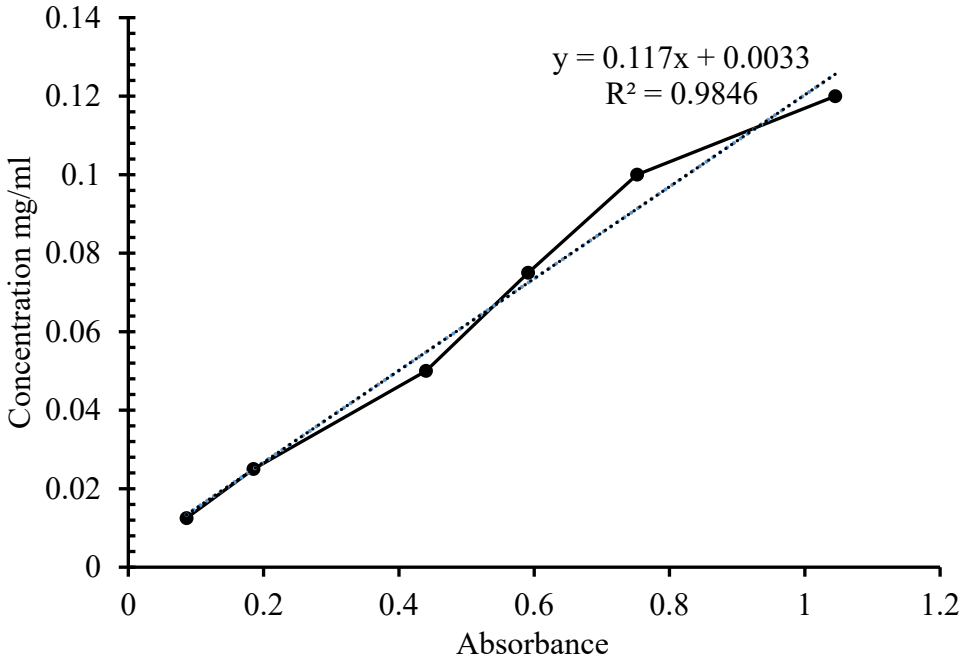

Fig. F.5 Calibration curve for tyrosine content determination for day 15

Appendix G

T-test for comparison of antioxidant activity of clove and *pakhanbedh* extract

For concentration 0.156 mg/ml

T-test: Two samples assuming unequal variances

	Cloves	*Pakhanbedh*
Mean	55.96	57.76
Variance	1.0201	0.81
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-2.304600872	
P(T<=t) one-tail	0.04126023	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.082520459	
t Critical two-tail	2.776445105	

For concentration 0.312 mg/ml

T-test: Two samples assuming unequal variances

	Cloves	*Pakhanbedh*
Mean	63.63	64.41
Variance	0.09	0.49
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.773949422	
P(T<=t) one-tail	0.087086714	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.174173429	
t Critical two-tail	3.182446305	

For concentration 0.625 mg/ml

T-test: Two samples assuming unequal variances

	Cloves	*Pakhanbedh*
Mean	78.63	83.82
Variance	1.5625	1.21
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-5.398737454	
P(T<=t) one-tail	0.002848497	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.005696995	
t Critical two-tail	2.776445105	

For concentration 1.25 mg/ml

T-test: Two samples assuming unequal variances

	Cloves	*Pakhanbedh*
Mean	87.78	89.7
Variance	1	1.21
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-2.236998636	
P(T<=t) one-tail	0.044458739	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.088917479	
t Critical two-tail	2.776445105	

Appendix H

Table G.1 Cost evaluation of herb extracts incorporated paneer

Ingredients	Rate	Quantity	Cost (NRs)
Milk	80/L	2L	160
Citric acid	900/kg	2g	1.8
Cloves	1850/kg	1.35g	2.5
Pakhanbedh	100/kg	0.45g	0.045
Total cost			164.345
Final cost with 10% overhead			180.78
Product prepared			315.6g
Cost per kg			NRs 572.82/kg

Appendix I

Date:…….

Sensory Evaluation 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 your 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 Attribute			
	Flavor	Body and texture	Color and appearance	Overall acceptability
A				
B				
C				
D				
E				
F				
G				
H				
I				
J				
K				

Comments:

Signature:……..

Appendix J

Table I.1 Materials used in product preparation

S. N.	Materials Used	Details
1	Wooden mould	
2	Van Gulik butyrometer	
3	Refrigerator	
4	Vacuum sealer	
5	Heating vessels	
6	Heating arrangement	
7	Thermometer	
8	Muslin cloth	
9	Packaging material PP	
10	Titration set	
11	Kjeldahl digestion and distillation set	
12	Digital electronic balance	
13	Hot air oven	
14	Muffle furnace	
15	Bacterial Incubator	
16	pH meter	HANNA HI 96017, Sensitivity ±0.01
17	Beakers	

18	Volumetric flasks	
19	Pipettes and micropipettes	
20	Petri plates	
21	Water bath	
22	Magnetic stirrer	
23	Spectrophotometer	UV-VIS Single Beam Spectrophotometer, Model no. 291
24	Rotary vacuum evaporator	IKA®RV 10 Model-HB 10D S96-2425W
25	Colony counter	
26	Autoclave	

Table I.2 Chemicals used in product analysis

S.N.	Chemicals used	Details
1	Citric acid	
2	L-tyrosine	
3	Methanol	Qualigens, Assay 99%
4	Ethanol	
5	Gallic acid	
6	Petroleum ether	
7	DPPH	
8	Diethyl ether	Qualigens, Assay 98%
9	Oxalic acid	Qualigens, Assay 99.5%
10	Sodium carbonate	Qualigens, Assay 99-101%
11	Sodium hydroxide	HIMEDIA, Assay 97-103.5%
12	Hydrochloric acid	Qualigens, Minimum assay 35-37%
13	Standard plate count agar	Himedia
14	Disodium Hydrogen Phosphate	Merck, Minimum assay 98%
15	Sodium Dihydrogen Phosphate	Merck, Minimum assay 98%
16	Folin-Ciocalteau reagent	

Color plates

Plate P.1 Clove and *pakhanbedh* extract

PlateP.2 Paneer samples for analysis

Plate P.3 Control paneer

Plate P.4 Herbal paneer

Plate P.5 Paneer samples unfit for consumption

Plate P.6 Microbial analysis

Plate P.7 Spice extract preparation

Plate P.8 Examination of paneer prepared

Plate P.9 Drainage of whey

Plate P.10 Concentration of spice extract

Plate P.11 Kjeldahl distillation set