EVALUATION OF THE SELECTED SPICES FOR
EXTENDING THE SHELF LIFE OF PANEER

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SUBMITTED TO THE
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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE

OF

Master of Science

IN

DAIRY CHEMISTRY

BY

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EVALUATION OF THE SELECTED SPICES FOR EXTENDING THE SHELF LIFE OF PANEER

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ABSTRACT

Study of evaluating the selected spices for extending shelf life of paneer was conducted. The entire study was divided into four parts viz. selection of spices, selection of rate of addition of spice, preliminary screening of the spices for their effectiveness in enhancing shelf life of paneer and final testing of the selected spices for their relative efficiency in improving shelf life of paneer.

Seven different spices, commonly used with paneer during culinary preparations viz black pepper, cardamom, cinnamon, clove, garlic, ginger and onion were incorporated in paneer at the rate of 0.5 per cent. All the samples containing these spices were found acceptable in sensory evaluation. Therefore, all the seven spices were selected for further study.

For selecting rate of addition in paneer, each spice was incorporated in the product at the rate of 0.0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 per cent. The prepared samples of paneer were subjected to sensory evaluation. Based on changes in sensory score of paneer with increase in rate of addition of spices, the rate of addition for black pepper, cardamom, clove, garlic, ginger and onion was selected as 0.6 per cent and for cinnamon the rate was selected as 0.4 per cent.

In preliminary screening of the selected spices for their effectiveness to enhance shelf life of paneer black pepper, cardamom, cinnamon, clove, garlic,
ginger and onion were incorporated in the coagulum at the rate of 0.6 per cent and cinnamon was incorporated in paneer at the rate of 0.4 per cent. The sample of paneer without addition of spice was used as a control. The samples were packed in PET/LDPE film pouches and stored at 7°C±1°C for 14 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges when fresh and after 14 days of storage. The overall acceptability score of black pepper, cardamom, cinnamon and clove declined slightly on 14th day of the storage but remained above acceptable level. However, the score of garlic, ginger, onion and that of the control declined sharply and went below acceptable level due to development of strong objectionable flavour. Therefore, black pepper, cardamom, cinnamon and clove were selected, whereas, garlic, ginger and onion were dropped from further study.

Finally, selected spices (black pepper, cardamom, cinnamon and clove) were tested for their relative efficiency in improving shelf life of paneer. For final screening these spices were incorporated in the product at their respective optimized rates. The sample of paneer without addition of spice was used as a control. The samples of paneer were stored at 7°C ± 1°C as well as at 32°C ± 1°C. The fresh and stored samples of paneer were analyzed for sensory attributes and chemical characteristics at an interval of 7 days during storage at 7°C. The samples of paneer stored at 32°C ± 1°C were to be analyzed at an interval of one day. However, in these samples very strong off flavour was developed and whey separation took place with in one day of storage. Therefore, their further storage study was terminated.

The overall acceptability score of control samples of paneer went below acceptable level on 14th day of the storage. The samples of paneer containing black pepper went slightly below the acceptable level on 21st day of the storage. The overall acceptability score of samples containing cinnamon or clove went slightly below acceptable level on 28th day of the storage. However, the overall
acceptability score of samples containing cardamom remained well above the acceptable level even on 28th day of the storage.

Results of changes in chemical characteristics indicate that the amongst black pepper, cardamom, cinnamon and clove, cardamom has maximum ability to control acidity, free fatty acids content and soluble nitrogen content of paneer during storage. The changes in these chemical characteristics are very well corroborated with changes in sensory attributes of paneer during storage.

The effect of cardamom on microbial counts of paneer viz. standard plate counts (SPC), yeast and mould counts and coliform counts was evaluated. The results obtained for changes in microbial counts of paneer during are well corroborated with the changes in sensory attributes and chemical characteristics of paneer during storage.

The present study entailed to conclude that addition of black pepper, cardamom, clove, garlic, ginger or onion in paneer at the rate greater than 0.6 per cent and cinnamon at the rate greater than 0.4 per cent results into sharp decline in sensory score of paneer. Addition of garlic, ginger or onion in paneer develops strong objectionable flavour in paneer up on storage at 70°C. Addition of black pepper, cardamom or clove at the rate of 0.6 per cent or cinnamon at the rate of 0.4 per cent improves shelf life of paneer on storage at 70°C. The order of the relative effectiveness in enhancing shelf life of paneer is cardamom > cinnamon > clove > black pepper. Thus, cardamom is the best spice to improve shelf life of paneer during storage.
Dr. K. D. Aparnathi  
Professor & Head  
Dairy Chemistry Department  
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Anand Agricultural University  
Anand-388110

CERTIFICATE

This is to certify that the thesis entitled “Evaluation of the Selected Spices for Extending the Shelf Life of Paneer.” submitted by Ms. Krishna Kumari Eresam in partial fulfillment of the requirement for the award of the degree of Master of Science in Dairy chemistry to the Anand Agricultural University is a record of bonafide research work carried out by her under my guidance and supervision.

Place : Anand  
Date:  
(K.D. Aparnathi)  
Major Advisor
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The great accomplishments of man have resulted from the transmission of ideas and enthusiasm.

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PLACE: ANAND

(Krishna kumari Eresam)

DATE : 25/06/2009
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1. INTRODUCTION

Dairying occupies a place of pride in the Indian food process industry. It is playing a remarkable role to consolidate our socio-economic growth. Milk production has shown rapid growth of 4-5 per cent per annum during the last 35 years, now reaching a level of about 100 million tonnes 2006-2007. India has already entered in the international market in milk products, exporting 59,746 MT of milk products during 2005-06. Under the WTO regime, Indian exports will have to adhere to stringent quality and food safety standards.

The milk utilization pattern in India indicates that a considerable portion of milk is utilized for production of traditional Indian dairy products (Baxi, 1994), out of total milk production only 50% of the milk produced is used for making indigenous products such as dahi, ghee, paneer, etc. An estimated one per cent of the country’s total milk production is converted into paneer, its annual production is estimated at 150,000 tonnes (Aneja et al. 2002).

Paneer is an important heat and acid coagulated milk product which is used as a base material for the preparation of a large number of culinary dishes. Paneer provides an easy means of conserving and preserving valuable milk solids. It contains whole of milk casein, part of denatured whey proteins, almost all fat, colloidal salts and soluble milk solids in proportion to the moisture content. It has firm, close, cohesive and spongy body and smooth texture (Kanawjia et al. 1990). The best quality paneer is made from buffalo milk.

Paneer, like other indigenous product is a highly perishable product and suffers from limited shelf life, largely because 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 in paneer occurs mainly due to the growth of micro-organisms (Thakral, 1986) which bring about various physico-chemical changes leading to the development of off-flavour in the product.
Introduction

Over the years, various attempts have been made to increase the shelf life of paneer. Food additives such as sorbic acid, potassium sorbate (Thakral, 1986), solutions of $\text{H}_2\text{O}_2$ and brine, and delvicid (Sachdeva, 1983) have been tried successfully to increase the shelf-life of paneer, though they are not permitted additives to paneer under PFA rules, 1955. Antioxidants like THBQ and BHA has also been tried as possible antimicrobial agents in paneer (Kumar and Bector, 1991).

There has been increasing concern of the consumers about foods free of chemical preservatives because of their possible toxic effect in human beings. Consumers are also demanding foods with long shelf life and absence of risk of causing food borne diseases. There is an increasing demand for foods containing natural ingredients. The consumers’ demand has forced the food industry to exploit potential of natural alternatives for synthetic antimicrobial compounds. Spices offer a promising alternative for food safety. Inhibitory activity of spices and derivatives on the growth of bacteria, yeasts, fungi and microbial toxins synthesis has been well reported, so they could be used in food conservation as main or as adjuvant antimicrobial compounds in order to assure the production of microbiologically stable foods. Spices have been well known for their medicinal, preservative and antioxidant properties (Souza et al., 2005). They are currently used mainly for enhancing the flavour of foods rather than extending shelf life (Almeida and Regitano, 2000). In addition to imparting flavour, certain spices prolong the shelf life of foods due to their bacteriostatic or bacteriocidal activity, and some prevent rancidity by their antioxidant activity (Shelef et al., 1980). Many plant essential oils of spices are active against various food borne bacteria and moulds (Aureli et al., 1992). However, no work has been reported about use of spices in extending shelf life of paneer.
Therefore the present the proposed study has been contemplated with the following objectives:

a) Preliminary screening of selected spices for their effectiveness in preservation of paneer.

b) Selection of the best spices for application in paneer.

c) Optimization for incorporation of the selected spices in paneer.

d) Analysis of the paneer for chemical, microbiological and sensory characteristics.

e) Storage study to evaluate the shelf-life of paneer.
Paneer is an indigenous dairy product prepared by the heat and acid coagulation of milk. It is an important base material for the preparation of various culinary dishes and snacks (Mathur et al., 1991). Paneer consists of the protein and usually all the fat, insoluble salts and colloidal materials, together with part of the moisture serum of the original milk, which contained lactose, whey proteins, soluble salts, vitamins and other milk components (Kanawjia et al., 1990). It is estimated that about 4-5% of the total milk in India is converted into paneer (Chandan, 2007).

Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Spices include flowers (clove), fruits (black pepper, cardamom, red chilli, and cumi), bulbs (garlic and onion), leaves (bay, mint, rosemary, coriander etc.), stem (cinnamon, coriander), rhizomes (ginger) and other plant parts (Shelef, 1984).

### 2.1 History Of Paneer

Historical development of paneer is not well documented. However, it is supposed that “Paneer khiki” a distinctive cheese variety was originally developed by the Iranian “Bakhtiari” tribe, in south-west Asian region. Paneer means container, “Khiki” means skin (Rennet form of goat or sheep was perhaps used to make it, hence the name). In earliest form of paneer was prepared by curdling milk by using sour milk, pieces of creepers called “putika” or bark of “palaska” tree (Singh et al., 1984).

Cottage cheese prepared by acid coagulation in western countries is similar product, Queso del pais, Queso Blanco, Queso criollo and Queso dela Tierra are the names of the similar products used in South and Central America (Bhattacharya et al., 1971). In certain parts of India, chhana is more commonly...
know as paneer. Traditionally paneer has been a variety of pressed chhana, used mainly for preparing cooked vegetable dishes. It was estimated in 1966 that nearly 1.2 per cent of Indian’s total milk production and 2.2 per cent of the quantity into milk products was utilized for the production of about 35 million kg of chhana. The preparation and use of chhana are confined mainly to the eastern region of the country, notably West Bengal, which produce the maximum quantity (De, 1983).

2.2 Paneer Market in India

Indian paneer market is estimated to be around one lakh metric tonnes per annum, 80% of which is sold as loose paneer by local milk vendors. It is the largest dairy product sold in terms of volume after liquid Milk. Paneer is a universally accepted product across Indian sub-continent and is the highest consumed dairy product. Paneer market can be divided into two major segments viz consumers and institutions. Institutional segment contribute to over 80% of the total Paneer market (Neha, 2007), its annual production is estimated at 150,000 tonnes (Aneja, et al. 2002).

2.3 Definition

According to Prevention of Food Adulteration Act (PFA), 1954, Paneer means the product obtained from cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 70.0 per cent moisture, and milk fat content shall not be less than 50.0 per cent of the dry matter.

According to Bureau of Indian Standards (BIS, 1983)- Paneer is “an important indigenous milk product prepared by the combined action of acid coagulation and heat treatment of buffalo or cow milk or a combination thereof (milk solids, subjected to the approval by the control committee for food standards suitably processed may also be used). The phenomenon of precipitation involves
the formation of large structural aggregates of proteins in which milk fat and other colloidal and soluble solids are entrained with whey”.

Table 2.1 BIS standards

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Requirements</th>
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</thead>
<tbody>
<tr>
<td>Moisture% by mass (max.)</td>
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</tr>
<tr>
<td>Milk fat% by mass on dry matter basis (min.)</td>
<td>50.00</td>
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<tr>
<td>Titratable acidity% lactic acid, (max.)</td>
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<tr>
<td>Standard plate count per g (max.)</td>
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<tr>
<td>Coliform per g (max.)</td>
<td>90</td>
</tr>
<tr>
<td>Yeast and Mould per g (max.)</td>
<td>250</td>
</tr>
</tbody>
</table>

2.4 Methods Of Manufacture

Although paneer can be made from cow, buffalo or mixed milk, buffalo milk is preferred. There are two methods for manufacturing of paneer.

2.4.1 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 minutes. Thereafter, the paneer is removed and cut into suitable sizes and immersed in chilled water for 3-4 hour to make it firm (Singh and Kanawjia, 1988; Sachdeva and Singh, 1988; Rao, et al., 1992).

2.4.2 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 $85^\circ$C to obtain a co- precipitate through a plate heat exchanger and pumped to a cheese vat and cooled to $75^\circ$C. Hot milk is coagulated by adding citric acid solution with proper mixing. The curd is left to
settle for 10 to 15 minutes 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 minutes 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 three hours for cooling and firming the paneer (Pruthi, 1999; Rao, et al., 1992).

2.5 Chemical Aspects

Chemical composition and chemical characteristics of paneer are summarized below.

2.5.1 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; Desai, 1988; Pal and Yadav, 1991; Singh, et al., 1991; Syed, 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 percent moisture, 23-26 percent fat, 17-18 percent protein, 2-2.5 percent carbohydrate and 1.5-2.0 percent 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 per cent 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.

Dhole et al., (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar city (M.S.) for chemical quality. The average moisture content of market samples of paneer was found in the range of 42.62 to 60.39 per cent. The average fat content in the market samples of paneer was found in the range of 21.60 to 23.50 per cent. Amongst the samples collected, 21 samples
(30.0 %) fulfilled the minimum standard fat content prescribed by BIS. The average protein content in market samples of paneer was found in the range of 15.06 to 20.33 per cent.

The mineral composition of market paneer and changes occurring in their profile during storage at ≤ 10°C for 30 days were studied by Boghra (1988). The average mineral content of market paneer sample for calcium, magnesium, phosphorous, citrate, sodium, potassium, chlorine, copper, iron and zinc were reported to be 27.00, 344.00, 123.00, 32.54, 53.75, 50.49, 0.52, 3.71 and 3.06 mg per 100g, respectively. There were no noticeable changes reported in the concentration of the minerals during storage except only for citrate, which declined significantly from initial concentration of 144.06 and 113.47 mg per 100g to 83.62 and 49.25 mg per 100g in buffalo milk and cow milk paneer respectively. The decrease was attributed perhaps to its rapid utilization by various microorganisms present in paneer.

2.5.2 Chemical characteristics

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

2.5.2.1 Acidity of paneer

Mistry (1988) prepared cow milk paneer samples from milk with 3.5 per cent fat by heating to 82°C for 5 min in the presence of either 0.02 or 0.05 per cent calcium sulphate, or disodium hydrogen phosphate, and coagulation at 82°C with 1 per cent citric acid solution. Test and control samples were packed and sealed in polyethylene bags, and stored in plastic boxes in a refrigerator (7-10°C). The author found that acidity of the control sample was 0.475 per cent which was different from that of the test samples. When calcium sulphate was used as an additive in milk, paneer made from such milk gave acidity of 0.457 and 0.464 per cent, respectively for $T_1$ (0.02 % calcium sulphate) and $T_2$ (0.05% calcium sulphate).
sulphate) treatment values of acidity of paneer were still higher than disodium hydrogen phosphate was used as an additive to milk. The value of acidity was 0.572 and 0.539 per cent, respectively for $T_3$ (0.02 % disodium hydrogen phosphate) and $T_4$ (0.05 % disodium hydrogen phosphate) treatment. The values of titratable acidity 0.483, 0.468, 0.449, 0.529 and 0.530 on 7th day, and 0.459, 0.455, 0.444, 0.540 and 0.580 at the end of 15 days of storage, respectively. During storage of paneer indicated an increase in $T_0$ and $T_1$ while decreased in $T_2$, $T_3$ and $T_4$.

Sachdeva and Singh (1990) reported the chemical changes occurring in paneer treated with different dipping materials were determined during storage at 8-10°C. Initial acidity of fresh paneer dipped in plain water was 0.20 per cent. Dipping in acidified water and acidified brine increased whereas that in buffered water decreased the titratable acidity. A gradual rise in titratable acidity followed by a sharp increase towards the end of storage marked the spoilage of paneer. The rate of increase in acidity was very slow in case of brine dipped samples. When hydrogen peroxide treatment was given, there was very little increase in acidity throughout the storage and eventually it was the development of mouldy flavour that rendered the product unacceptable. However, when the storage period was prolonged further by using delvocid, there was a sharp rise in acidity towards the end of 34 days of storage.

Kumar and Bector (1991) reported that when paneer stored at 5°C, initial titratable acidity, 0.54 per cent, of control samples increased slowly during storage and reached 0.9 per cent on day four and thereafter declined and reached a value of 0.59 per cent on day ten. However, the titratable acidity of paneer samples containing 0.05 per cent TBHQ and BHA, individually or in combination, remained constant up to two days and then started increasing slowly. The change in acidity was found to proceed at a lower rate in BHA added samples.

Gohian (1996) studied that the market sample of paneer collected from 18 different producers showed the mean acidity values ranging from 0.45 to 0.74 per
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cent. The mean values of acidity for paneer sample from 4 different markets and an organized dairy were 0.61, 0.69, 0.66, 0.59 and 0.61 per cent respectively, with general mean value for all the 30 market paneer samples 0.63 per cent.

Goel (2000) studied that the acidity of the laboratory made paneer increased from initial average value of 0.51 to 0.69 per cent in T₁ and 0.79 per cent in T₃ on the day 4 at 22 ± 1°C. The minimum change exposed to air, which received treatment T₂ (air washing with enhancing with enclosure effect and UV rays treatment) followed by T₁ (LAFU) and treatment T₃ (outside exposure). The change in the acidity was found to be statistically significant (p < 0.05). Treatment T₁ and T₂ are at par while treatment T₃ is significant different from these two treatment. The analysis indicates that the level of aerial contamination significantly influences the change in acidity. The acidity of market sample of paneer (Treatment, T₄) increased from an average initial value of 0.54 to 1.17 per cent lactic acid on the day 4. The market sample of paneer showed a very rapid change in acidity as compared to the laboratory made paneer samples.

Goel (2000) also studied that laboratory made paneer at 7 ± 2°C showed increase in per cent lactic acidity from 0.49 to 0.524 per cent in T₁ and 0.556 per cent in T₃ on the day 12 maximum change in acidity was observed in paneer exposed to the outside environment whereas paneer exposed to superior level of air sanitation showed minimum changes. The difference in the development of acidity was found to be statically significant (P < 0.05). Similarly, the changes in the acidity observed on the day 0, 6 and 12 were also found statically significant. The market samples of paneer with its initial average acidity of 0.553 increased to 0.64 per cent lactic acidity. Paneer stored at two different temperatures indicating the development of acidity in paneer stored at 7 ± 2°C was very slow in comparison to paneer store at 22 ± 1°C.

Venkateswarlu et al., (2003) observed the acidity of coconut milk paneer during storage at refrigeration with and without preservatives. The acidity of
paneer preserved with 0.2 per cent H₂O₂, 0.1 per cent potassium sorbate and 5 per cent brine were found increase from 0.228, 0.224 and 0.216 to 0.410, 0.478 and 0.482 per cent lactic acid at the end of storage period 27th, 21st and 12th day respectively. While the changes in acidity of control paneer increased gradually from 0.224 to 0.472 per cent lactic acid at the end of 9th day storage.

Yellamanda et al., (2006) reported that initial acidity of all pickled paneer samples were higher (0.42-0.43 per cent) than the acidity of fresh paneer samples. Titratable acidity levels in pickled paneer increased significantly during storage up to 60 days, probably due to the effect of slight fermentation in the paneer cubes and also due to infiltration of acidity of pickle base.

Gokhale and Pandya (2009) reported that paneer was prepared by using standardized milk and acidification, vacuum drying and salting treatments were given to the paneer cubes of approximately 1.5 cubic cm sizes and were packed in LDPE (90 micron) poly bags and was stored at 8 ± 2°C. The titratable acidity of the treated paneer (0.29 per cent LA) was more compared to control sample and it was due to the treatments it received.

2.5.2.2 Free fatty acid (FFA) content of paneer

Kumar and Bector (1991) reported that the initial level of FFA in control samples was slightly higher than the samples containing 0.05 per cent TBHQ and BHA, individually or in combination, and increased during storage indicating lipolytic changes, however, the rate of increase in FFA was lower in samples containing TBHQ and BHA as compared to control.

Pal et al., (1993) reported that low-fat paneer prepared from 1:1 ratio of cow and buffalo milk was coated with paraffin wax and stored at 10°C, together with non-waxed control paneer. The initial thiobarbituric acid value of 0.33 ± 0.02 (mg mal/100g) at 0 day in the control sample increased to 0.72 ± 0.01 (mg mal/100g) on 15th day storage, where as the initial value of 0.35 ± 0.03 (mg mal/100g) increased to only 0.46 ± 0.03 (mg mal/100g) in paraffined sample indicating lesser degree of oxidative deterioration of the product.
Gohian (1996) analyzed the market sample of paneer collected from 18 different producers and reported that the mean acid degree value ranging from 0.583 to 2.250. The mean acid degree value for 4 different markets and the organized dairy were 1.147, 1.337, 1.901, 1.218 and 1.097 respectively. The general mean obtained of acid degree for over all 30 sample of market paneer was 1.308.

Goel (2000) studied the laboratory made paneer and reported that the free fatty acidity content of expose paneer increase from its initial value of 0.0665 to 0.1383 ($T_1$) and 0.209 ($T_3$) per cent oleic acid on the day 4 at 22 ± 2°C. The paneer exposed to the outside atmosphere observed the maximum change, while paneer exposed to controlled level of air-borne contamination had low level of change. The initial FFA content of market sample of paneer increased from its initial value of 0.074 to 0.447 per cent on the day 4, where as on the day 2, with a value of 0.249 per cent pronounced rancid flavour was perceived at 22 ± 2°C.

The free fatty acidity of laboratory made paneer sample with its initial value of 0.0626 increased 0.0698 ($T_1$) and 0.0773 ($T_3$) per cent on the day 12. Higher rate of changes in the paneer content was observed in the paneer exposed to the outside atmosphere, while treatment $T_1$ and $T_2$ were at par. Market sample of paneer shows a rise from its initial value of 0.699 to 0.080 per cent at 7 ± 2°C.

Venkateswarlu et al., (2003) reported that FFA content of the paneer preserved with 0.2 per cent $H_2O_2$ was observed to be increased from 0.13 to 0.38 per cent oleic acid during storage at refrigeration. While the changes in the FFA content of the control sample and experimental samples preserve with 0.1 per cent potassium sorbate and 5 per cent brine was found to increase gradually from the initial value of 0.14 on the day of production to 0.38, 0.43 and 0.39 at the day of storage periods 9th, 21st, 12th day at refrigeration respectively.

Gokhale and Pandya (2009) reported that paneer was prepared by using standardized milk and acidification, vacuum drying and salting treatments were given to the paneer cubes of approximately 1.5 cubic cm sizes and were packed in
LDPE (90 micron) poly bags and was stored at 8 ± 2°C. The free fatty acids content of fresh sample was 0.04 per cent which increased to 0.175.

2.5.2.3 Soluble nitrogen content of paneer

Mistry (1988) prepared cow milk paneer samples from milk with 3.5 per cent fat by heating to 82°C for 5 min in the presence of either 0.02 or 0.05 per cent calcium sulphate, or disodium hydrogen phosphate, and coagulation at 82°C with 1 per cent citric acid solution. Test and control samples were packed and sealed in polyethylene bags, and stored in plastic boxes in a refrigerator (7-10°C). The author found that soluble protein content of paneer varied from 2.55 to 3.26 per cent on wet basis and from 5.82 to 7.14 per cent on dry matter basis. The values of soluble protein during storage 3.13, 3.94, 4.03, 4.07 and 4.07 per cent on 7th day, and 4.40, 4.26, 4.26, 4.35 and 4.63 per cent at the end of 15 days of storage respectively.

Sachdeva and Singh (1990) reported that chemical changes occurring in paneer treated with different dipping materials were determined during storage at 8-10°C. The soluble nitrogen content of the paneer dipped in plain, chlorinated, buffered and acidified water increased considerably after 6 days of storage. The rate of increase was higher in case of paneer dipped in acidified water followed by those dipped in buffered, chlorinated and plain water, respectively.

The soluble nitrogen content of paneer dipped in brine and acidified brine increased at very slow rate till the 18th day of storage and accelerated thereafter. Of the two treatments, the rate of increase in soluble nitrogen was higher for the acidified brine dipped paneer. The paneer sample dipped in brine showed an increase from 0.093 to 0.217 per cent on the 24th day of storage whereas the soluble nitrogen of acidified brine increased from 0.095 to 0.295 on 22nd day itself.

Sachdeva and Singh (1990) also reported the soluble nitrogen content of samples treated with potassium sorbate and devocid increased slowly in the beginning and rapidly upwards the end of storage on 10th day. The per cent soluble nitrogen increased from an initial value of 0.105 to 0.217 on the 8th day in case of
potassium sorbate treated paneer and to 0.25 on the 10th day of storage in case of delvocid treated sample.

Gohian (1996) studied that the soluble nitrogen content in 30 market samples of paneer examined from 18 different producers varied from 0.154 to 0.578% with a general mean 0.334%. The mean values obtained for 4 different markets and the organized dairy were 0.276, 0.375, 0.439, 0.371 and 0.270% respectively.

Gokhale and Pandya (2009) reported that paneer was prepared by using standardized milk and acidification, vacuum drying and salting treatments were given to the paneer cubes of approximately 1.5 cubic cm sizes and were packed in LDPE (90 micron) poly bags and was stored at 8 ± 2°C. Soluble nitrogen content of fresh paneer was 0.02 per cent which increased to 0.12 per cent. All the samples were coliform negative (i.e. less than 10 per g)

2.6 Nutritional Aspects

Paneer has a high nutritive value because of the higher concentration of fat and protein present in the product. Paneer provides a high calorific value of approx. 308.9 to 332.8 kcal per 100g of which fat and protein individually contribute 218.5 to 247 and 73.1 to 77.4 kcal per 100g respectively, i.e. around 95 per cent of total calories (values calculated as suggested by Lehninger, 1982).

Soni (1979) studied nutritive value of paneer and found that the protein efficiency ratio (PER) of paneer made from cow milk was 2.5, while that from buffalo milk was 3.4. The PER value of mixed milk was found low 2.3, which was identical to that of cow skim milk powder taken as control. The biological value of cow milk paneer was 80.38, while that of buffalo milk paneer was 86.56. Digestibility co-efficient values were shown to be identical for all three types of paneer made from cow, buffalo and mixed milk. Net protein utilization values for cow milk paneer and mixed milk paneer were reported to be 78.28 and 77.17 respectively, while it was higher (83.10) in case of buffalo milk paneer.
Sharda (1980) studied the bioavailability of minerals mainly calcium, magnesium and phosphorous of paneer using albino rats. The retention of calcium was 78.8 per cent while that of magnesium was 68.3 per cent.

### 2.7 Microbiological Aspects

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 gain 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; Ghodeker, 1989). The type and number of microorganisms including yeast and mould and their distribution in the product, may vary depending upon the location of the ‘halwai’ shops, extent of the exposure of the product to the atmosphere, temperature and period of storage etc. some of these organisms even could be pathogenic in nature and may be hazardous for the health of consumer (Gupta, 1985).

#### 2.7.1 Standard plate counts

Sachdeva (1983) prepared paneer samples under controlled conditions in laboratory and reported low total viable counts of 4.838 and 4.0 log cfu per g, respectively.

The incidences of staphylococci in paneer were reported by Kphlon and Grover (1984). Out of 30 samples of paneer from Ludhiana market analyzed, 80 per cent (24 samples) were contaminated with staphylococci with average count 10,000 cfu per g, 25 per cent of the total samples were also found to be positive for coagulase and TDNase tests.

Gupta (1985) studied the presence of the total bacterial load of paneer. The standard plate count in fresh sample of market paneer ranged from $2.5 \times 10^4$ to $3.5 \times 10^5$ cfu per g (average initial count of $3.0 \times 10^5$). The standard plate count of
NDRI dairy made paneer sample ranged from $5.0 \times 10^3$ to $1.8 \times 10^5$ (average $7.9 \times 10^9$) and for laboratory prepared paneer the SPC was cfu per g as low as $1.0 \times 10^4$ cfu per g. The laboratory prepared product also showed absence of coliform and staphylococci.

Sachdeva and Singh (1990) reported that total plate count of paneer treated with different dipping materials during storage. The fresh paneer had a total plate count of $10^1$ to $10^3$ per g which increased to the number of $10^4$ to $10^6$ per g regardless of treatment. The initial count of the samples ranged from $230 \times 10^2$ to $90 \times 10^3$ cfu per g being lowest for the samples dipped in chlorinated water. The total count of the spoiled samples ranged from $158 \times 10^4$ to $45 \times 10^6$. The total plate count of paneer treated with 2 per cent potassium sorbate increased steadily during the early storage from an initial value of $36 \times 10^3$ to $200 \times 10^3$ on the $10^{th}$ day and then showed a sharp increase to $165 \times 10^4$ on the $12^{th}$ day of storage. The sample treated with delvocid showed a linear increase in total count from an initial value of $52 \times 10^3$ to $125 \times 10^4$. The total count of paneer dipped in hydrogen peroxide treated water was reduced markedly. Initial count of $237 \times 10^1$ decreased to $160 \times 10^1$ at the end of $8^{th}$ day and increased thereafter to $33 \times 10^3$ at the end of 24 days. The total count of paneer that was dipped in hydrogen peroxide treated water followed by a delvocid dip, the initial count of $150 \times 10^1$ to $120 \times 10^1$ till the $6^{th}$ day and increased thereafter reaching $95 \times 10^5$ on the $32^{nd}$ day of storage. The count further increased to $180 \times 10^5$ on the $34^{th}$ day of storage.

Kumar and Bector (1991) reported there was progressive increase in the total plate counts of paneer samples during storage. However, the rate of increase in total plate count of control was more rapid as compared to the ones containing TBHQ and BHA individually or in combination. The initial counts of control, $3.0 \times 10^3$ increased to $2.8 \times 10^5$ per g on day four and $9.0 \times 10^6$ per g on day seven during storage. Paneer samples containing 0.05 per cent TBHQ were having initial counts less than $10^3$ per g increased to $1.96 \times 10^6$ per g on day four and $2.0 \times 10^6$ per g on day seven during storage. The corresponding values for paneer sample
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containing 0.05 per cent BHA were less than $10^3$, $2.0 \times 10^5$ and $7.0 \times 10^5$ per g, respectively. Similar trend was also observed in samples containing 0.05 per cent TBHQ and BHA in combination. These results showed that the TBHQ and BHA reduced (about 70 per cent) the initial level of microorganisms and kept the rate of increase in their number low during storage, as compared to control samples.

Pal et al., (1993) reported that low-fat paneer prepared from 1:1 ratio of cow and buffalo milk was coated with paraffin wax and stored at $10^0$C, together with non-waxed control paneer. Total plate count of control paneer sample showed the increase from $3.03 \pm 0.09$ to $6.27 \pm 0.07$ 15th day storage, while in paraffined sample the corresponding count were $3.12 \pm 0.06$ to $5.91 \pm 0.03$.

Gohian (1996) revealed that the SPC of market paneer samples from 18 different producers varied significantly from 4.6989 to 7.5549 log cfu per g, the mean total plate count values obtained for 4 different markets and an organized dairy plant were 5.8266, 6.2288, 6.2425, 6.0278 and 5.4835 log cfu per g, respectively and the values were at par. The general mean for all the 30 samples was 5.9424 log cfu per g, about 80 per cent of the total 30 samples, contain SPC $\geq 5.0$ log cfu per g and only 46.6 per cent of the market samples were falling with in the prescribed BIS limit for SPC. Vaishnavi et al. (2001) reported total plate count of $3 \times 10^3$ to $9.7 \times 10^{10}$ cfu per g in the samples of paneer collected from Chandigarh market.

Goel (2000) studied the microbiological changes in market and laboratory made paneer samples during storage at $22 \pm 2^0$C and $7 \pm 2^0$C. The initial average count of laboratory made paneer showed an effect of exposure to different levels of air-borne contamination. The sample, exposure to outside atmosphere with higher microbial load had higher initial count as compared to the sample exposed to a low level of air-borne contamination. During storage, this difference increased at higher order on the day 4, the count of outside exposed paneer sample increased to $8.9$ log cfu per g. The initial average count in market samples of paneer was higher as compared to the laboratory made paneer. Moreover, the rate of increase
was also found to be higher in the market sample stored at 22 ± 2°C. The initial total viable count of paneer exposed to different level of air-borne contamination indicated a higher count when exposed to higher level of air-borne contamination while the market sample exhibited the highest number of count. The increase in total viable count under refrigerated storage showed a very slow growth in all the samples.

Venkateswarlu et al., (2003) reported that total count of all the samples of coconut milk paneer increased during storage. Total count of paneer (log SPC values of 2.48) dipped in hydrogen peroxide treated water was markedly reduced in initial stages of storage (2.43) and increased thereafter (4.93 at the end of 27 days). Similarly, there was a gradual increase of standard plate count during the entire storage period at refrigeration in the coconut milk paneer samples preserved with 0.1 per cent potassium sorbate, 5 per cent brine and control paneer.

Dhole et al., (2009) evaluated seventy samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The average standard plate counts of market samples of paneer were ranged from 1 x 10^4 to 224 x 10^5 cfu per g amongst the seventy samples, only four samples (5.7 per cent) met the BIS specification.

Gokhale and Pandya (2009) reported that paneer was prepared by using standardized milk and acidification, vacuum drying and salting treatments were given to the paneer cubes of approximately 1.5 cubic cm sizes and were packed in LDPE (90 micron) poly bags and was stored at 8 ± 2°C. Fresh paneer had total viable count of 12.54 x 10^2 per g which increased to 29-36 x 10^3 per g within 90 days.

2.7.2 Coliform counts

Gupta (1985) studied the presence of coliform in NDRI Karnal dairy made and laboratory prepared sample of paneer. The ranges of coliforms in paneer samples were 1.0 x 10^1 to 5.5 x 10^3 (average 2.7 x 10^3), count of NDRI dairy
made paneer sample ranged from $< 1.0 \times 10^1$ to $1.5 \times 10^2$ (average $1.5 \times 10^3$) cfu per g, for laboratory prepared paneer coliform could not be detected in the laboratory prepared sample.

Viswesharaiah and Ananthakrishnan (1985) while assessing the quality of 8 to 24 h old fifty four market samples of paneer found mean coliform count 2 to 86 cfu per g only. Rajorhia et al. (1984) found mean coliform count as high as above 25 million cfu per g with range of $116 \times 10^3$ to $17 \times 10^7$ cfu per g for seven Delhi market samples. Kumar and Sinha (1989) analyzed 31 NDRI made paneer sample along with other indigenous milk product and found 7.3 to 10,000 per g. The IMVIC test carried out on isolation further confirmed the presence of fecal coliforms ranging between 0 to 10,000 per g, and based on this 84 per cent paneer sample were classified by them as ‘unsatisfactory’.

Sachdeva and Singh (1990) determined coliform count of paneer treated with different dipping materials and reported that the initial coliform count was not more than 3 to 4 in the first dilution of all the paneer samples and this increased to a maximum of 30 – 50 over the storage period. No colonies of *Escherichia coli* were detectable in any of the paneer samples either initial or during the storage of paneer.

Kumar and Bector (1991) reported the initial level of coliform counts of control increased from 90 per g to $3.5 \times 10^3$ per g after four days and $8.0 \times 10^6$ per g after seven days of storage, however, in case of paneer samples containing 0.05 per cent TBHQ, the initial count was much lower, almost 1/3 (30 per g), as compared to control and it increased to $1.36 \times 10^3$ per g after four days and $5.5 \times 10^5$ after 7 days of storage.

Gohian (1996) studied the coliform count in market samples of paneer and found that the count vary from 2.0 to 4.9642 log cfu per g among 18 producers. For 4 different market and the organized dairy the mean count were 3.704, 3.265, 2.740 and 3.160 log cfu per g, respectively which were at par. The general mean for 30 samples had the BIS limit, about 36.6 per cent of the sample had the
coliform count in the range 2.0 to < 4.0 and another 33.4 per cent sample had count even $\geq 4.0 \text{ log } \text{cfu per g.}$

Dhole et al., (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The average coliform counts in the market samples of paneer were found in the range of $12.6 \times 10^3$ to $23.2 \times 10^3 \text{ cfu per g.}$ amongst the samples collected only two samples (2.8 per cent) fulfilled the BIS specification.

2.7.3 Yeast and mould counts

Thakral (1986) studied the status of yeast in market, dairy and laboratory made paneer during storage at $37^0\text{C}$ when the product got spoiled, market paneer showed more increase in number of yeasts $(3.1 \times 10^4 \text{ cfu per g})$ as compared to dairy $(6.0 \times 10^3 \text{ cfu per g})$ and laboratory $(3.0 \times 10^1 \text{ cfu per g})$ made paneer sample. In case of laboratory made paneer, yeasts could be detected only after one day of storage, their growth was reported to be very slow and the number increased to $1.0 \times 10^1 \text{ cfu per g}$ only after 2 day, and reached a population of $3.1 \times 10^3 \text{ cfu per g}$ after 5 days of storage. After storage at a temperature of 5-7$^0\text{C}$, no significant increase was observed in yeast and mould counts of the laboratory prepared paneer.

According to Thakral (1986) the keeping quality for all the 3 types of paneer at $37^0\text{C}$ were found to be different i.e. on an average less than 1 day for market, more than 1 day for dairy and 2 days for laboratory made paneer sample. It was thus concluded that the early spoilage of market samples of paneer might be due to higher number of yeast contained in it.

The survival and growth pattern of artificially inoculated yeast culture viz. *Saccharomyces cervisiae var ellipsoïds*-522 and *Candida gulliermondii*-3124 strains individually at two different levels $1.0 \times 10^3 - 5.0 \times 10^3$ and $1.0 \times 10^5$ to $5.0 \times 10^5 \text{ cfu per ml}$ to paneer milk. Growth and changes in number of yeast artificially inoculated showed that *S. ceervisia*-522 when inoculated at low level but at higher
temperature of 70°C the yeast cells could not be recovered. However, when inoculated at lower temperature of 60°C in milk, they were less than 1.0 x 10^1 cfu per g of paneer. When it was stored at 22°C the yeast population showed considerable increase in number within 4 days. Similar trend of increase in yeast population for 22°C and 5°C stored paneer were observed at the higher rate of inoculum. This indicated that through a small number of organisms survived in paneer, they can multiply and achieve sufficiently high number during storage and could thus, lead to deterioration of the product (Thakral, 1986).

Sachdeva and Singh (1990) reported the initial count of the paneer sample dipped in plain, chlorinated, buffered and acidified water and brine and acidified brine varied over a narrow range of 35 x 10^1 to 52 x 10^1. The yeast and mould count at the time of spoilage in the respective samples ranged from 53 x 10^2 to 63 x 10^3. The count of paneer treated with 2 per cent potassium sorbate solution increased from an initial value of 12 x 10^1 to 75 x 10^2 on the 12th day of storage, samples given a devocid dip showed a very initial count of 6 x 10^1. Dipping in hydrogen peroxide treated water resulted in paneer with an initial yeast and mould count of 19 x 10^1 which decreased to 7 x 10^1 till the 8th day and thereafter increased steadily to as high as 190 x 10^2 on the 24th day of storage.

Kumar and Bector (1991) analyzed the yeast and mould counts of control paneer sample and found that initial count increased from 10 per g to 50 per g after 4 days and 250 per g after 7 days of storage, however, there was no change in the yeast and mould of samples containing 0.05 per cent TBHQ and BHA individually or in combination up to four days but thereafter it increased to 50, 180 and 90 per g, respectively. It shows that TBHQ and BHA have a considerable inhibitory effect on yeasts and moulds and check the growth of these organisms during storage; however, TBHQ seems to be more effective as compared to BHA.

Gohian (1996) observed that the yeast count in the paneer sample was higher (general mean 3.1950 log cfu per g) then the moulds (general mean 2.2363 log cfu per g). Yeast and mould count from 18 different producers indicated the
count varying from 1.6990 to 5.1746 log cfu per g, showing highly significant difference in counts. The mean of the counts for 4 different markets and the organized dairy plant were 2.9409, 2.7631, 3.7415, 4.0869 and 2.1878 log cfu per g respectively, and the variation in counts were significant. The general total yeast and mould for all the 30 market paneer sample was 3.1101 log cfu per g. More than half (63.3 per cent) of total 30 samples showed yeast and mould count in the range of 2.0 to <4.0 log cfu per g, whereas, only 13.3 per cent had count <2.0, about 23.3 per cent paneer sample had the count exceeding 4.0 log cfu per g only seven sample (23.3 per cent) out of total 30 were falling within the BIS prescribed limit for yeast and mould count.

Goel (2000) studied that the initial average yeast and mould count of laboratory made exposed paneer samples indicate lower counts in samples exposed to superior to the outside environment at 22 ± 2°C. Market sample of paneer contained high initial counts of 3.84 log cfu per g, which is much higher than the laboratory made paneer sample. On day 4, the yeast and mould counts in market sample increased to 6.77 log cfu per g. A rapid growth in yeast and mould count of outside exposed sample was also observed. It increased from low level of 1.42 log cfu per g to 5.07 log cfu per g in 4 days at 22 ± 2°C. The initial yeast and mould count of outside exposed paneer is higher as compared to the paneer exposed to low level of air-borne contamination on the day 12, it increased to a population of 2.43 log cfu per g from its initial population of 1.56 log cfu per g at 7 ± 2°C. The market samples of paneer with its initial population of 3.817 log cfu per g increased to 5.85 log cfu per g on the day 12 at 7 ± 2°C. The yeast and mould in the market sample was very high as compared to the laboratory made exposed paneer samples.

Venkateswarlu et al. (2003) reported that yeast and mould count of coconut milk paneer preserved with H2O2, the log values of yeast and mould counts initially declined from 1.93 to 1.79 in 6 days of storage and later increased to 4.54 at the end of 27 days storage. While the paneer preserved with 0.1 per cent
potassium sorbate and 5 per cent brine the log values of yeast and mould increased from initial values of 2.41 and 2.30 to 4.84 and 4.70 in 21 days and 12 days of storage at refrigeration respectively. Similarly, in the control paneer the log values on the day of production increased from 2.49 to 4.64 at the end of 9 days storage at refrigeration temperature.

Dhole et al. (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The yeast and mould counts in market samples of paneer were ranged between $1 \times 10^2$ to $99 \times 10^2$ cfu per g. Amongst the samples collected only four (5.7 per cent) samples, met the standards prescribed by BIS for the yeast and mould counts for paneer.

Gokhale and Pandya (2009) reported that paneer was prepared by using standardized milk and acidification, vacuum drying and salting treatments were given to the paneer cubes of approximately 1.5 cubic cm sizes and were packed in LDPE (90 micron) poly bags and was stored at $8 \pm 2^\circ$C. The yeast and mould of fresh paneer samples were lower (73 cfu per g) and did not increase much (117 cfu per g).

2.8 Measures To Improve Shelf Life Of Paneer

Paneer is rich in nutrients and there is also enough moisture content in product to permit growth of variety of micro organisms. The ambient storage conditions are generally favorable for their survival and rapid growth. Even through the initial heat treatment is sufficient to take care of spoilage microflora, the spoilage generally occurs due to post process contamination of the product, which is mainly responsible for restricting the shelf life of paneer.

Bhattacharya et al. (1971) showed that shelf life of laboratory made paneer is only one day or even less at room temperature. At $10^\circ$C although its freshness was lost in just 3 days and the reported shelf life was about 6 days, therefore, short shelf life of paneer is one of the major handicaps for its industrial production.
Several methods have been tried so far by a few workers to enhance the 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 below.

2.8.1 Antimicrobial agents

Various preservatives have been tried to improve shelf life of paneer. Certain antioxidants are also known to possess qualities to act as inhibitors of microbial growth when present in aqueous solution (Chang and Branen, 1975; Erickson and Tompkin, 1977; Fung et al., 1977; Klindworth et al., 1979).

Kumar and Bector (1991) studied the changes in paneer samples during storage at 5, 15 and 25°C with and without addition of two antioxidants viz., TBHQ and BHA or their combination at 0.05 per cent level. Paneer samples markedly reduced the initial counts of microorganisms and subsequently checked their growth during storage. The lipolytic and proteolytic changes in paneer during storage were also checked to a great extent as revealed by changes in titratable acidity, free fatty acid and soluble nitrogen contents. Paneer samples showed that the shelf-life of treated samples increased by two times as compared to control samples.

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 seconds (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 9th day of storage, after that control were rated lower for appearance, flavour and overall acceptability. Paneer samples of group 4 remained quite acceptable during the entire storage period.

### 2.8.1.1 Sorbic acid / Potassium sorbate

Thakral (1986) studied the impact on shelf life of paneer by using 0.1 per cent potassium sorbate. The surface of the butter paper was also impregnated uniformly with 10 per cent potassium sorbate solution and the treated wrappers were used for packing the paneer. It was reported that the keeping quality of treated paneer was extended by one more day as compared to the control at 37°C.

The mould could not be detected in the control as well as treated paneer. However the yeast was not found to be inhibited in stored treated paneer samples. In treated samples it was noticed only at the end of 6 day, similarly up to 2day yeast did not increase in number in treated paneer. Whereas, it increased from $1.0 \times 10^1$ to $4.2 \times 10^4$ cfu per g after 2 day in control samples. Incorporation of 0.1 per cent potassium sorbate and storing paneer at 5-7°C was reported to increase the shelf life nearly 3 fold (up to 18 days) as against 6 day in the control samples.

Singh et al. (1989) reported that sorbic acid at the rate of 0.15 per cent was added to milk before preparing paneer. The paneer was subsequently wrapped with sorbic acid coated butter paper the storage of paneer at 25-35°C extended the shelf life up to 36 days, the same shelf life was claimed at 5°C but by use of about 1/3 amount i.e. 0.05 per cent sorbic acid.

Sachdeva and Singh (1990) reported by dipping of paneer samples in various antimicrobial agents and stored at 8-10°C. Compared with control paneer, which had a shelf-life of 10 days, that dipped in chlorinated, buffered or acidified water, or delvocid had a shorter shelf-life and dipping in potassium sorbate had no effect, whilst dipping in brine, acidified brine, H$_2$O$_2$ or H$_2$O$_2$ with delvocid extended the shelf-life to 22, 20, 22 and 32 days respectively.
Pashupati et al. (2007) reported the addition of sorbic acid decreased the microbial load and enhanced the shelf life of paneer in descending order 150 ppm, 100 ppm, 50 ppm and without affecting the quality of paneer up to 7 days during room temperature.

2.8.1.2 Hydrogen peroxide

Nayak and Bector (1999) reported the results which showed that addition of $\text{H}_2\text{O}_2$ to milk significantly decreased the yield of paneer which was very soft, fragile and more white in appearance. Approximately 20-25 per cent of the $\text{H}_2\text{O}_2$ added to milk appeared in paneer which decreased as storage period increased.

2.8.1.3 Nisin

Thakral (1986) carried out studies on yeasts in paneer. Recently, Mane et al. (2007) have conducted study on application of nisin for improving shelf life of paneer. They found that addition of nisin at the rate of 250 RU per g was superior and showed no significant difference in flavour scores of paneer. However, no data regarding sensory attributes and chemical changes during storage are reported by these authors.

2.8.3 Herbs

Kaur et al. (2003) studied the effect of pre-treatments of green leafy vegetables on the quality attributes of vegetable impregnated paneer. The effect of blanching, leaf size reduction and stage of incorporation of coriander and mint leaves on yield composition and sensory attributes of vegetable impregnated paneer was studied. Incorporation of unblanched leaves caused discolouration. However, blanching in steam or hot water improved colour, increased moisture content and caused loss of ascorbic acid and flavouring compounds. Reducing the size of leaves by chopping or grinding and stage of incorporation significantly affected the yield and moisture content of vegetable impregnated paneer. The uniformity of distribution of vegetables in paneer was best attained on incorporation of coriander leaves to milk before coagulation.
Bajwa et al. (2004) studied that paneer samples were prepared incorporating 10 per cent coriander (Coriandrum sativum) or mint (Mentha piperita) leaves. Salted and unsalted paneer samples were stored at room and refrigeration temperatures for shelf-life studies. All the samples were acceptable for one and 10 days, respectively, at room and refrigeration temperatures.

2.8.4 Lactoperoxidase (LP) system

Shive Kumar and Mathur (1994) reported that paneer were manufactured from buffalo milk that had been preserved by the lactoperoxidase system (70 : 30 SCN-: H₂O₂) and stored at 30°C for 12 h. No differences in composition or organoleptic properties were detected between paneer manufactured from LP-treated milk and fresh milk. It were concluded that preservation of buffalo milk by 70 : 30 LP-system for 12 h at 30°C did not affect the quality of paneer during storage for 6 months.

2.8.5 Effect of paraffining

Pal et al. (1993) reported that low-fat paneer prepared from 1:1 ratio of cow and buffalo milk was coated with paraffin wax and stored at 10°C, together with non-waxed control paneer. Treatment with paraffin wax extended the shelf life of low-fat paneer by more that 10 days during refrigerated storage.

2.8.6 Heat sterilization

Sachdeva (1983) studied the effect of heat sterilization particularly to enhance the shelf life of paneer at room temperature. The paneer cubes (approximately size 1” x 3/4” x ½”) packed in tins were sterilized by auto claving at a steam pressure of 15psi for 15 min. The paneer kept well over a period of 50 days under ambient temperature thereafter, the perception of a mouldy character rendered the paneer organoleptically unacceptable.

2.8.7 Deep fat frying

Sachdeva (1983) also tried to increase shelf life by deep fat frying of paneer cubes in vegetable oil. The treatment had an adverse effect on keeping quality of paneer. An oxidized flavour developed on 6th day of storage in treated samples. In
stored sample, body and texture also deteriorated and the product became hard and chewy.

2.8.8 Dipping of paneer in treated water

Different materials known for their antimicrobial activity were used to treat the water, in which, paneer was subsequently dipped prior to packaging. These were (i) potassium sorbate (2 per cent), (ii) chlorine water (35ppm), (iii) buffered water (pH 7.5), (iv) acidified water (pH 5.5) (v) brine (5%), (vi) acidified brine (pH 5.5), (vii) delvocid (netamycin 0.5 per cent), (viii) hydrogen proxide (0.2 per cent) and (ix) combination of H$_2$O$_2$ and delvocid (Sachdeva, 1983).

Potassium sorbate solution (2 per cent) was used for dipping paneer before packaging, there was no marked improvement in shelf life at 5-8°C storage temperature. The final log count of 6.4 cfu per g indicated inadequate inhibitory effect on the micro organisms present. It also imparted an unclean sorbate flavour and slight bitterness to the product. The use of 35 ppm chlorinated water as dipping medium for paneer blocks for 2 h before packaging did not show any significant improvement in shelf life of paneer, on the contrary, the flavour deteriorated due to the treatment.

Similarly, use of buffered water (pH 7.5) containing sodium bicarbonate and calcium phosphate, acidified water (pH 5.5), acidified brine (pH 5.5 and delvocid (0.5 per cent) alone as dipping media for paneer did not show significant increase in shelf life. It was also observed that some of the dipping media, such as buffered solution, acidified water and acidified brine even induced faster deterioration of the product during storage and hence, many not be use full in hence the shelf life of paneer.

Use of 5 per cent brine and 0.2 per cent H$_2$O$_2$ individually when tried as a dipping medium for 2 h resulting in marked improvement in shelf life of paneer and the product was acceptable up to 22 days of storage at 5-8°C. It was reported that the deterioration in the flavour of treated samples got spoiled after about 8h of
refrigerated storage. However when delvocid was used in combination with germicide ($H_2O_2$), excellent results was obtained as the paneer sample thus treated kept good for a period of about 32 days.

Sachdeva and Singh (1990) found that dipping of paneer in brine (5 per cent) not only improved the shelf life markedly but also made it more palatable. Kaur et al. (2003) studied the effect of immersion of plain and vegetable impregnated paneer samples in 1 to 5 per cent brine on NaCl. Sodium chloride content of 1.7 to 1.8 per cent in paneer samples was found to be optimum with respect to improvement in sensory attributes of paneer samples with or without vegetables. Incorporation of higher salt on immersion in brine of higher concentration might increase its shelf life but adversely affect the sensory attributes.

2.8.9 Modified atmosphere packaging (MAP)

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

2.9.10 Low temperature

Arora and Gupta (1980) observed the storage of paneer at sub zero temperature -13 and -32°C for 120 days, the flavour and appearance is not affected but its body and texture deteriorated and the product become crumbly and fluffy on thawing. Visweshwaraiah (1987) had tried extension of the shelf-life of paneer by dehydration or freezing. Paneer was extruded to increase surface area and then dried for up to 2 h. Also it was frozen at -9 and -15°C. Although dehydrated paneer had a shelf life of up to 2 months, rehydration characteristics were poor and it lacked cohesive properties. Frozen paneer was having a shelf life of up to 8 days, although surface hardening was observed.

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-flavour after 4 months renders the paneer unacceptable (Kanawjia, et al., 1990).

2.8.11 Other treatments

Mini et al. (1995) reported that the effect of clean and sanitized air and hygienic practices on shelf life of paneer stored at 22 ± 1°C and 7 ± 2°C. Anil Kumar (1997) reported that the drying of paneer was one of the attempts made to extend the shelf life. Goel (2000) has evaluated the performance of air washing system with and without enclosure and UV sanitization treatment. Air washing with UV sanitation treatment showed a shelf life of 2 days and 12 days at 22 ± 1°C and 7 ± 2°C respectively.

Venkateswarlu et al. (2003) reported the process of manufacture of paneer from skim milk by incorporating coconut milk. The shelf life of paneer under experimental condition ranged from 1 to 2 days at room temperature and 9 to 24 days at refrigeration temperature by using preservatives.

Yellamanda et al. (2006) has tried pickling of low fat paneer by using tomato, tamarind, base with spices and oils at a ratio of 1:3 (w/w), during the storage period of three months at room temperature. Oven dried pickled paneer samples scored significantly higher overall acceptability scores compared to oil fried pickled paneer samples.

2.8.12 Hurdle technology

The limiting water activity for growth of microorganisms is influenced by environmental factors. If the environmental factors are favorable, the minimum water activity requirement for growth of microorganisms is lower. However, if the environmental factors are unfavorable, the minimum water activity requirement for growth of microorganisms is higher. Based on this fact so-called hurdle technology is developed for preservation of food. According to hurdle concept, each preservation parameter is termed as hurdle. Thus, all the parameters like
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water activity, pH, redox potential, heat treatment, temperature of storage, availability of nutrients, preservatives etc., which are included as “hurdles”

Singh et al. (1991) reported that treatment with sorbic acid and or gamma-radiation reduced the microbial load. Sorbic acid added to milk at 70°C before coagulation so that final concentration in milk came to be 0.10 per cent. After packing the paneer in polyethylene pouches, the product was γ-irradiated at the dose level of 2.5 kGy. The treatment enhances the shelf life of paneer up to 30 days at ambient temperature

Rao et al. (1992) observed the application of hurdle technology to the manufacture of paneer stable for ≥14 days at 30°C is described. Paneer samples with this shelf life may be prepared by mild heat treatment, a small reduction in a_w and acidification. Rao and Patil (1999) developed a paneer curry using hurdle technology. The product was so formulate as to have a water activity of 0.95, pH 5.0, potassium sorbate 0.1 per cent and processed at F-value of 0.8 in tins. The changes in rheological properties of paneer portion as well as chemical and sensory changes during storage at 30°C were studied. The product kept well for about one month and was found to have better quality than the heat-sterilized (15.0 F-value) product stored under similar condition.

Gokhale and Pandya (2009) reported that paneer was prepared by using standardized milk and acidification, vacuum drying and salting treatments were given to the paneer cubes of approximately 1.5 cubic cm sizes and were packed in LDPE (90 micron) poly bags and was stored at refrigerators (8 ± 2°C) temperature for 90 days. Paneer was soaked in different concentrations of vinegar solutions and were dried under vacuum till the moisture content reduced to 35 per cent. The moisture content in the fresh sample of paneer was 59.6 per cent and treated samples were 35 per cent lower in moisture content. The total plate count and yeast and mould count did not increased significantly during storage period of 90 days. Paneer sample was given for sensory evaluation after soaking in warm water
for 25-30 min. All the paneer samples remained acceptable even after storage period of more than 90 days.

Karthikeyan et al. (2009) made an attempt to utilized long recognized potential of microwave technology (425 watts for 5 min) with chemical preservative (sorbic acid 0.1 per cent and nisin) in combination and alone in order to extend shelf life of paneer under 35 ± 1°C temperature. Sorbic acid treated samples either individually or in combination with nisin, microwave treatment had an acceptable flavour up to 4th day, but their score was slightly < 7. Major flavour defects noted in treated paneer during storage was putrid/cheese flavour which was mostly due to microbial growth. Body & texture was significantly affected on all days of storage. Because of microwave heating moisture content in paneer slightly reduced making it little harder and rubbery. No sample was rejected due to damage caused to the body and texture of paneer. Considerable decreases in colour appearance score of paneer samples was observed as storage period prolonged. Microwave treated samples had significantly lower score due to slight dull colour and uneven surface while rest of sample scored better. As regards overall acceptability of the paneer samples, it was noticed that on fourth day, microwave + sorbic and microwave + sorbic acid + nisin had highest scores as 7.22 and 7.23 respectively. In general, it is seen that paneer could be stored up to four days at room temperature when treated with sorbic acid cum microwave heating; similar results for all three treatments given together were observed.

2.8.13 Trends toward use of natural ingredients

There has been increasing concern of consumers about food free of chemical preservatives because these could be toxic for humans. 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). Preservative agents are
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required to ensure that manufactured foods remain safe and unspoiled (Rasooli, 2007).

Being natural foodstuffs, spices appeal to consumers who tend to question the safety of synthetic additives. Antimicrobial properties of spices have been documented in recent years and interest continues to the present. In addition spices appear to be the most potential ingredients to improve shelf life of paneer.

2.9 SPICES

Spices and herbs have been used for thousands of centuries by many cultures to enhance the flavour and aroma of foods. Early cultures also recognized the value of using spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have documented the antimicrobial properties of some spices, herbs, and their components (Zaika, 1988).

Madsen and Grypa (2000) reported that spices are desirable food ingredients to create and explore new tasty products. Lewis (1984) reported that spices are derived from parts of the plants; the aroma is due to volatile essential oils of different chemical composition such as terpenes, sesquiterpenes, aldehydes, ketones, phenols, esters, ethers, oxides, etc. Achinewhu et al. (1995) reported the approximate composition of spices on dry weight basis, the crude protein content ranged from 4.6 to 22.1 percent, the fat (ether extract) ranged from 7.5 to 36.0 percent, total carbohydrate content ranged from 34.6 to 71.9 percent. The levels for peroxide value and free fatty acids (as percent oleic acid) of the spices are generally low indicating good storage stability of these plant materials. The flavour imparting essential oils (as percent oleoresin) content of the spices/herbs were fairly high and ranged from 0.1 to 5.2 per cent.

2.9.1 Classification of spices

There is no method to classify spices. However, the most common classification (Clark 1970) is based on the flavour and colour, i.e., hot (pepper), pungent (garlic), aromatic (cinnamon, clove, and cardamom), colouring
Review of literature

(turmeric), and herbaceous (rosemary, sage). Lewis (1984) reported that spices are also classified according to their taste such as sweet, spicy, sour, bitter and astringent.

Table 2.2 Main constituents and botanical name of spices

<table>
<thead>
<tr>
<th>Name</th>
<th>Botanical name</th>
<th>Main constituents of essential oil/oleoresin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td><em>Piper nigrum</em></td>
<td>Monoterpenehydrocarbons, piperine</td>
</tr>
<tr>
<td>Cardamom seed</td>
<td><em>Elettaria cardamomum</em></td>
<td>Cineole, α-terpinyl acetate</td>
</tr>
<tr>
<td>Cinnamon</td>
<td><em>Cinnamomum zeylanicum</em></td>
<td>Cinnamaldehyde</td>
</tr>
<tr>
<td>Cloves</td>
<td><em>Syzygium aromaticum</em></td>
<td>Eugenol</td>
</tr>
<tr>
<td>Garlic</td>
<td><em>Allium sativum</em></td>
<td>Diallylthiosulfinate</td>
</tr>
<tr>
<td>Ginger</td>
<td><em>Zingiber officinale</em></td>
<td>Zingiberene, cineole, borneol, geraniol, zingerone</td>
</tr>
<tr>
<td>Onion</td>
<td><em>Allium cepa</em></td>
<td>Trans-S-1-propenyl cysteine sulfoxide</td>
</tr>
</tbody>
</table>

Ceylan and Fung (2004)

2.9.2 Antimicrobial properties of spices

Spices and its essential oils/oleoresins are one of the components of the extremely effective naturally occurring antimicrobial systems that include products derived from animals as well as plants. Since the time of pasture antimicrobial properties of onion and garlic have been observed and recorded.

Hoffman and Evans (1911) were amongst the earliest to describe the preservative action of cinnamon, cloves, mustard, allspice, nutmeg, ginger, and
black pepper. Huhtanen (1980) reported the inhibition of *Clostridium botulinum* by white and black pepper.


Zaika (1988) found that cinnamon and cloves have antimicrobial potential, due to water infusion of cinnamon and clove inhibited the growth of eight species of yeast, including *Saccharomyces cervisiae*. Nakatani (1994) reported that the antimicrobial activity of 27 spices against all microorganisms and found that garlic was the inhibitory against all microorganisms tested, followed by onion, nutmeg and clove. Shibasaki (1982) reported that the major antimicrobial components of spices are in their essential oils. Raccach (1984) reported that the protein and fat component of foods bind essential oil compound, reduce their availability and protect microorganisms from their antimicrobial action.

Renata and Vanassalelsch (2003) reported that antimicrobial activity of 1 per cent (w/v) fresh garlic, ground clove and red dried chilli on *Listeria monocytogenes* was tested in broth systems at 37°C and at 4°C for 7 h. The initial cell concentration in the broth system was between 2 x 10^6 and 4 x 10^6 cfu per ml. At 37°C, growth to viable numbers of 3 x 10^8 cfu per ml in 7 h was measured. Clove had bactericidal activity and reduced the count to 1 cfu per ml. Garlic displayed bacteriostatic properties, and a count of 4x10^6 cfu per ml was maintained. Red chilli displayed an inhibitory effect and resulted in 50 per cent lower counts than the control.

**Table 2.3 Inhibitory effects of spices**

<table>
<thead>
<tr>
<th>Spices</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cloves</td>
<td><em>Mycotoxigenic Aspergillus</em></td>
</tr>
<tr>
<td>Cinnamon</td>
<td><em>Mycotoxigenic Aspergillus, Aspergillus parasiticus</em></td>
</tr>
<tr>
<td>Garlic</td>
<td><em>Salmonella typhymurium, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Mycotoxigenic</em></td>
</tr>
</tbody>
</table>
2.9.3 Antioxidant activity of spices

Farag et al. (1990) used thyme and cumin essential oils to prevent butter deterioration during storage at room temperature. Butter oxidation and lipolysis were followed by measuring the acid, peroxide and thiobarbituric acid (TBA) values. During butter storage, there was very little change in the peroxide and TBA value. The addition of cumin and thyme oil at 200 mg per kg to butter caused very little increase in the acid value. The data for lipolytic bacterial counts were in general agreement with essential oils showed a greater anti-hydrolytic effect and were better preservatives than butylated hydroxyl toluene.

Shobana and Naidu (2000) reported that relative antioxidant activities decreased in the order of cloves, cinnamon, pepper, ginger, garlic, mint and onion. Spice mix namely ginger, onion and garlic; onion and ginger; ginger and garlic showed cumulative inhibition of lipid peroxidation thus, exhibiting their synergistic antioxidant activity. The antioxidant activities of spice extracts were retained even after boiling for 30 min at 100°C, indicating that the spice constituents were resistant to thermal denaturizing.

2.9.4 Use of spices in food to extend shelf life

Spices are aromatic vegetable materials, have long been used in food not only for their flavouring, but also for their medicinal and preservative properties (Davidson et al., 1983). Spices also stimulate appetite by increasing salivation, carminative action, and preserve the food by their antimicrobial and antioxidant
properties (Lewis, 1984). Spices and oils were distinguishably used for their therapeutic action, for flavouring and much used in popular medicine (Taylar and Robbers, 1999).

An increasing number of such natural systems are being deliberately utilized for food preservation, or being explored for such use. The future potential is substantial; particularly as the efficacy of these systems demonstrated in additive or synergistic combinations with some of the other antimicrobial factors that we can employ to improve the safety and shelf life stability of foods.

While naturalness alone is not necessarily a sufficient objective for these developments, the use of natural inhibitors as components of systems that can together enhance the effectiveness of preservation, with advantages in product quality and safety, justifies pursuit (Subbulakshmi and Naik, 2002). Several investigators used natural flavouring additives in some dairy products as flavours, preserving and antibacterial materials. (El-Nemer, et al., 2003; Hussein, 2004).

2.9.4.1 Black Pepper

Lee et al. (1995) reported that peppers are rich in polyphenols, particularly the flavonoids, quercetin and luteolin, and also excellent source of antioxidants, ascorbic acid and other phytochemicals (Chuah, et al., 2008). Nair et al. (1996) have proved bacteriostatic properties of black pepper on *E. coli*, *Salmonella virochow* and *Staphylococcus aureus* at the concentration used in meat preparation. Ilondu and Iloh (2007) carried out the study on the inhibition of three fungi isolated from sorrel drink (Zobo) using water extracts of *Zingiber officinale* (Ginger), *Piper guineense* (West African Black Pepper) as well as heat as hurdles.

2.9.4.2 Cardamom

Cardamom (*Eletteiral cardamomum*) is known for centuries for its enhancing taste and flavour, it imports to the food. Cardamom is aptly called as ‘queen of spice’. The world production of cardamom is 28,000 metric tons (Spices Board 1997). Cardamom contains volatile oil (6-14 per cent) which imparts to the cardamom its characteristic aroma. The major compounds are 1, 8 cineole and $\alpha$-
terpinyl acetate. Monoterpene compounds have been found in cardamom (Menon, 2000).

Sen and Rajorhia (1996) conducted a study to find out the efficacy of cardamom powder in preservation of sandesh. They reported that the cardamom powder added at the rate 0.1 per cent enhance the shelf life of sandesh to 90 days as against 60 days for control sample at 7°C. This can attributed to the presence of several antimicrobial phenolics such as limonene, nerolidon, cineol, terpene, sabinine, pinene etc.

Ratiba et al. (2006) studied effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat’s milk. White soft cheese was made from heated goat’s milk (75°C) and cardamom powder (0.10 and 0.20%), thyme powder (0.10 and 0.15%), and clove powder (0.10 and 0.20%) were added to the cheese curd after whey drainage. The obtained cheese were stored in brain at 6 ±1°C and analyzed when fresh and after 15, 30 and 45 days. The author concluded that the use of some spices powders improved the quality of the resultant goat’s cheese, especially cardamom or clove powder at concentration of 0.10 and 0.2 per cent and storage at 6 ±1°C for 45 days.

Choudhary and Sandey (2009) studied whey based mango herbal (Cardamom) beverage. Various trials were carried out at the laboratory and the level of sugar (8 per cent) and mango pulp (12 per cent) with respect to whey in the product development was standardized. Edible medicinal plant of cardamom extract (0-3 per cent levels) was incorporated in the beverage in order to prepare health conscious as well as improve flavour of the whey beverage. They were subjected to sensory evaluation to test the acceptability on storage for one month. Among the beverage containing various level of cardamom extract, 2 per cent level of cardamom beverage were found to be highly acceptable in sensory characteristics up to 30 days at refrigerated temperature.

2.9.4.3 Cinnamon
Menon et al. (2002) studied the cinnamon powder for antilisteric acity in meat and cheese. It exhibited bacteriostatic action on \textit{L. monocytogenes} in both the foods. Foods treated with 6 per cent cinnamon showed 1-2 $\log_{10}$ less \textit{Listeria} counts per g than in the untreated controls on holding the foods at 30\degree C for 7 days. Treatment with 3 per cent cinnamon also showed down the growth of the microorganisms significantly in meat but to a lesser extent in cheese at 30\degree C.

Agaoglu et al. (2007) reported the antimicrobial activity of some spices used in meat products against some microorganisms. Tested were carried out in vitro with \textit{Staphylococcus aureus}, \textit{Klebsiella pneumoniae}, \textit{Pseudomonas aeruginosa}, \textit{Escherichia coli}, \textit{Enterococcus faecalis}, \textit{Mycobacterium smegmatis}, \textit{Micrococcus luteus}, and \textit{Candida albicans} as test strains by using disc diffusion method they found that cinnamon was the most effective spice against tested microorganisms. The antimicrobial activity of cinnamon may be explained by its volatile oil components. The most important active substances found in cinnamon oil are cinnamic aldehyde and eugenol.

2.9.4.4 Clove

Ouattara et al. (1997) reported that essential oils that contain carvacrol and eugenol have been shown to exhibit the strongest antimicrobial activity. Kramer (1985) analyzed the amounts of gallic acid and eugenol was determined to be 1.26 g and 3.03 g respectively in 100 g of clove. Helle and Bertelsen (1995) reported that the clove showed the highest antioxidant activity in an oil-in water emulsion.

Souhair and Nefisa (1980) studied the effects of black pepper, cinnamon, peppermint, cumin, ginger and clove on growth and aflatoxin formation of \textit{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 per cent.
Maraghy (1995) reported the high antimycotic activity of four kinds of spices, viz. Chinese cassia, cinnamon, clove and thyme, were tested as preservatives for agricultural commodities. Clove, thyme and a mixture of the four kinds of spice completely inhibited aflatoxin production on lentil seeds during eight weeks of incubation.

Rajkumar and Berwal (2003) studied the inhibitory effect of clove against *Penicillium chrysogenum*, *Penicillium verrugosum*, *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 per cent inhibition level was also calculated. Clove exhibited minimum inhibitory concentration of 0.86, 1.12, 1.08, 1.30 and 0.92 per cent (w/v) against *P. chrysogenum*, *P. verrugosum*, *Asp. flavus* and *Asp. parasiticus*, respectively. At 5 per cent level of clove all spoilage and toxigenic mould growth was inhibited during 25 days of study period.

Sharma and Verma (2006) reviewed the efficiency of four plant essential oils bay, clove, cinnamon and thyme as natural food preservatives. The effect of the plant oils at concentrations of 0.1, 0.5 and 1 per cent was studied in low-fat and full-fat and full-fat soft cheese against *Listeria monocytogenes* and *Salmonella enteritidis* at 4°C and 10°C, respectively, over a 14 days period. In low-fat cheese, all four oils at 1 per cent reduced L. monocytogenes to 1.0 log_{10} cfu per ml. In full-fat cheese, oil of clove was the only oil to achieve this reduction.

### 2.9.4.5 Garlic

Shelf life of chicken, beef and mutton cuts sprayed with water extracts of ginger, garlic and onion were extended at ambient temperature. Garlic had higher antimicrobial activity with longer shelf life than ginger and onion treatments. Addition of 1 per cent garlic extract completely inhibited the growth of *E.coli*, *Salmonella Typhosa*, *S. dysenteriae* and *S. aureus* (Ziauddin et al., 1996).

Banerjee and Sarkar (2004) reported that aqueous extracts of garlic were found to possess a potent bacteriostatic against gram-positive as well as gram-
negative food borne bacterial pathogens. Most of the tested strains were resistant to penicillins, although sensitive to garlic. While the growth of *B. cereus* and *Cl. perfringens* was completely inhibited at 10 and 70 mg garlic, respectively, per ml test broth, their respective enterotoxin production ceased at 10 and 50 mg garlic per ml.

Sanwal and Payasi (2007) studied the ripe banana fruits which were subjected to dip treatment for 4 h using chemicals, plant extracts or their combinations, and shelf life of treated fruits stored at room temperature was monitored by assaying fruit firmness. The treatment of fruit with 1 per cent garlic extract or 10 per cent onion extract and adjusting the pH to 4.5 extended the shelf life by 4-5 days when compared with untreated fruit. Treatment with a combination of 1 per cent garlic extract and sodium metabisulphite and adjusting the pH to 4.5 prolonged the shelf life of ripe banana to 13-14 days when compared with 6-7 days for the untreated fruit. The treatment delayed the fruit softening by diminishing the rate of starch and pectin degradation. The rate of increase in [beta]-amylase and polygalacturonase an activity along with softness was also retarded in treated fruits when compared with control fruits.

2.9.4.6 Ginger

Helle and Bertelsen (1995) by using chromatographic techniques separated out eight diarylheptanoids, including cm'cumin, and five gingerol-related compounds, [6] - gingerol and [6] - sbogaul being two of the identified substances. Pinto *et al.* (2004) has evaluated the suitability of ginger juice as a flavouring agent in developing ginger ice cream. Ice cream was prepared in an ‘ice and salt’ type of hand freezer using ginger in the form of juice at the selected levels of 3, 4 and 5 per cent. Addition of ginger juice resulted in a progressive decrease in all the compositional attributes and pH, and an increase in acidity, which was significant, especially at higher levels of ginger juice addition. The incorporation of ginger juice at 4 per cent rate is advocated in manufacture of ice cream having superior flavour characteristics over vanilla ice cream.
Bandyopadhyay et al. (2007) reported the antioxidant activities of beet (*Beta vulgaris*), mint (*Mentha spicata* L.) and ginger (*Zingiber officinale* L.) alone or in combination were assessed after their fortification in sandesh using Randox's total antioxidant level determining chemicals and ultimately it was compared with the synthetic antioxidants like TBHQ, BHA and BHT. Addition of beet or mint alone in sandesh showed lower antioxidant level than the addition of ginger alone. But according to sensory characteristics, sandesh containing beet, ginger or combination of beet with ginger or mint was more acceptable to panelist than control sandesh. Patel *et al.* (2007) studied the whey based mango herbal (ginger) beverages were prepared with different levels of ginger extract ranging from 1 to 3 per cent (v/v) using three different varieties of mango pulp. Beverages were evaluated for physico-chemical properties and overall acceptability. Whey based mango herbal beverage with pulp of langra variety and 2 per cent ginger extract was liked most by the panel of judges.

### 2.9.4.7 Onion

Shelf life of chicken, beef and mutton cuts sprayed with water extracts of ginger, garlic and onion were extended at ambient temperature. Addition of as little as 4 per cent onion extract completely inhibited the growth of *S. dysenteriae* and *S. aureus* and reduced the number of *E. coli* and *S. Typhosa* by 48.3 and 95.3 per cent, respectively (Al-Delaimy and Ali, 1970)

Serdaroglu and Felekoglu (2009) reported that sardine (*Sardina pilchardus*) mince was treated with rosemary extract (300 ppm) and onion juice (1 ml per 100 g) then stored at −20°C for 5 months. Proximate composition, thiobarbituric acid (TBA), free fatty acids (FFA) and peroxide value (PV) were determined on 0 and 15 days and 1, 2, 3, 4 and 5 months of storage. Fatty acid composition was also determined on 0 and 5 months of frozen storage. TBA, PV and FFA levels increased on all experimental groups due to the lipid oxidation. Rosemary showed antioxidative effect on sardine mince during frozen storage as indicated by TBA, PV and FFA levels. Oxidation was delayed for 3 months by onion juice treatment.
At the end of 5 months’ storage, the TBA values in onion juice treatment and control treatment were out of consumable limits. After frozen storage of 5 months polyunsaturated fatty acid level decreased and saturated fatty acid level increased in the control treatment. No significant change was observed in fatty acid composition in samples of rosemary and onion juice treatments.
3. MATERIALS AND METHODS

This chapter covers details on the manufacturing of paneer, selection of best spices and rate of addition of spices in paneer. It also covers details regarding storage and analysis schedule of paneer. It also encompasses details of the method 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.

3.1 Collection Of Raw Materials

For preparation of paneer milk, citric acid and spices were procured.

3.1.1 Milk

For the preparation paneer full cream milk (6.0%Fat, 9.0% SNF) and standardized milk (4.5%Fat, 8.5%SNF) were collected from Vidya Dairy, Anand.

3.1.2 Spices

The below listed seven of spices were collected from local market of Anand city from a retailer.

1. Black pepper
2. Cardamom
3. Cinnamon
4. Clove
5. Garlic
6. Ginger
7. Onion

3.1.3 Packaging material

Composite polyethylene terephthalate (PET)/low density polyethylene (LDPE) film (50μ thickness) was used for packaging of samples during storage study. The material was obtained from Vidya Dairy, Anand.

3.1.4 Chemicals

All the chemicals used in the study were of analytical grade.
3.2 Preparation Of Spices For Paneer

The dry spices (black pepper, cardamom, cinnamon and clove) were grinded in to powder form and fresh spices (garlic, onion and ginger) were converted into paste by using domestic mixer.

3.3 Sterilization Of Utensils

All utensils were washed with boiling water and muslin cloth was boiled in water for 5min.

3.4 Method Of Manufacturing Of Paneer

The paneer was prepared in the laboratory using method described by De (1983).

Milk for preparation of paneer was standardized to 5.5 per cent fat by mixing the full cream milk and the standardized milk in calculated amount. The milk standardized for preparation of paneer was subjected to heat treatment of 95°C for 5 minutes. The milk was subsequent by cooled to 70°C. Citric acid was added at the rate of 1 per cent by weight of milk in form of 1 per cent solution. The solution was added with continuous agitation until the coagulation was complete. The curd was allowed to settle for 5 minutes. Whey was drained through a muslin cloth by gentle squeezing with hand and curd was collected. Each sample of curd was then filled in a round shaped per sterilized stainless steel hoop lined with clean muslin cloth. The curd was pressed for 20 minutes followed by immersing in chilled water (4 to 6°C) for 2 hours. The samples were removed from chilled water and blocks on wooden planks for allowing the water to drain off for 15 minutes.

3.5 Incorporation Of Spices In Paneer
The spices were incorporated in the product at the stage after removal of whey and before pressing the curd. The prepared sample of spice was incorporated in to curd at the required rate and mixed by stirring with sterilized stainless steel spoon. The subsequent steps in the preparation of paneer remained same as described 3.4.

3.6 Selection Of Spices
For selecting spices, the seven different spices listed under 3.1.2 were incorporated in paneer at the rate of 0.5 per cent and resultant samples were subjected to sensory evaluation for acceptability.

3.7 Selection for Rate Of Addition Of The Spices
For selecting rate of addition of each spice the curd was divided in to six equal parts and the spice was incorporated in the product at the rate of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 per cent by weight of expected yield of paneer as per the method described under 3.5. Total three replications were taken for each spice. The prepared samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale.

3.8 Preliminary Screening Of The Spices
In preliminary screening of spices in paneer the curd was divided in to eight equal parts and the seven spices were incorporated in the product at the selected rate (black pepper 0.6%, cardamom 0.6%, cinnamon 0.4%, clove 0.6%, garlic 0.6%, ginger 0.6%, onion 0.6%). In one of the sample of paneer none of the spice was added. Total three replications were conducted.

Each block of paneer was cut in to four equal parts and packed separately in to the composite PET/LDPE film pouches and stored at 7°C±1°C for 14 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges when fresh and after 14 days of storage.
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The prepared samples of paneer were also analyzed for acidity, free fatty acids and soluble nitrogen content using the methods as described under 3.13.2, 3.13.1 and 3.13.3 respectively.

3.9 Final Screening Of The Spices

In final screening of spices for their suitability in paneer the curd was divided into five equal parts and the four spices (black pepper, cardamom, cinnamon and clove) were incorporated in the product at the selected rate (black pepper 0.6%, cardamom 0.6%, cinnamon 0.4%, clove 0.6%). In one of the sample of paneer none of the spice was added. Total three replications were conducted.

Each block of paneer was cut into four equal parts and packed separately into the composite PET/LDPE film pouches and stored at 7\(^0\)C±1\(^0\)C for 28 days. The samples were also stored at 32\(^0\)C±1\(^0\)C in BOD incubator. The prepared samples of paneer were subjected to sensory evaluation by panel of judges when fresh and at regular interval of 7 days during storage. The prepared samples of paneer were also analyzed for acidity, free fatty acids and soluble nitrogen content the methods as described under 3.13.2, 3.13.1 and 3.13.3 respectively.

3.10 Evaluation Of The Best Spice For Its Effect On Microbial Counts In Paneer

The prepared samples of fresh and stored paneer containing the best spice were also analyzed for microbial counts viz. standard plate count, coliform count and yeast and mold count at regular interval of 7 days.

3.11. Preparation Of Chemicals

Various chemicals required for chemical analysis, were prepared by using standard methods.

3.11.1. Preparation of 0.1N HCl

Take 8.4 ml of concentrated hydrochloric acid in clean and dry beaker. Take approximately 500ml water in 1000ml volumetric flask and pour slowly acid from side to the flask and mix, give several washing of beaker with distilled water.
and transfer quantitatively to volumetric flask. Cool using ice bath, make volume and standardization against 0.1 N sodium carbonate.

### 3.11.2 Preparation of 0.1N \( \text{H}_2\text{SO}_4 \)

Take 3 ml of concentrated \( \text{H}_2\text{SO}_4 \) in clean and dry beaker. And take approximately 500ml water in 1000 ml volumetric flask or in measuring cylinder. Pour slowly acid from side to the flask and mix, give several washing of beaker with distilled water and transfer quantitatively to volumetric flask. Cool by ice bath and use for standardization against 0.1 N sodium carbonate.

### 3.11.3 Preparation of 0.1N NaOH

Weigh 4.4 g of NaOH pellets in clean and dry beaker dissolve in little glass distilled water and shake it with glass rod transfer in volumetric flask (1000ml). Give several washing of beaker with distilled water and transfer quantitatively volumetric flask and use for standardization against 0.1N oxalic acid.

### 3.11.4 Sharp’s extraction solution

Stock solution was prepared by dissolving 57.5 ml glacial acetic acid, 136.1g of sodium acetate trihydrate, 47.0 g sodium chloride and 8.9 gm of anhydrous calcium chloride in about 750 ml distilled water and finally adjusting the volume up to 1 lit. From the stock solution 250 ml was diluted to 1 lit with distilled water to obtain the working solution.

### 3.12 Evaluation Of Paneer For Sensory Attributes

Each block of paneer was cut in to rectangular pieces of approximately 1cm x 2cm. The prepared samples of paneer were subjected to sensory evaluation by panel of six judges using 9 point hedonic scale (Amerine et al., 1967).
Table 3.1: Score card for sensory evaluation on 9 points hedonic scale

<table>
<thead>
<tr>
<th>Hedonic Rating</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Like extremely</td>
<td>9</td>
</tr>
<tr>
<td>Like very much</td>
<td>8</td>
</tr>
<tr>
<td>Like moderately</td>
<td>7</td>
</tr>
<tr>
<td>Like slightly</td>
<td>6</td>
</tr>
<tr>
<td>Neither like nor dislike</td>
<td>5</td>
</tr>
<tr>
<td>Dislike moderately</td>
<td>4</td>
</tr>
<tr>
<td>Dislike slightly</td>
<td>3</td>
</tr>
<tr>
<td>Dislike very much</td>
<td>2</td>
</tr>
<tr>
<td>Dislike moderately</td>
<td>1</td>
</tr>
</tbody>
</table>

Evaluate the given sample of Paneer using above guidelines

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>Flavor</td>
<td></td>
</tr>
<tr>
<td>Color &amp; Appearance</td>
<td></td>
</tr>
<tr>
<td>Body &amp; Texture</td>
<td></td>
</tr>
<tr>
<td>Overall Acceptability</td>
<td></td>
</tr>
</tbody>
</table>

Comments (if any):

Name  Date  Signature

3.13 Analysis Of Paneer For Proximate Composition

3.13.1 Determination of moisture

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

About 3gm of paneer sample was weighed in a previously dried and tarred aluminum dish, mixed thoroughly with about 5ml 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

\[
\text{Moisture (\%w/w)} = 100 \times \left( \frac{W_1 - W_2}{W_1 - W} \right) 
\]

\( W = \text{Weight in gm of the empty dish} \)
\( W_1 = \text{Weight in gm of the dish with paneer sample before drying} \)
\( W_2 = \text{Weight in gm of the dish with paneer sample after drying.} \)

3.13.2 Determination of fat

Fat content of paneer was estimated by following the method described for cheese (Anon, 1972).

Exactly 3g of paneer was weighed and add 10 ml of H\(_2\)SO\(_4\) (sp. gr. 1.820 – 1.825 at 20\( ^\circ \)C) in cheese butyrometer and 1ml of iso amyl alcohol (Sp.gr.0.815 – 0.825 at 20\( ^\circ \)C). The butyrometer was lock stoppered and the contents were vigorously shaken to digest non-fat substances. Liquid level in the butyrometer was brought to calibration by addition of required amount of water. The butyrometer was then placed in the Gerber centrifuge machine and centrifuged for about 10 min at 1100 rpm. The butyrometer was tempered in a hot water bath maintained at 65 ± 2\( ^\circ \)C for 30 min and the fat per cent was read from the fat column.

3.13.3 Determination of protein

Protein content was determined by Kjeldahl method as described by Horwitz (1980).

Accurately weighed paneer sample (0.5 gm) was transferred into 800ml digestion flask. To this 25ml of concentrated H\(_2\)SO\(_4\) and 10g digestion mixture (consisting of copper and potassium sulphate, 1: 10 w/w) was added and preceded for digestion. The mixture was digested over flame till it became transparent. Mixture was allowed to cool, diluted with 200ml distilled water and neutralized
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with approximately 80ml of 50% w/v sodium hydroxide solution. The mixture was distilled and the distillate collected in a conical flask containing 50ml of saturated boric acid solution and 1 drop of mixed indicator (equal volume of saturated solution of methyl red in ethanol and 0.1% solution of methyl blue in ethanol). About 150ml of the distillate was collected and then titrated against 0.1 N H$_2$SO$_4$.

Calculation of protein content:

\[
\text{Total nitrogen (}\%\text{w/w)} = \frac{V}{W} \times 0.14
\]

Where, \(V\) = Volume of 0.1 N H$_2$SO$_4$ required for titration

\(W\) = Weight in g of the sample.

Protein (\% w/w) = Total nitrogen (\%) \times 6.38.

### 3.13.4 Determination of Lactose

Lactose content in paneer was estimated by difference.

### 3.13.5 Determination of ash

The ash content of the paneer sample was estimated by the method of BIS (1981).

About 5g of the sample was weighed in a previously dried and tarred silica crucible. The contents were subjected to heat until the combustion was complete. The crucible was then transferred to a Muffle furnace, temperature until the ignited material was free from carbon. Contents were cooled in desiccators and weighed. The process of ignition, cooling and weighing was repeated at half hourly intervals until the difference between two successive weighing was less than 1mg.

Calculation of ash content:

\[
\text{Total ash (}\%\text{w/w)} = 100 \times \frac{(W_2 - W)}{(W_1 - W)}
\]

Where, \(W\) = Weight in g of the empty crucible

\(W_1\) = Weight in g of the crucible with sample

\(W_2\) = Weight in g of the crucible with ash.
3.14 Analysis Of Paneer For Chemical Characteristics

The chemical changes taking place in the paneer are analyzed by using different methods are described below.

3.14.1 Determination of free fatty acids

Free fatty acids content of paneer was estimated by following the method described for cheese (Thomas, 1954).

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

Calculation of free fatty acids:

Free fatty acids (% Oleic acid) = \( \frac{2.82 \times T}{5 \times W} \)

\( T \) = Volume in ml of 0.02N NaOH required for titration, and
\( W \) = Weight in g of the sample taken.

3.14.2 Determination of acidity

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

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

Calculation of acidity:

Acidity (% lactic acid by weight) = \( \frac{10 - V}{M} \times 0.9 \)

\( V \) = Volume in ml of standard acid used in titration
\( M \) = Mass in g of sample taken for the test.
3.14.3 Determination of soluble nitrogen

The soluble nitrogen content of the paneer sample was estimated by Kjeldahl method as described by Kosikowski (1970) for cheese.

Three grams of paneer was ground into a smooth paste warm (50°C) sharp’s extraction solution. Additionally using more solution, the paste was further diluted and the same was quantitatively transferred to 100 ml volumetric flask. The final volume was adjusted to 100 ml using the same extraction solution.

The content was tempered at 50°C ± 1°C for 1 hr with intermittent shaking, followed by filtering through Whatman No 40 filter paper. Twenty milliliters of the filtrate was used for estimation of soluble nitrogen. Digestion and distillation was done by the Kjeldahl method.

Calculation of soluble nitrogen:

\[ \% \text{ soluble nitrogen} = 2.333 \times (B-A) \times N \]

where:
- \( A \) = Volume in ml of standard \( \text{H}_2\text{SO}_4 \) required for blank
- \( B \) = Volume in ml of standard \( \text{H}_2\text{SO}_4 \) required for sample
- \( N \) = Normality of standard \( \text{H}_2\text{SO}_4 \)

3.15 Analysis Of Paneer For Microbial Counts

The standard methods of standard plate count, coliform count and yeast and mould count are described below.

3.15.1 Preparation of sample

The samples for microbiological analyses were prepared under aseptic conditions. A sanitized set of pastle and mortar was taken for macerating the sample.

Eleven gram of the paneer sample was weighed aseptically in a sterile 100ml glass beaker and it was transferred aseptically to the sanitized mortar with the help of a sterile stainless steel spatula. The sample was then macerated thoroughly by making a paste using small quantity of previously warmed (45°C)
99ml of 2% sterile diluents and the remaining sterile diluents and the contents transferred to the same conical flask, to obtain first dilution (1:10).

Further dilutions were the prepared using 9 ml quantity of citrate buffer sterile blank from the first dilution as per requirements. The dilutions were used immediately for plating purpose.

3.15.2 Enumeration of microorganisms

The pour plate method was adopted for the enumeration of different groups of microorganisms in paneer. Working table was previously sanitized with Lysol solution (3% phenol solution).

3.15.2.1 Standard plate count (SPC)

Total viable count of Paneer was determined by following the method was described by Messer et al., (1985) except that the diluents used was 2 per cent sodium citrate.

One ml each of the required dilutions was transferred into sterile Petri plates (bottom diameter 90 mm) in duplicate. To each plate 10 to 15 ml of sterilized SPC agar medium previously melted and cooled to 45 degree centigrade was added. The contents were mixed thoroughly by gently tilting and rotating the plates. Agar was then allowed to cool and solidify undisturbed at 37°C for 48 h. to check sterility and asepsis, a control plate using the same agar (but without the addition of sample) was prepared and incubated similarly. At the end of 48h of incubation, plates showing the colonies within the range of 25-250 were selected for counting purpose. The average value of the counts per ml was obtained and finally the SPC of paneer sample was expressed in terms of colony forming units (cfu) per gram.

3.15.2.2 Yeast and Mold count

The method of plating, incubation and counting for the enumeration of yeast and mold was described by Frank, et al. (1985).

The procedure of plating was similar as described in 3.3.2.1 except that potato dextrose agar medium (aseptically acidified to pH 3.5 with sterile 10%
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tartaric acid) was used for pouring the plates. After solidification the plates were inverted and incubated at 22±1°C for 3 to 5 days. Average value of the counts was taken and the results expressed in terms of cfu per gram of paneer.

3.15.2.3 Coliform count

The plating, incubation and counting method to enumerate coliforms in paneer samples were followed as described by Hartman and Lagrange, (1985).

The procedure of plating was similar as described in 3.3.2.1 except that violet red bile agar medium was used for pouring the plates and an additional second layer with 3 to 4ml of the same medium was over layered completely on the previously solidified first layer. After complete solidification of the second layer, the plates were incubated at 37±1°C for 24 h. Dark red colonies measuring 0.5mm or more in diameter were counted and the results expressed in the terms of cfu per gram of Paneer.

3.16 Experimental Design And Statistical Analysis

The values of each attributes under study were subjected to statistical analysis using Completely Randomized Design with equal number of observations. The statistical modal of Steel and Torrie (1980) was adopted, which is illustrated below.

\[ Y_{ij} = M + T_i + E_{ij} \]

Where, \( Y_{ij} \) = response due to \( j^{th} \) observation in the \( i^{th} \) treatment

\( M \) = general mean

\( T_i \) = effect of \( i^{th} \) treatment

\( E_{ij} \) = error due to \( j^{th} \) observation in the \( i^{th} \) treatment.
4. RESULTS AND DISCUSSION

Paneer is an important indigenous dairy product; it is used in the preparation of various dishes. It is an intermediate moisture food, a highly favorable medium for the growth of various changes. Under ordinary condition, paneer shows signs of deteriorations after one day of storage. Such a short shelf life of paneer hinders its storage and acceptance for trade by organized sector. Measure to extend the shelf life of paneer will also make it commercially viable and will be welcomed by both the organized and the small scale sectors. Thus, several attempts were made to enhance the shelf life of paneer. This study is another step in this direction.

Recently, there has been more focused interest in discovering new natural antimicrobials. Plant products with natural antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacterial and fungal growth. Although, spices have been well known for their medicinal, preservative and antioxidant properties, they are currently used mainly for enhancing the flavour of foods rather than extending shelf life.

Several attempts were made by various workers to enhance shelf life of paneer. However, no attempts have been reported so far to exploit potential of spices for this purpose. This study is a step in this direction and is aimed at evaluating suitability of selected spices to extend shelf life of paneer.

The study was divided into four phases. In the first phase selection of spices was carried. In the second phase, selection for rate of addition of the spices was studied. In third phase preliminary screening of the spices was carried out. Final screening of spices was investigated in the fourth phase. In the fourth phase, evaluation of the finally selected spices for its effectiveness at room temperature (32±1°C) as well as at refrigerated temperature (5±2°C) was evaluated.
4.1 Selection Of Spices

Appropriate food additive can play an important role in extending the shelf life of any product. Now a days natural compounds, such as essential oils, chitosan, nisin or lysozyme, are investigated to replace chemical preservatives and to obtain ‘green label’ products, a wide variety of spices are available. Spices are indispensable components of Indian cuisines. These are aromatic materials, have long been used in foods not only for their flavouring, but also for their medicinal (Davidson et al. 1983) and also stimulate appetite by increasing salivation, carminative action, and preserve the food by their antimicrobial and antioxidant properties (Lewis 1984). These are used in small amounts and their contribution to nutrient intake is very limited. Some of the spices are rich in iron, trace metals and potassium.

In selection of spices for enhancing the shelf life of paneer the most important point for consideration was the compatibility of the spice for use in the paneer. Therefore, from an array of available spices, eight different spices viz. black pepper (BP), cardamom (Ca), cinnamon (Ci), clove (Cl), garlic (Ga), ginger (Gi) and onion (On) were selected for the study. All the selected spices are commonly used in our day to day food that we commonly consumed. These spices are especially added during various culinary preparations from paneer.

The above mentioned spices are used in paneer during culinary preparation. In the present study these spices were to be incorporated in paneer it self. Therefore, compatibility of the added spices in the paneer necessitated some preliminary work for their selection. For selecting spices, the seven different spices were incorporated in paneer at the rate of 0.5 per cent and resultant samples were subjected to sensory evaluation for acceptability. The results indicated that all the samples of paneer were acceptable in organoleptic evaluation. Hence, all the seven spices were selected for further study.
Results and Discussion

The selected spices reported to have antimicrobial properties. The majority of the antimicrobial components of spices are phenol compounds with a hydroxyl group. Ahmed and Stoll (1996) reported that the main active principles in black pepper are alkaloids: methylpyroline, piperovatine, chavicine, pipieridine and piperine which control various pests including fungi. Sen and Rajorhia (1996) reported that cardamom contain several antimicrobial phenolics such as limonene, nerolidon, cineol, terpene, sabinine, pinene etc. Cardamom inhibit the growth of *S. aureus*, *B. stearothermophilus* and *B. coagulans* etc. (Liu and Nakano, 1996). Cinnamon and clove inhibited the growth of eight species of yeast, including *Saccharomyces cerevisiae* (Shelef 1984). Garlic were found to possess a potent bacteriostatic principle against Gram-positive as well as Gram-negative foodborne bacterial pathogens. Garlic inhibit the growth of *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli* etc. (Banerjee and Sarkar 2004). Indu et al. (2006) reported the extracts of ginger showed inhibitory activity against two serogroups of *E. coli* (enterotoxigenic *E. coli*). Addition of as little as 4 per cent onion extract completely inhibited the growth of *S. dysenteriae* and *S. aureus* and reduced the number of *E. coli* and *S. Typhosa* by 48.3 and 95.3 per cent, respectively (Al-Delaimy and Ali 1970).

In addition to compatibility, acceptability and antimicrobial properties of the spices they also have several health benefits. Many health benefit attributes of these common food adjuncts have been recognized in the past few decades by pioneering experimental research involving both animal studies and human trials. These studies documented digestive stimulant action, hypolipidemic effect, antidiabetic influence, antilithogenic property, antioxidant potential, anti-inflammatory property, antimutagenic, and anticarcinogenic potential of spices. Among these, the hypocholesterolemic and antioxidant properties of a few specific spices have far-reaching nutraceutical value.
Results and Discussion

These beneficial physiological effects also have the potential of possible therapeutic application in a variety of disease conditions. Wide range of non-nutrient bioactives and phytochemicals such as flavonoids and other phenolics which are also found in spices (Srinivasan 2005).

4.2 Selection For Rate Of Addition Of Spices In Paneer

In second phase of the study work was carried out to decide rate of addition of the 7 selected spices viz. black pepper, cardamom, cinnamon, clove, garlic, ginger and onion in paneer.

For selecting rate of addition of each spice the coagulum obtained by removal of 90 per cent whey, was divided in to 6 equal parts and the spice was incorporated in the product at the rate of 0.0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 per cent by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control. Each block of paneer was cut in to rectangular pieces of approximately 1cm x 2cm. Three replications were conducted for the each selected spice.

The prepared samples of paneer were subjected to sensory evaluation by panel of six judges using 9 point hedonic scale. The results obtained for sensory evaluation of sample of paneer added with the selected spices at different rates are presented below.

4.2.1 Black pepper

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of black pepper in paneer are presented in Table 4.1 and the trend is depicted in Figure 4.1

4.2.1.1 Flavour

The acceptability of any food product is influenced predominantly by its flavour score. Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when black pepper was added at different rates. The changes in flavour score presented in Figure 4.1 revealed that the score declined slightly on addition of black pepper up
Results and Discussion

to 0.6 per cent. However, further addition resulted into sharp decline in the flavour score.

Table 4.1: Effect of rate of addition of black pepper on sensory score of Paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Sensory attribute</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavour</td>
<td>Body &amp; Texture</td>
<td>Colour &amp; Appearance</td>
<td>Overall acceptability</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>7.34</td>
<td>8.20</td>
<td>8.22</td>
<td>7.75</td>
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<tr>
<td>0.2</td>
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<td>7.72</td>
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<tr>
<td>0.4</td>
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<td>7.87</td>
<td>7.22</td>
<td>7.31</td>
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</tr>
<tr>
<td>0.6</td>
<td>7.61</td>
<td>7.87</td>
<td>6.90</td>
<td>7.33</td>
<td></td>
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<tr>
<td>0.8</td>
<td>6.89</td>
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</tr>
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<td>1.0</td>
<td>6.60</td>
<td>7.17</td>
<td>6.17</td>
<td>6.39</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Em</th>
<th>C. D</th>
<th>C V %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.117</td>
<td>0.360</td>
<td>2.829</td>
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<td>0.160</td>
<td>0.492</td>
<td>3.569</td>
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<td>0.134</td>
<td>0.414</td>
<td>3.272</td>
</tr>
<tr>
<td></td>
<td>0.205</td>
<td>0.633</td>
<td>4.984</td>
</tr>
</tbody>
</table>

Figure 4.1: Effect of rate of addition of black pepper on sensory score of paneer
4.2.1.2 Body and texture

Changes in body and texture score of paneer revealed that there was a significant (P<0.05) difference between body and texture score of the paneer, when black pepper was added at different rates. The changes in body and texture score presented in Figure 4.1 revealed that the score declined slightly on addition of black pepper up to 0.6 per cent. However, further addition resulted into sharp decline in the body and texture score.

4.2.1.3 Colour and appearance

Colour and appearance is an important attribute in determining the acceptability of a product on visual perception. Changes in colour and appearance score of paneer revealed that there was a significant (P<0.05) difference between colour and appearance score of the paneer, when black pepper was added at different rates. The changes in colour and appearance score presented in Figure 4.1 revealed that there was a sharp decline in the colour and appearance score of paneer right from the lowest rate of addition of black pepper. The sharp decline in the colour and appearance for from beginning was due to brown colour of black pepper imparted to the paneer, which is very uncommon for the paneer.

4.2.1.4 Overall acceptability

The overall acceptability of product generally goes parallel with the flavour score of the product. Changes in overall acceptability score of paneer revealed that there was a significant difference between overall acceptability score of the paneer, when black pepper was added at different rates. The changes in overall acceptability score presented in Figure 4.1 revealed that the score declined gradually on addition of black pepper up to 0.6 per cent. However, further addition resulted into sharp decline in the overall acceptability score of the paneer.

No data are reported in the literature for effect of added black pepper on sensory attributes of paneer.
4.2.2 Cardamom

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of cardamom in paneer are presented in Table 4.2 and the trend is depicted in Figure 4.2

Table 4.2: Effect of rate of addition of cardamom on sensory score paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Flavour</th>
<th>Body &amp; Texture</th>
<th>Colour &amp; Appearance</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.41</td>
<td>8.39</td>
<td>8.18</td>
<td>7.42</td>
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<tr>
<td>0.2</td>
<td>7.44</td>
<td>8.20</td>
<td>8.03</td>
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</tr>
<tr>
<td>0.4</td>
<td>7.79</td>
<td>8.20</td>
<td>7.89</td>
<td>7.83</td>
</tr>
<tr>
<td>0.6</td>
<td>7.83</td>
<td>8.04</td>
<td>7.30</td>
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<tr>
<td>0.8</td>
<td>6.99</td>
<td>7.64</td>
<td>6.68</td>
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</tr>
<tr>
<td>1.0</td>
<td>6.79</td>
<td>7.46</td>
<td>6.27</td>
<td>6.99</td>
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</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Em</th>
<th>C. D</th>
<th>C V %</th>
</tr>
</thead>
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<tr>
<td>*</td>
<td>0.088</td>
<td>0.271</td>
<td>2.065</td>
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<tr>
<td>*</td>
<td>0.137</td>
<td>0.423</td>
<td>2.975</td>
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<td>*</td>
<td>0.235</td>
<td>0.723</td>
<td>5.499</td>
</tr>
<tr>
<td>*</td>
<td>0.205</td>
<td>0.633</td>
<td>4.984</td>
</tr>
</tbody>
</table>

Figure 4.2: Effect of rate of addition of cardamom sensory score of paneer
4.2.2.1 Flavour

Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when cardamom was added at different rates. The changes in flavour score presented in Figure 4.2 revealed that the score remained same as that of the control at the lowest rate (0.2%) of the addition. Then the score improved slightly on addition of cardamom up to 0.6 per cent. However, further addition resulted in sharp decline in score.

4.2.2.2 Body and Texture

Changes in body and texture score of paneer revealed that there was a significant (P<0.05) difference between body and texture score of the paneer, when cardamom was added at different rates. The changes in body and texture score presented in Figure 4.2 revealed that the score declined gradually on addition of cardamom up to 0.6 per cent. However, further addition resulted into sharp decline in the body and texture score.

4.2.2.3 Colour and Appearance

Colour and appearance is an important attribute in determining the acceptability of a product on visual perception. Changes in colour and appearance score of paneer revealed that there was a significant (P<0.05) difference between colour and appearance score of the paneer, when cardamom was added at different rates. The changes in colour and appearance score presented in Figure 4.2 revealed that the score declined gradually on addition of cardamom up to 0.4 per cent. However, further addition resulted into sharp decline in the body and texture score.

4.2.2.4 Overall acceptability

Changes in overall acceptability score of paneer revealed that there was a significant (P<0.05) difference between overall acceptability score of the paneer, when cardamom was added at different rates. The changes in overall acceptability score presented in Figure 4.2 revealed that the score remained same as that of the control at the lowest rate (0.2%) of the addition.
Results and Discussion

Then the score improved slightly on addition of cardamom up to 0.6 per cent. However, further addition resulted in to sharp declined the score. No data are reported in the literature for effect of added cardamom on sensory attributes of paneer.

4.2.3 Cinnamon

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of cinnamon in paneer are presented in Table 4.3 and the trend is depicted in Figure 4.3.

Table 4.3: Effect of rate of addition of cinnamon on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Sensory attribute</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavour</td>
<td>Body &amp; Texture</td>
</tr>
<tr>
<td>0.0</td>
<td>7.40</td>
<td>8.20</td>
</tr>
<tr>
<td>0.2</td>
<td>7.46</td>
<td>7.81</td>
</tr>
<tr>
<td>0.4</td>
<td>7.75</td>
<td>7.79</td>
</tr>
<tr>
<td>0.6</td>
<td>7.21</td>
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<tr>
<td>0.8</td>
<td>6.87</td>
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</tr>
<tr>
<td>1.0</td>
<td>6.63</td>
<td>7.07</td>
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</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th></th>
<th>S. Em</th>
<th>C. D</th>
<th>Test</th>
<th>C V %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavour</td>
<td>0.142</td>
<td>0.439</td>
<td>*</td>
<td>3.415</td>
</tr>
<tr>
<td>Body &amp; Texture</td>
<td>0.196</td>
<td>0.603</td>
<td>*</td>
<td>4.483</td>
</tr>
<tr>
<td>Colour &amp; Appearance</td>
<td>0.285</td>
<td>0.879</td>
<td>*</td>
<td>7.195</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>0.139</td>
<td>0.427</td>
<td>*</td>
<td>3.368</td>
</tr>
</tbody>
</table>

4.2.3.1 Flavour

Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when cinnamon was added at different rates. The changes in flavour score presented in Figure 4.3 revealed that the score remained same as that of the control at the lowest rate (0.2%) of the addition. Then the score improved slightly on addition of cinnamon up to 0.4 per cent. However, further addition resulted in sharp decline in score.
Results and Discussion

Figure 4.3: Effect of rate of addition of cinnamon sensory score of paneer

4.2.3.2 Body and Texture

Changes in body and texture score of paneer revealed that there was a significant ($P<0.05$) difference between body and texture score of the paneer, when cinnamon was added at different rates. The changes in body and texture score presented in Figure 4.3 revealed that the score declined gradually on addition of cinnamon up to 0.6 per cent. However, further addition resulted into sharp decline in the body and texture score.

4.2.3.3 Colour and Appearance

Changes in colour and appearance score of paneer revealed that there was a significant ($P<0.05$) difference between colour and appearance score of the paneer, when cinnamon was added at different rates. The changes in colour and appearance score presented in Figure 4.3 revealed that there was a sharp decline in the colour and appearance score of paneer right from the lowest rate of addition of cinnamon. The sharp decline in the colour and appearance from beginning was due
to brown colour of cinnamon imparted to the paneer, which is very uncommon for the paneer.

4.2.3.4 Overall acceptability

Changes in overall acceptability score of paneer revealed that there was a significant (P<0.05) difference between overall acceptability score of the paneer, when cinnamon was added at different rates. The changes in overall acceptability score presented in Figure 4.3 revealed that the score declined gradually on addition of cinnamon up to 0.4 per cent. However, further addition resulted into sharp decline in the overall acceptability score of the paneer. No data are reported in the literature for effect of added cinnamon on sensory attributes of paneer.

4.2.4 Clove

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of clove in paneer are presented in Table 4.4 and the trend is depicted in Figure 4.4

Table 4.4: Effect of rate of addition of clove on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Sensory attribute</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavour</td>
<td>Body &amp; Texture</td>
</tr>
<tr>
<td>0.0</td>
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<td>7.70</td>
<td>8.18</td>
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<td>7.70</td>
<td>8.07</td>
</tr>
<tr>
<td>0.6</td>
<td>7.68</td>
<td>7.87</td>
</tr>
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<td>0.8</td>
<td>6.89</td>
<td>7.28</td>
</tr>
<tr>
<td>1.0</td>
<td>6.70</td>
<td>7.11</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Em</th>
<th>C. D</th>
<th>Test</th>
<th>C V %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.216</td>
<td>0.665</td>
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<td>5.039</td>
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<td></td>
<td>0.222</td>
<td>0.684</td>
<td>*</td>
<td>4.905</td>
</tr>
<tr>
<td></td>
<td>0.186</td>
<td>0.574</td>
<td>*</td>
<td>4.367</td>
</tr>
<tr>
<td></td>
<td>0.106</td>
<td>0.327</td>
<td>*</td>
<td>2.493</td>
</tr>
</tbody>
</table>
Results and Discussion

4.2.4.1 Flavour

Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when clove was added at different rates. The changes in flavour score presented in Figure 4.4 revealed that the score declined gradually on addition of clove up to 0.6 per cent. However, further addition resulted into sharp decline in the flavour score.

4.2.4.2 Body and Texture

Changes in body and texture score of paneer revealed that there was a significant (P<0.05) difference between body and texture score of the paneer, when clove was added at different rates. The changes in body and texture score presented in Figure 4.4 revealed that the score declined gradually on addition of clove up to 0.6 per cent. However, further addition resulted into sharp decline in the body and texture score.

4.2.4.3 Colour and Appearance

Changes in colour and appearance score of paneer revealed that there was a significant (P<0.05) difference between colour and appearance score of the paneer, when clove was added at different rates.
Results and Discussion

The changes in colour and appearance score presented in Figure 4.4 revealed that there was a sharp decline in the colour and appearance score of paneer right from the lowest rate of addition of clove. The sharp decline in the colour and appearance from beginning was due to brown colour of clove imparted to the paneer, which is very uncommon for the paneer.

4.2.4.4 Overall acceptability

Changes in overall acceptability score of paneer revealed that there was a significant (P<0.05) difference between overall acceptability score of the paneer, when clove was added at different rates. The changes in overall acceptability score presented in Figure 4.4 revealed that the score declined gradually on addition of clove up to 0.6 per cent. However, further addition resulted into sharp decline in the overall acceptability score of the paneer.

No data are reported in the literature for effect of added clove on sensory attributes of paneer.

4.2.5 Garlic

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of garlic in paneer are presented in Table 4.5 and the trend is depicted in Figure 4.5

4.2.5.1 Flavour

Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when garlic was added at different rates. The changes in flavour score presented in Figure 4.5 revealed that the score declined slightly on addition of garlic up to 0.6 per cent. However, further addition resulted into sharp decline in the flavour score.
Results and Discussion

Table 4.5: Effect of rate of addition of garlic on sensory score paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Sensory attribute</th>
<th></th>
<th></th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavour</td>
<td>Body &amp; Texture</td>
<td>Colour &amp; Appearance</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>7.62</td>
<td>8.24</td>
<td>7.57</td>
<td>7.78</td>
</tr>
<tr>
<td>0.2</td>
<td>7.32</td>
<td>7.98</td>
<td>7.01</td>
<td>7.61</td>
</tr>
<tr>
<td>0.4</td>
<td>7.31</td>
<td>7.98</td>
<td>6.95</td>
<td>7.53</td>
</tr>
<tr>
<td>0.6</td>
<td>7.37</td>
<td>7.89</td>
<td>7.01</td>
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</tr>
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<td>7.02</td>
<td>7.86</td>
<td>6.93</td>
<td>7.28</td>
</tr>
<tr>
<td>1.0</td>
<td>6.85</td>
<td>7.84</td>
<td>6.84</td>
<td>7.20</td>
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</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Em</th>
<th>C. D</th>
<th>C V %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.097</td>
<td>0.205</td>
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<tr>
<td></td>
<td>0.274</td>
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<td>6.051</td>
</tr>
</tbody>
</table>

Figure 4.5: Effect of rate of addition of garlic on sensory score of paneer

4.2.5.2 Body and Texture

Changes in body and texture score of paneer revealed that there was no significant difference between body and texture score of the paneer, when garlic was added at different rates.
Results and Discussion

The changes in body and texture score presented in Figure 4.5 revealed that declined slightly on addition of 0.2 per cent garlic. However, on further addition of garlic the body and texture score of paneer remained almost constant up to the highest (1.0%) rate of addition. The data indicated that addition of garlic paste (1.0%) had no adverse effect on body and texture of the paneer.

4.2.5.3 Colour and Appearance

Changes in colour and appearance score of paneer revealed that there was no significant difference between colour and appearance score of the paneer, when garlic was added at different rates. The changes in colour and appearance score presented in Figure 4.5 revealed that the score declined sharply on addition of cardamom up to 0.2 per cent. However, on further addition of garlic the colour and appearance score of paneer remained almost constant up to the highest (1.0%) rate of addition. The data indicated that addition of garlic paste (1.0%) had no adverse effect on colour and appearance of the paneer.

4.2.5.4 Overall acceptability

Changes in overall acceptability score of paneer revealed that there was no significant difference between overall acceptability score of the paneer, when garlic was added at different rates. The changes in overall acceptability score presented in Figure 4.5 revealed that the score declined gradually on addition of garlic up to 0.6 per cent. However, further addition resulted into sharp decline in the overall acceptability score of the paneer. No data are reported in the literature for effect of added garlic on sensory attributes of paneer.

4.2.6 Ginger

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of ginger in paneer are presented in Table 4.6 and the trend is depicted in Figure 4.6
Table 4.6: Effect of rate of addition of the ginger on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Flavour</th>
<th>Body &amp; Texture</th>
<th>Colour &amp; Appearance</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.47</td>
<td>8.14</td>
<td>7.85</td>
<td>7.53</td>
</tr>
<tr>
<td>0.2</td>
<td>7.24</td>
<td>7.89</td>
<td>7.75</td>
<td>7.42</td>
</tr>
<tr>
<td>0.4</td>
<td>7.36</td>
<td>7.87</td>
<td>7.67</td>
<td>7.44</td>
</tr>
<tr>
<td>0.6</td>
<td>7.26</td>
<td>7.84</td>
<td>7.67</td>
<td>7.61</td>
</tr>
<tr>
<td>0.8</td>
<td>7.07</td>
<td>7.70</td>
<td>7.57</td>
<td>7.25</td>
</tr>
<tr>
<td>1.0</td>
<td>6.92</td>
<td>7.70</td>
<td>7.52</td>
<td>7.22</td>
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</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th></th>
<th>S. Em</th>
<th>C. D</th>
<th>Test</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.094</td>
<td>-</td>
<td>*</td>
<td>2.259</td>
</tr>
<tr>
<td>C. D</td>
<td>0.290</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CV %</td>
<td>2.259</td>
<td>2.791</td>
<td>5.041</td>
<td>2.787</td>
</tr>
</tbody>
</table>

Figure 4.6: Effect of rate of addition of ginger on sensory score of paneer

4.2.6.1 Flavour

Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when ginger was added at different rates.
Results and Discussion

The changes in flavour score presented in Figure 4.6 revealed that the score remained almost same as that of the control up to 0.4 per cent of the addition. However, further addition resulted into slight decline in the flavour score over the remaining range of concentration.

4.2.6.2 Body and Texture

Changes in body and texture score of paneer revealed that there was no significant difference between body and texture score of the paneer, when ginger was added at different rates. The changes in body and texture score presented in Figure 4.6 revealed slight decline in the body and texture of paneer score of paneer right from the lowest rate of addition of ginger. The data indicated that addition of garlic paste (1.0%) had no adverse effect on body and texture of the paneer.

4.2.6.3 Colour and Appearance

Changes in colour and appearance score of paneer revealed that there was no significant difference between colour and appearance score of the paneer, when ginger was added at different rates. The changes in colour and appearance score presented in Figure 4.6 revealed that there was a slight decline in the colour and appearance score of paneer right from the lowest rate of addition of ginger. The data indicated that addition of ginger paste (1.0%) had no adverse effect on colour and appearance of the paneer.

4.2.6.4 Overall acceptability

Changes in overall acceptability score of paneer revealed that there was no significant difference between overall acceptability score of the paneer, when ginger was added at different rates. The changes in flavour score presented in Figure 4.6 revealed that the score remained almost same as that of the control up to 0.6 per cent of the addition. However, further addition resulted into slight decline in the flavour score over the remaining range of concentration.

No data are reported in the literature for effect of added ginger on sensory attributes of paneer.
4.2.7 Onion

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of onion in paneer are presented in Table 4.7 and the trend is depicted in Figure 4.7.

4.2.7.1 Flavour

Changes in flavour score of paneer revealed that there was a significant (P<0.05) difference between flavour score of the paneer, when onion was added at different rates. The changes in flavour score are presented in Figure 4.7 revealed that the score declined slightly on addition of onion up to 0.2 per cent. Then the flavour score improved slightly up to 0.6 per cent addition. However, further addition resulted into sharp decline in the flavour score.

4.2.7.2 Body and Texture

Changes in body and texture score of paneer revealed that there was no significant difference between body and texture score of the paneer, when onion was added at different rates. The changes in body and texture score presented in Figure 4.7 revealed that declined gradually on addition of onion all through out the concentration range, however, the rate of decline was very slow. The data indicated that addition of ginger paste (1.0%) had no adverse effect on body and texture of the paneer.

4.2.7.3 Colour and Appearance

Changes in colour and appearance score of paneer revealed that there was no significant difference between colour and appearance score of the paneer, when onion was added at different rates. The changes in colour and appearance score presented in Figure 4.7 revealed that the score declined gradually on addition of onion all through out the concentration range, however, the rate of decline was very slow. The data indicated that addition of ginger paste (1.0%) had no adverse effect on colour and appearance of the paneer.
### Table 4.7: Effect of rate of addition of onion on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Sensory attribute</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavour</td>
<td>Body &amp; Texture</td>
</tr>
<tr>
<td>0.0</td>
<td>7.73</td>
<td>8.41</td>
</tr>
<tr>
<td>0.2</td>
<td>7.43</td>
<td>8.11</td>
</tr>
<tr>
<td>0.4</td>
<td>7.56</td>
<td>8.04</td>
</tr>
<tr>
<td>0.6</td>
<td>7.67</td>
<td>7.93</td>
</tr>
<tr>
<td>0.8</td>
<td>7.11</td>
<td>7.87</td>
</tr>
<tr>
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<td>6.94</td>
<td>7.84</td>
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</table>

#### ANOVA TABLE

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<th>Test</th>
<th>C V %</th>
</tr>
</thead>
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<td>3.786</td>
</tr>
<tr>
<td>0.164</td>
<td>-</td>
<td>NS</td>
<td>3.532</td>
</tr>
<tr>
<td>0.137</td>
<td>-</td>
<td>NS</td>
<td>3.089</td>
</tr>
<tr>
<td>0.050</td>
<td>0.154</td>
<td>*</td>
<td>1.139</td>
</tr>
</tbody>
</table>

### Figure 4.7: Effect of rate of addition of onion on sensory score of paneer

#### 4.2.7.4 Overall acceptability

Changes in overall acceptability score of paneer revealed that there was a significant (P<0.05) difference between overall acceptability score of the paneer, when onion was added at different rates.
The changes in overall acceptability score presented in Figure 4.7 revealed that the score declined slightly on addition of onion up to 0.2 per cent. Then the overall acceptability score improved slightly up to 0.6 per cent addition. However, further addition resulted into sharp decline in the overall acceptability score.

No data are reported in the literature for effect of added onion on sensory attributes of paneer.

**4.2.8 Selected rate of spices for addition in paneer**

For further work in the study rate of addition of the selected spices was determined by sensory evaluation of the paneer. The most important factor in deciding the rate of addition of each spice in paneer is the acceptability of the paneer in organoleptic test.

It is evident from the careful observations of data for overall acceptability score of paneer reveal that the score declined gradually, remained constant or improved slightly on addition of black pepper, cardamom, clove, garlic, ginger or onion up to 0.6 per cent depending up on individual spices added. However, their further addition resulted in to sharp decline in the score. In case of cinnamon the gradual decline in overall acceptability score was up to 0.4 per cent addition and its further addition resulted in to sharp decline in the score. During preliminary study it was found that flavour of the added spices was slightly intensified during storage of the paneer.

Therefore, it was decided use black pepper, cardamom, clove, garlic, ginger and onion in paneer at the rate of 0.6 per cent and cinnamon at the rate of 0.4 per cent for further study.
4.3 Preliminary Screening Of The Spices For Their Effectiveness For Enhancing Shelf Life Of Paneer

In third phase of the study, work was carried out for preliminary screening of the selected spices for their effectiveness in enhancing shelf life of paneer.

For preliminary screening of the selected spices for their effectiveness to enhance shelf life of paneer the coagulum obtained by removal of 90 per cent whey, was divided into 8 equal parts and black pepper, cardamom, cinnamon, clove, garlic, ginger and onion were incorporated in the coagulum at the rate of 0.6 per cent and cinnamon was incorporated in the coagulum at the rate of 0.4 per cent by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control. Three replications were conducted for the selected concentration of each spice.

Each block of paneer was cut into four equal parts and packed separately in PET/LDPE film pouches and stored at 7°C±1°C for 14 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after 14 days of storage.

4.3.1 Changes in sensory attributes

The results obtained for sensory evaluation of fresh and stored samples of paneer are presented below.

4.3.1.1 Flavour

The data obtained for changes in flavour score of paneer during storage at 7°C are presented in Table 4.8 and the trend is depicted in Figure 4.8. As indicated earlier the acceptability of any food product is influenced predominantly by its flavour score. The changes in flavour score revealed that both spice and storage period had significant effect on flavour score of the paneer. The interaction between spice and storage period was also significant.

The changes in flavour score presented in Figure 4.8 revealed that the score of black pepper, cardamom, cinnamon and clove decline slightly on 14th day of the storage but remained above acceptable level (6.0).
Table 4.8: Effect of spices on flavour of paneer during storage (7ºC) at selected concentration

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
<th>0</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.31</td>
<td>4.02</td>
</tr>
<tr>
<td>BP</td>
<td></td>
<td>7.28</td>
<td>6.55</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>7.75</td>
<td>6.97</td>
</tr>
<tr>
<td>Ci</td>
<td></td>
<td>7.50</td>
<td>7.08</td>
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<tr>
<td>Cl</td>
<td></td>
<td>7.14</td>
<td>6.67</td>
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</tr>
<tr>
<td>Gi</td>
<td></td>
<td>7.17</td>
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<tr>
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<td>6.69</td>
<td>2.66</td>
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</table>

ANOVA TABLE

<table>
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<tr>
<th>Source of Variation</th>
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<th>Storage period x Spice</th>
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</thead>
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<td>0.342</td>
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<td>C. D</td>
<td>0.698</td>
<td>0.349</td>
<td>0.987</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>C V %</td>
<td></td>
<td>9.59</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.8: Effect of spices on flavour of paneer during storage (7ºC) at selected concentration

On the other hand the flavour score of garlic, ginger, onion and that of the control decline sharply and went below acceptable level. It is evident that the
Results and Discussion

flavour score of fresh paneer decreases on incorporation of garlic, ginger and onion. The incorporation of these spices drastically reduces the flavour score of the paneer up on storage of the samples. The sharp decline in the flavour score was attributed to development of objectionable flavour. In the samples containing garlic, ginger and onion very strong unpleasant flavour was noticed.

4.3.1.2 Body and texture

The data obtained for changes in body and texture score of paneer during storage at 70°C are presented in Table 4.9 and the trend is depicted in Figure 4.9. The changes in body and texture score revealed that both spice and storage period had significant effect on body and texture score of the paneer. The interaction between spice and storage period was also significant.

Table 4.9: Effect of spices on body & texture of paneer during storage (70°C) at selected concentration

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.75</td>
<td>6.50</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>7.61</td>
<td>7.03</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
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<td></td>
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<td>Ci</td>
<td>7.53</td>
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</tr>
<tr>
<td>Gi</td>
<td>7.47</td>
<td>6.36</td>
<td></td>
</tr>
<tr>
<td>On</td>
<td>7.70</td>
<td>6.25</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Spice</th>
<th>Storage period</th>
<th>Storage period x Spice</th>
</tr>
</thead>
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<td>0.039</td>
<td>0.111</td>
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<td>C. D</td>
<td>0.228</td>
<td>0.114</td>
<td>0.322</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>C V %</td>
<td></td>
<td>2.71</td>
<td></td>
</tr>
</tbody>
</table>
Results and Discussion

Figure 4.9: Effect of spices on body & texture of paneer during storage (7°C) at selected concentration

The changes in body and texture score presented in Figure 4.9 revealed that the score of all the samples of paneer declined on 14th day of the storage but remained above acceptable level. The relative decline in the score was much greater in case of the samples containing garlic, ginger, onion and that of the control compared to the samples containing black pepper, cardamom, cinnamon and clove.

4.3.1.3 Colour and appearance

The data obtained for changes in colour and appearance score of paneer during storage at 7°C are presented in Table 4.10 and the trend is depicted in Figure 4.10. The changes in colour and appearance score presented in Table 4.10 revealed that both spice and storage period had significant effect on colour and appearance score of the paneer. However, interaction between spice and storage period was non-significant.

The changes in colour and appearance score presented in Figure 4.10 revealed that the score of all the samples of paneer declined on 14th day of the storage but remained above acceptable level. The colour and appearance score was low in the samples containing cinnamon and clove was much lower and remained...
Results and Discussion

lower on storage compared to the samples containing black pepper, cardamom, garlic, ginger and onion and that of the control.

Table 4.10: Effect of spices on colour & appearance of paneer during storage ($7^\circ C$) at selected concentration

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
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</tr>
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<td>BP</td>
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<tr>
<td>Ca</td>
<td>7.44</td>
</tr>
<tr>
<td>Ci</td>
<td>6.86</td>
</tr>
<tr>
<td>Cl</td>
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<td>Ga</td>
<td>7.28</td>
</tr>
<tr>
<td>Gi</td>
<td>7.22</td>
</tr>
<tr>
<td>On</td>
<td>7.30</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Spice</th>
<th>Storage period</th>
<th>Storage period x Spice</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.086</td>
<td>0.043</td>
<td>0.121</td>
</tr>
<tr>
<td>C. D</td>
<td>0.248</td>
<td>0.124</td>
<td>NS</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>C V %</td>
<td></td>
<td></td>
<td>3.03</td>
</tr>
</tbody>
</table>

Figure 4.10: Effect of spices on colour & appearance of paneer during storage ($7^\circ C$) at concentration

4.3.1.4 Overall acceptability
Results and Discussion

The data obtained for changes in overall acceptability score of paneer during storage at 7°C are presented in Table 4.11 and the trend is depicted in Figure 4.11.

**Table 4.11: Effect of spices on overall acceptability of paneer during storage (7°C) at selected concentration**

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
<th>0</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.75</td>
<td>5.39</td>
</tr>
<tr>
<td>BP</td>
<td></td>
<td>7.25</td>
<td>6.56</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>7.86</td>
<td>7.11</td>
</tr>
<tr>
<td>Ci</td>
<td></td>
<td>7.47</td>
<td>6.86</td>
</tr>
<tr>
<td>Cl</td>
<td></td>
<td>7.28</td>
<td>6.47</td>
</tr>
<tr>
<td>Ga</td>
<td></td>
<td>6.92</td>
<td>4.25</td>
</tr>
<tr>
<td>Gi</td>
<td></td>
<td>6.72</td>
<td>2.44</td>
</tr>
<tr>
<td>On</td>
<td></td>
<td>6.50</td>
<td>3.25</td>
</tr>
</tbody>
</table>

**ANOVA Table**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Spice</th>
<th>Storage period</th>
<th>Storage period x Spice</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.174</td>
<td>0.087</td>
<td>0.246</td>
</tr>
<tr>
<td>C. D</td>
<td>0.502</td>
<td>0.251</td>
<td>0.709</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>C V %</td>
<td></td>
<td>6.80</td>
<td></td>
</tr>
</tbody>
</table>

The changes in overall acceptability score presented in Table 4.11 revealed that both spice and storage period had significant effect on overall acceptability score of the paneer. The interaction between spice and storage period was also significant.

The changes in overall acceptability score presented in Figure 4.11 revealed that the score of black pepper, cardamom, cinnamon and clove declined slightly on 14th day of the storage but remained above acceptable level (6.0). On the other hand the overall acceptability score of garlic, ginger, onion and that of the control declined sharply and went below acceptable level.
Results and Discussion

Figure 4.11: Effect of spices on overall acceptability of paneer during storage (7\(^0\)C) at selected concentration

It is evident that the overall acceptability score of fresh paneer decreases on incorporation of garlic, ginger and onion. The incorporation of these spices drastically reduced the overall acceptability score of the paneer up on storage of the samples. The sharp decline in the overall acceptability score was attributed to development of strong objectionable flavour.

No data are reported in the literature for effect of black pepper, cardamom, cinnamon, clove, garlic, ginger and onion on sensory attributes of paneer during storage.

4.3.2 Spices selected on the basis of preliminary screening

On the basis of performance of various spices in preliminary screening black pepper, cardamom, cinnamon and clove were selected for further study. Garlic, ginger and onion were dropped from the further study due to their deleterious effect on acceptability of paneer, especially up on storage.
4.4 Final Screening Of The Spices For Their Effectiveness For Enhancing Shelf Life Of Paneer

In fourth phase of the study work was carried out for final screening of the spices selected on the basis of preliminary screening for their effectiveness in enhancing shelf life of paneer.

For final screening of the selected spices (black pepper, cardamom, cinnamon and clove) for their effectiveness to enhance shelf life of paneer the coagulum obtained by removal of 90 per cent whey, was divided into 5 equal parts and black pepper, cardamom, and clove were incorporated in the coagulum at the rate of 0.6 per cent and cinnamon was incorporated in the coagulum at the rate of 0.4 per cent by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control. Three replications were conducted for the selected spices.

Each block of paneer was cut into four equal parts and packed separately in PET/LDPE film pouches and stored at 7\(^\circ\)C ± 1\(^\circ\)C for 28 days. The samples of paneer were also stored at 32\(^\circ\)C ± 1\(^\circ\)C. The fresh control samples of paneer were analyzed for the chemical composition, sensory attributes and chemical characteristics.

The prepared samples of fresh paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale. The fresh samples of paneer were also analyzed for chemical characteristics viz. acidity, free fatty acidity and soluble nitrogen. The samples stored at 7\(^\circ\)C ± 1\(^\circ\)C were subjected to the sensory evaluation and analysis for the chemical characteristics at a regular interval of 7 days during the storage.

Visible mould growth was observed in control samples of paneer on 21\(^{st}\) day of storage. Therefore, sensory and chemical analysis of these samples was not carried out and their further storage study was terminated.
The samples of paneer stored at $32^\circ C \pm 1^\circ C$ were to be analyzed at an interval of one day. However, in the samples stored at $32^\circ C \pm 1^\circ C$ very strong off flavour was developed and whey separation took place within one day of storage. Therefore, the sensory evaluation and analysis for the chemical characteristics of the samples stored at $32^\circ C \pm 1^\circ C$ were not carried out and their further storage study was terminated.

Thakral (1986) observed that paneer turned acidic and sour after one day at $37^\circ C$ and after 2 days at $22^\circ C$. Kanawjia (1990) reported that at room temperature paneer does not keep well for more than one day. The author noticed that the spoilage of paneer occurs due to growth of microorganisms on the surface. A greenish yellow slime formation on the surface of paneer and the discoloration is accompanied by an off-flavor. According to Pal et al. (1993) on storage of paneer at room temperature, it becomes unfit for consumption usually after one day. However, Venkateswarlu et al. (2003) the shelf life of paneer under the experimental condition ranged from 1 to 2 days at room temperature. The results obtained in the present study for storage of paneer at $32^\circ C \pm 1^\circ C$ are in accordance with those reported by Kanawjia (1990). The variation in the results compared to those reported by Venkateswarlu et al. (2003) may be attributed to variation in quality of the raw milk used in the manufacture of paneer and post production contamination of the product.

### 4.4.1 Chemical composition of paneer

The data obtained for chemical composition of control sample of fresh paneer revealed that the paneer contained 50.22 per cent moisture, 29.30 per cent fat, 17.50 per cent protein, 1.85 per cent ash and 1.13 per cent lactose. The per cent fat on dry matter basis was 58.60. In preparation of paneer samples for the study, steps followed for different samples was common up to the stage of coagulation and removal of whey. The one common coagulum was divided into different parts for incorporation of the selected spices and one of the part without any addition of spice was used as control.
Results and Discussion

The rate of addition of the spices was very small (0.4 to 0.6 %). Therefore, analysis for chemical composition of paneer was carried out only for the control sample.

The literature on chemical composition of paneer indicates that the moisture, fat, protein, lactose and ash content of paneer vary from 47.68 to 59.70, 22.90 to 27.00, 16.81 to 33.27, 2.07 to 2.61 and 1.30 to 2.18 per cent respectively (Bhattacharya et al. 1971; Pal and Yadav, 1991; Singh et al. 1991; Goel, 2000; Dhole, et al. 2009). Therefore, in the present study data obtained for chemical composition of paneer are well within those reported in the literature.

According to PFA Act (1954), paneer shall not contain more than 70.0 per cent moisture, and milk fat content shall not be less than 50.0 per cent of the dry matter. According to BIS (1983) moisture content in paneer shell be 60.0 per cent by mass (max) and milk fat content shall be 50.0 per cent by mass on dry matter basis (min). Therefore, the samples of paneer prepared in the present study fullfiled both the PFA and the BIS requirements for the chemical compostion.

4.4.2 Changes in sensory attributes

The results obtained for sensory evaluation of fresh and stored samples of paneer are presented below.

4.4.2.1 Flavour

The data obtained for changes in flavour score of paneer during storage at 7°C are presented in Table 4.12 and the trend is depicted in Figure 4.12.

The results indicated that the flavour score of fresh samples of paneer was in the order of cardamom > control > black pepper > cinnamon > clove. The flavour score of paneer containing cardamom was higher than that of the control sample of paneer. Thus addition of cardamom improved the flavour score of paneer.
Results and Discussion

The flavour score of control sample of paneer declined sharply from beginning of the storage. Amongst the samples of paneer containing spices the highest rate of decrease in flavour score was observed in case of black pepper and the lowest rate was observed in case of cardamom. The changes in flavour score in case of cinnamon and clove was almost similar and remained intermediate to that of the black pepper and cardamom.

**Table 4.12: Effect of selected spice on flavour score of paneer during storage at 7°C**

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>7.61</td>
</tr>
<tr>
<td>BP</td>
<td>7.53</td>
</tr>
<tr>
<td>Ca</td>
<td>8.12</td>
</tr>
<tr>
<td>Ci</td>
<td>7.43</td>
</tr>
<tr>
<td>Cl</td>
<td>7.07</td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth

**Figure 4.12: Effect of selected spices on flavour of paneer during storage at 7°C**

The flavour score of control samples of paneer went below acceptable level (6.0) on 7th day of the storage. The control sample became unacceptable due to
putrid odour and acidic and bitter taste. The samples of paneer containing black pepper had acceptable flavour score up to 14\textsuperscript{th} day of the storage and it went below the acceptable level on 21\textsuperscript{st} day of the storage. Similarly flavour score of samples containing cinnamon and clove remained acceptable up to 21\textsuperscript{st} day of the storage and went below acceptable level on 28\textsuperscript{th} day of the storage. However, the flavour score of samples containing cardamom remained well above the acceptable level even on 28\textsuperscript{th} day of the storage.

4.4.2.2 Body and texture

The data obtained for changes in body and texture score of paneer during storage at 7\textdegree C are presented in Table 4.13 and the trend is depicted in Figure 4.13

The results indicated that the body and texture score of fresh samples of paneer was in the order of control > black pepper > cardamom > cinnamon > clove. The body and texture score of paneer containing control was highest. The addition of all the spices used in the study found to reduce body and texture score of paneer. This adverse effect may be attributed to interference of spice particles in development of body and texture of the paneer.

The body and texture score of control sample of paneer declined sharply from beginning of the storage. Amongst the samples of paneer containing spices the rate of decrease in body and texture score was almost same up to 14\textsuperscript{th} day of the storage. On further storage the rate of decrease was greater in case of samples containing black pepper and clove. In case of cinnamon the rate of decrease became sharp after 21 days of storage. In the sample containing cardamom body and texture score decreased at slower rate up to 14\textsuperscript{th} day of the storage and then remained almost constant during the remaining storage period.

The body and texture score of control samples of paneer went slightly below acceptable level (6.0) on 14\textsuperscript{th} day of the storage. The samples of paneer containing black pepper, cinnamon and clove had acceptable body and texture score up to 21\textsuperscript{st} day of the storage and it went below the acceptable level on 28\textsuperscript{th} day of the storage. However, the body and texture score of samples containing
Results and Discussion

cardamom remained well above the acceptable level even on 28th day of the storage.

Table 4.13: Effect of selected spice on body and texture score of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td>7.87</td>
<td>7.16</td>
<td>5.95</td>
<td>VMG*</td>
<td>-</td>
</tr>
<tr>
<td>BP</td>
<td>7.74</td>
<td>7.38</td>
<td>6.86</td>
<td>6.50</td>
<td>5.41</td>
</tr>
<tr>
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<td>7.69</td>
<td>7.16</td>
<td>7.11</td>
<td>7.00</td>
</tr>
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<td>Ci</td>
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<td>7.11</td>
<td>7.11</td>
<td>5.80</td>
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<td>Cl</td>
<td>7.53</td>
<td>7.40</td>
<td>6.96</td>
<td>6.64</td>
<td>5.87</td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth

Figure 4.13: Effect of spices on body and texture of paneer during storage at 7°C

4.4.2.3 Colour and appearance

The data obtained for changes in colour and appearance score of paneer during storage at 7°C are presented in Table 4.14 and the trend is depicted in Figure 4.14.
Table 4.14: Effect of selected spice and period on colour and appearance of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.02</td>
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<td>7.42</td>
<td>VMG*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>7.42</td>
<td>6.95</td>
<td>7.03</td>
<td>6.42</td>
<td>6.27</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>7.89</td>
<td>7.47</td>
<td>7.43</td>
<td>7.29</td>
<td>7.27</td>
<td></td>
</tr>
<tr>
<td>Ci</td>
<td>7.37</td>
<td>7.01</td>
<td>6.63</td>
<td>6.38</td>
<td>6.30</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>6.82</td>
<td>6.56</td>
<td>6.31</td>
<td>6.12</td>
<td>6.07</td>
<td></td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth

Figure 4.14: Effect of spices on colour and appearance of paneer during storage at 7°C

The results indicated that the colour and appearance score of fresh samples of paneer was in the order of control > cardamom > black pepper > cinnamon > clove. The colour and appearance score of paneer containing control was highest. The addition of all the spices used in the study found to reduce colour and appearance score of paneer. This adverse effect on colour and appearance score of
the paneer may be attributed to the brown to black colour imparted by respective spices to the paneer.

The colour and appearance score of all the samples of paneer declined at almost same rate all through out the storage period. However, amongst all the samples of paneer minimum rate of decrease in colour and appearance score was observed in case of samples containing cardamom. The colour and appearance score of all the samples of paneer containing spices remained above the acceptable level (6.0) all through out the storage period.

### 4.4.2.4 Overall acceptability

The data obtained for changes in overall acceptability score of paneer during storage at 7°C are presented in Table 4.15 and the trend is depicted in Figure 4.15.

The results indicated that the overall acceptability score of fresh samples of paneer was in the order of cardamom > control > black pepper = cinnamon > clove. The overall acceptability score of paneer containing cardamom was higher than that of the control sample of paneer. Thus addition of cardamom improved the overall acceptability score of paneer.

The overall acceptability score of control sample of paneer declined sharply from beginning of the storage. Amongst the samples of paneer containing spices the highest rate of decrease in overall acceptability score was observed in case of black pepper and the lowest rate was observed in case of cardamom. The changes in overall acceptability score in case of cinnamon and clove was almost similar and remained intermediate to that of the black pepper and cardamom.

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>7.77</td>
</tr>
</tbody>
</table>

Table 4.15: Effect of selected spice and period on overall acceptability of paneer during storage at 7°C
Results and Discussion

<table>
<thead>
<tr>
<th></th>
<th>BP</th>
<th>Ca</th>
<th>Ci</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.37</td>
<td>7.92</td>
<td>7.37</td>
<td>6.91</td>
</tr>
<tr>
<td>6.93</td>
<td>7.78</td>
<td>7.22</td>
<td>6.83</td>
<td>6.83</td>
</tr>
<tr>
<td>6.58</td>
<td>7.27</td>
<td>6.88</td>
<td>6.88</td>
<td>6.88</td>
</tr>
<tr>
<td>5.95</td>
<td>7.27</td>
<td>6.75</td>
<td>6.62</td>
<td>6.62</td>
</tr>
<tr>
<td>5.03</td>
<td>7.10</td>
<td>6.17</td>
<td>5.87</td>
<td>5.87</td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth

![Figure 4.15: Effect of spices on overall acceptability of paneer during storage at 7°C](image)

The overall acceptability score of control samples of paneer went below acceptable level (6.0) on 14th day of the storage. The samples of paneer containing black pepper had acceptable overall acceptability score up to 14th day of the storage and it went slightly below the acceptable level on 21st day of the storage.

The overall acceptability score of samples containing clove remained acceptable up to 21st day of the storage and went slightly below acceptable level on 28th day of the storage. Similarly overall acceptability score of samples containing cinnamon remained acceptable up to 21st day of the storage and went below acceptable level on 28th day of the storage. However, the overall acceptability score of samples containing cardamom remained well above the acceptable level even on 28th day of the storage.
Reports in literature indicate that paneer can be kept good for only 6 days although it starts losing its freshness on the 3rd day (Bhattacharya et al., 1971; Sachdeva et al., 1990). Arora and Gupta (1980) also reported that paneer, like other indigenous product is a highly perishable product and suffers from limited shelf life, largely because of its high moisture content. Therefore, data obtained for effect of storage on sensory attributes for control sample of paneer is exactly in accordance with those reported in the literature.

Nair et al. (1996) have used black pepper in traditional meat products. Sen and Rajorhia (1996) conducted a study to find out the efficacy of cardamom powder in preservation of sandesh. They reported that the cardamom powder added at the rate 0.1 per cent enhance the shelf life of sandesh to 90 days as against 60 days for control sample at 7°C.

Agaoglu, et al. (2007) reported the use of cinnamon in meat products. Sharma and Verma (2006) investigated the efficiency of four plant essential oils bay, clove, cinnamon and thyme as natural food preservatives. The effect of the plant oils at concentrations of 0.1, 0.5 and 1% was studied in low-fat and full-fat and full-fat soft cheese.

Ratiba et al. (2006) studied effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat’s milk. The author found that the control cheese gained the maximum total score as compared to cheese containing the different spices. In general, the results indicated that the use of spice powders improved the flavour of goat’s milk, also the score were affected by the type and concentration of spice powders. The cheese samples containing 0.10 and 0.2 per cent cardamom gained the highest scores throughout the storage period compared to other treatments, also clove powder at each concentration were better than cheese treated with thyme.

Choudhary and Sandey (2009) studied the whey based mango herbal (Cardamom) Beverage. Edible medicinal plant of cardamom extract (0-3% levels) was incorporated in the beverage. Among the beverage containing various level of
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cardamom extract, 2 per cent level of cardamom beverage were found to be highly acceptable in sensory characteristics up to 30 days at refrigerated temperature.

However, no data are reported in the literature for effect of black pepper, cardamom, cinnamon and clove on sensory attributes of paneer during storage.

4.4.3 Changes in chemical characteristics

The fresh and store samples of paneer were analyzed for chemical characteristics viz. acidity, free fatty acids content and soluble nitrogen content.

4.4.3.1 Acidity

The data obtained for changes in acidity of paneer during storage at 7°C are presented in Table 4.16 and the trend is depicted in Figure 4.16

The results indicated that the acidity of fresh samples of paneer was in the order of cardamom > black pepper > control > clove > cinnamon. The acidity of paneer containing cardamom as well as black pepper was lower than that of the control sample of paneer. Thus addition of cardamom or black pepper decreases the acidity of paneer.

The average acidity of the fresh control sample of paneer was 0.49. The acidity of paneer was reported to vary from 0.20 (Sachdeva and Singh, 1990) to 1.17 (Goel, 2000). However, this is the extreme range of the values, but most of the reports (Mistry, 1988; Kumar and Bector, 1991; Gohian, 1996; Venkateswarlu, et al, 2003; Yellamanda et al, 2006) suggest the normal range of 0.47 to 0.59. Therefore, acidity value of paneer in the present study is well with in the reported normal range.

Table 4.16: Effect of selected spice on acidity (% lactic acid) of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.49</td>
</tr>
<tr>
<td>BP</td>
<td>0.38</td>
</tr>
<tr>
<td>Ca</td>
<td>0.33</td>
</tr>
<tr>
<td>Ci</td>
<td>0.65</td>
</tr>
</tbody>
</table>

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Results and Discussion

<table>
<thead>
<tr>
<th></th>
<th>Cl</th>
<th>0.52</th>
<th>0.87</th>
<th>0.58</th>
<th>0.70</th>
<th>0.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMG</td>
<td>Visible mould growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It is surprising to notice that addition of cardamom or black pepper to paneer decreased the acidity of paneer. On the other hand addition of cinnamon or clove increased the acidity of paneer. To substantiate such effects of the spices on acidity of paneer some trials were taken to determine acidity of the spice samples, taking distilled water as control by following the method used for the paneer.

The negative values were obtained for cardamom and black pepper when compared with the value obtained for the water. Similarly, positive values were obtained for cinnamon and clove when compared with the value obtained for the water. The observations of the trials supports the results obtained for the samples of paneer. No data are reported in the literature regarding effect of addition of these spices on acidity of paneer or other food products.

The results depicted in Figure 4.16 shows trend of changes in acidity of paneer samples during storage. The acidity of all the samples tended to rise.
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sharply up to 7\textsuperscript{th} day of the storage, except cinnamon. On further storage acidity continued to rise in case of control sample, however, in case of the samples containing spices acidity was found to decrease up to 14\textsuperscript{th} day of the storage. The decrease was marginal in case of black pepper, it was substantial in case of cardamom, cinnamon and clove. On further progress of storage, acidity values increased slowly in case of black pepper, cinnamon and clove, but acidity remained constant in case of cardamom. Thus, cardamom proved to be the best spice to control acidity of paneer during storage. These observations are well correlated with highest flavour score (4.4.2.1) of the paneer containing cardamom during storage.

Mistry (1988) observed that acidity of paneer increased from 0.475 to 0.483 per cent on 7\textsuperscript{th} day of storage at 7 to 10\textdegree C and it decreased to 0.459 per cent on 15\textsuperscript{th} day of the storage. Kumar and Bector (1991) reported that the initial titratable acidity, 0.54 per cent, of control samples increased slowly during storage and reached 0.9 per cent on day four and thereafter declined and reached a value of 0.59 per cent on day ten. However, Venkateswarlu \textit{et al.} (2003) found that the acidity of paneer increased gradually from 0.224 to 0.472 per cent at the end of ninth day storage, followed by sharp increase towards end of storage.

According to Sachdeva and Singh (1990) the cycle of increase and decrease in acidity value of paneer during storage is similar to that observed in case of feed back inhibition in biological systems. It involves 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. The observations of present study corroborate with those reported by Mistry (1988) and Kumar and Bector (1991).

Bajwa \textit{et al.} (2004) prepared paneer samples by incorporating 10\% coriander (\textit{Coriandrum sativum}) or mint (\textit{Mentha piperita}) leaves. The acidity increased significantly ($p<0.01$) with advancement in storage period; the rate of increase was lower in samples containing coriander or mint leaves and was further
reduced on incorporation of salt. While studying the effect cardamom, thyme and clove powder on quality of white cheese made from goat’s milk Ratiba et al. (2006) observed that the development of acidity was slower in cheese containing added spices especially that containing 0.2 per cent cardamom which showed the lowest development acidity.

However, no data are reported in the literature for effect of black pepper, cardamom, cinnamon and clove on acidity of paneer during storage

4.4.3.2 Free fatty acids

The data obtained for changes in free fatty acids content of paneer during storage at 7°C are presented in Table 4.17 and the trend is depicted in Figure 4.17.

The results indicated that the free fatty acids content of all fresh samples of paneer was same (0.06%), except cinnamon which has slightly higher value (0.07%). Therefore, it is evident that addition of these spices have no effect on free fatty acids content of paneer.

Kumar and Bector (1991) found difference in free fatty acids content of control samples of paneer and those added with BHA and TBHQ. No data are reported in the literature for effect of addition of spices on free fatty acids content of paneer.

<table>
<thead>
<tr>
<th>Spices added</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
<td>VMG*</td>
<td>-</td>
</tr>
<tr>
<td>BP</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
<td>0.15</td>
<td>0.27</td>
</tr>
<tr>
<td>Ca</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>Ci</td>
<td>0.07</td>
<td>0.05</td>
<td>0.05</td>
<td>0.11</td>
<td>0.17</td>
</tr>
<tr>
<td>Cl</td>
<td>0.06</td>
<td>0.05</td>
<td>0.06</td>
<td>0.12</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth
Kumar and Bector (1991) reported free fatty acid content of 0.28 (% oleic acid). Gohian (1996) analyzed the market sample of paneer collected from 18 different producers and reported that the acid degree value ranged from 0.583 to 2.250. The general mean obtained of acid degree value for over all 30 sample of market paneer was 1.308. Goel (2000) studied the laboratory made paneer and reported that the free fatty acidity content of paneer was 0.0665 (% oleic acid). The values reported for the market sample of paneer was 0.0699 (% oleic acid). Venkateswarlu, et al. (2003) found free fatty acid content of 0.11 (% oleic acid).

Gokhale and pandya (2009) reported that paneer prepared by using standardized milk and acidification, vacuum drying and salting treatments were
given to the paneer cubes of approximately 1.5 cm³ sizes and were packed in LDPE (90micron) poly bags and was stored at refrigerators (8 ± 2°C) temperature for 90 days. The free fatty acids content of fresh acids content of fresh sample was 0.04 per cent. Therefore, values obtained for free fatty aid content of paneer in present study was in agreement with those reported by Goel (2000) and Gokhale and pandya (2009). However, effect of addition of spice on free fatty acid content of paneer has not be reported.

The results depicted in Figure 4.17 shows trend of changes in free fatty acids content of paneer samples during storage. There was slight increase in The free fatty acids content of the control and black pepper containing samples up to 7th day of the storage, whereas, in case of the samples containing cardamom, cinnamon and clove there was a slight decrease the values. On further storage free fatty acids content in case of control sample further increased at a slow rate, however, in case of the samples containing spices free fatty acids content tended to remain almost unchanged up to 14th day of the storage. On further storage free fatty acids content increased at greater rate. The maximum rate of increase was observed in case of black pepper and the rate was minimum in case of cardamom. The rate of increase of in case of cinnamon and clove was intermediate to that of the black pepper and cardamom.

The free fatty acids content in samples containing cardamom remained stagnant during storage between 21 to 28 days. Thus, cardamom proved to be the best spice to control free fatty acids content of paneer during storage. These observations are well correlated with highest flavour score (4.4.2.1) of the paneer containing cardamom during storage.

Kumar and Bector (1991) reported that the free fatty acids content of paneer increased from 0.28 to 0.5 (% oleic acid) on 22 days of storage at 5°C. Goel (2000) found that the free fatty acids content of laboratory made paneer increased from 0.066 to 0.077 (% oleic acid) on 12 days of storage at 7°C. Author also reported that market sample of paneer has a rise its initial value of 0.0699 to
0.080 (% oleic acid) on 12 days of storage at 7°C. The similar increase was observed in the present study.

While evaluating the effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat’s milk Ratiba et al. (2006) found that total volatile fatty acids (TVFA) of all cheese samples increased significantly throughout the storage period. There were some slight difference between the control and all other treatments in TVFA at the first stage of storage and the rate of increase in TVFA varied considerably among the treatments during storage. Cheese treated with 0.15 per cent thyme had the lowest value throughout the storage period (45 days) while, control had the highest value of TVFA than other treatments. This suggested that added spices to the curd of cheese led to a slight decrease in TVFA as a result of inhibition of lipolytic microbial enzymes. However, effect of addition of spice on free fatty acid content of paneer during storage has not been reported.

4.4.3.3 Soluble nitrogen

The data obtained for changes in soluble nitrogen content of paneer during storage at 7°C are presented in Table 4.18 and the trend is depicted in Figure 4.18.

Table 4.18: Effect of selected spices on soluble nitrogen (%) of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Spice added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>0.202</td>
</tr>
<tr>
<td>BP</td>
<td>0.232</td>
</tr>
<tr>
<td>Ca</td>
<td>0.146</td>
</tr>
<tr>
<td>Ci</td>
<td>0.214</td>
</tr>
<tr>
<td>Cl</td>
<td>0.143</td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth
The results indicated that the soluble nitrogen content of fresh samples of paneer was in the order of clove > cardamom > control > cinnamon > black pepper. The soluble nitrogen content of paneer containing cardamom as well as clove was lower and cinnamon and black pepper was higher compared to the control sample of paneer.

Thus addition of cardamom or clove decreases the soluble nitrogen content, whereas, addition of black pepper or cinnamon increases the soluble nitrogen content in fresh sample of paneer. This effect may be attributed to effect of added spices on solubility of nitrogenous compounds in paneer. Kumar and Bector (1991) reported differences between soluble nitrogen content of control samples of paneer and that of the samples added with BHA and TBHQ. The lower values were reported by the authors in the samples containing BHA and TBHQ. Similar effects were also observed by Ratiba et al. (2006). However, effect of addition of spice on soluble nitrogen content of paneer has not be reported.

Mistry (1988) prepared cow milk paneer and found that the soluble protein content of paneer varied from 2.55 to 3.26 per cent, which is equivalent to 0.399 to...
Results and Discussion

0.510 per cent soluble nitrogen. Sachdeva and Singh (1990) reported the soluble nitrogen content of 0.10 per cent. Kumar and Bector (1991) found the soluble nitrogen content of 0.06 per cent in laboratory made paneer. Gohian (1996) studied that the soluble nitrogen content in 30 market samples of paneer examined from 18 different producers, the values varied from 0.154 to 0.578 per cent with a general mean 0.334 per cent. Therefore, values obtained for soluble nitrogen content of paneer in the present study were in general agreement with those reported in the literature.

The results depicted in Figure 4.18 shows trend of changes in soluble nitrogen content of paneer samples during storage. The sharp, continuous and the highest increase in soluble nitrogen content was observed in control samples of paneer. In the samples containing black pepper soluble nitrogen content increased at a slow rate up to 7th day of the storage, the rate became higher between 7th and 14th day and again became slower between 14th to 28th day. In the samples containing clove soluble nitrogen content increased at a slow rate up to 14th day of the storage, the rate became higher between 14th and 21st day and again became slower between 21st to 28th day.

In the samples containing cinnamon soluble nitrogen content increased gradually up to 21st day of the storage then the rate became slightly slower. In the samples containing cardamom soluble nitrogen content increased at a slow rate up to 7th day of storage, the values remained almost unchanged between 7th and 14th day and then increased at a higher rate. At the end of the storage minimum increase in soluble nitrogen content was found in the samples of paneer containing cardamom. Thus, cardamom proved to be the best spice to control soluble nitrogen content of paneer during storage.

Mistry (1988) reported that soluble protein content of paneer increased from 2.55 to 3.13 and 4.40 on 7th and 15th day respectively on storage of samples at 7 to 10°C, which are equivalent to 0.399 to 0.49 and 0.733 per cent soluble nitrogen content on 0, 7th and 15th day respectively. Sachdeva and Singh (1990)
observed considerable increase in soluble nitrogen content of paneer up on storage for 6 days at 8 to 10\(^{\circ}\)C, where in the initial value of 0.1 per cent increased to 0.24 per cent. Kumar and Bector (1991) found that the control soluble nitrogen content increased from 0.06 to 0.08 on 22 days of storage at 5\(^{\circ}\)C. Increase in soluble nitrogen content of control sample of paneer was collaborated with increased reported by Mistry (1988).

In the study of white cheese made from goat’s milk Ratiba et al. (2006) observed that the incorporation of cardamom, thyme and clove powder had significant effect on the changes in total nitrogen (TN) during storage. Slight but not significant differences were found in cheese with level of spices as compared to control. Also shows that the soluble nitrogen and SN/TN of cheese from different treatments increased with progressive storage suggesting protelysis. However, the addition of tested spices decreased slightly the development of SN and SN/TN.

### 4.4.4 Microbial counts of paneer containing cardamom

From the results obtained for changes in sensory attributes (4.4.2) and chemical characteristics (4.4.3) during storage suggested cardamom as the best spice to extend shelf life of paneer. Therefore, effect of cardamom on microbial counts of paneer was evaluated. The fresh and stored samples of paneer were analyzed for microbial counts viz. standard plate counts (SPC), yeast and mould counts and coliform counts. The data obtained for changes in SPC, yeast and mould counts and coliform counts are presented in Table 19.

**Table 19 : Changes in microbial counts during storage of paneer at 7\(^{\circ}\)C**

<table>
<thead>
<tr>
<th>Storage period</th>
<th>SPC</th>
<th>Yeast and Mould</th>
<th>Coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Ca</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>3.4 x 10(^3)</td>
<td>3.1 x 10(^3)</td>
<td>1.7 x 10(^2)</td>
</tr>
</tbody>
</table>
Results and Discussion

The same data were converted into log values and presented in Figure 19.

<table>
<thead>
<tr>
<th></th>
<th>7</th>
<th>15 x 10^5</th>
<th>8.6 x 10^5</th>
<th>13 x 10^2</th>
<th>3 x 10^1</th>
<th>2 x 10^1</th>
<th>1 x 10^1</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>57 x 10^5</td>
<td>1.5 x 10^4</td>
<td>45 x 10^3</td>
<td>16 x 10^2</td>
<td>5.5 x 10^2</td>
<td>2 x 10^1</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>VMG*</td>
<td>7.8 x 10^5</td>
<td>-</td>
<td>25 x 10^3</td>
<td>-</td>
<td>1 x 10^1</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>15 x 10^5</td>
<td>-</td>
<td>32 x 10^3</td>
<td>-</td>
<td>3 x 10^1</td>
<td></td>
</tr>
</tbody>
</table>

* VMG = Visible mould growth

The standard plate count of fresh sample of market paneer ranged from 2.5 x 10^4 to 3.5 x 10^5 cfu per g (average initial count of 3.0 x 10^5). The standard plate count of

4.4.4.1 Standard plate count

It is evident from the trend depicted in Figure 19 that there was a sharp increase in SPC on 7th day of the storage, both in control as well as cardamom added samples. On further storage the count continued to increase on 14th day of the storage in control sample, but the count decreased considerably in case of cardamom containing samples. In the cardamom containing sample again count increase up to 21st day of storage, followed by marginal increase on 28th day of the storage.

Gupta (1985) studied the presence of the total bacterial load of paneer.
Results and Discussion

NDRI dairy made paneer sample ranged from $5.0 \times 10^3$ to $1.8 \times 10^5$ (average $7.9 \times 10^4$), for laboratory prepared paneer contain count was as low as $1.0 \times 10^4$ cfu per g. Sachdeva and Singh (1990) reported that total plate count of paneer treated with different dipping materials during storage. The fresh paneer had a total plate count of $10^4$ to $10^3$ per g which increased to the number of $10^4$ to $10^6$ per g regard less of treatment. Kumar and Bector (1991) reported the initial counts of control, $3.0 \times 10^3$ increased to $2.8 \times 10^5$ per g on day four and $9.0 \times 10^6$ per g on day seven during storage at 15°C. Gohian (1996) studied that the microbiological analysis and revealed that the SPC of market paneer samples from 18 different producers varied from $4.6989$ to $7.5549$ log cfu per g. The general mean for all the 30 samples was $5.9424$ log cfu per g. Vaishnavi et al., (2001) reported total plate count of $3 \times 10^2$ to $9.7 \times 10^10$ in the samples of paneer collected from Chandigarh market.

Ratiba et al. (2006) studied effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat’s milk. The author found that the total viable count (TVC) of control significant increased throughout the storage period while, count of different treatments decreased up to 15 days, then slightly increased up to 15 days.

Then slightly increased till the end of storage period. Cardamom, thyme and clove concentration of 0.20, 0.15 and 0.20 per cent respectively had the highest effect on TVC especially 0.20 per cent cardamom.

The data obtained for SPC for control sample of paneer and changes in the count during storage were in accordance with those reported in the literature. Fresh samples of both control and cardamom containing paneer met the standards prescribed by BIS for the yeast and mould counts. Up on storage only in cardamom containing samples SPC remained with in BIS requirement up to 21 day of storage. However, effect of addition of spice on SPC during storage of paneer has not be reported.

4.4.4.2 Yeast and Mould count
Results and Discussion

It is evident from the trend depicted in Figure 19 that yeast and mould count increases sharply in case of control sample of paneer. In case of the samples containing cardamom the count increased gradually and at a slower rate all through out the storage period.

Thakral (1986) reported the yeast and mould count of $1.9 \times 10^2$ in laboratory made paneer. Sachdeva and Singh (1990) found that the initial count of the paneer sample dipped in plain, chlorinated, buffered and acidified water and brine and acidified brine varied over a narrow range of $35 \times 10^1$ to $52 \times 10^1$. The yeast and mould count at the time of spoilage in the respective samples ranged from $53 \times 10^2$ to $63 \times 10^3$. Kumar and Bector (1991) analyzed the yeast and mould counts of control paneer sample and found that initial count increased from 10 per g to 50 per g after 4 days and 250 per g after 7 days of storage at 15°C. Gohian (1996) studied the yeast and mould count in market sample of paneer. The author reported that samples from 18 different producers had count varying from 1.6990 to 5.1746 log cfu per g. The general total yeast and mould for all the 30 market paneer sample was 3.1101 log cfu per g. Venkateswarlu et al. (2003) reported that yeast and mould count of paneer 2.45 per g.

Dhole et al. (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The yeast and mould counts in market samples of paneer were ranged between $1 \times 10^2$ to $99 \times 10^2$ cfu per g.

While studying the effect of cardamom, thyme and clove powder on quality of white cheese made from goat’s milk Ratiba et al. (2006) observed the significant differences in TVC as affected by addition and storage period. The changes in psychrophillic bacterial count and yeasts and moulds followed similar trends as the TVC. They increased all through the storage reaching a maximum after 45 days of storage. Addition of the used spices was found to decrease the counts of yeasts and moulds and the decrease ran parallel to the concentration of added spices.
The data obtained for yeast and mould count for control sample of paneer and changes in the count during storage were in accordance with those reported in the literature. Fresh samples of both control and cardamom containing paneer met the standards prescribed by BIS for the yeast and mould counts. Up on storage only in cardamom containing samples yeast and mould count remained within BIS requirement up to 21 day of storage. However, effect of addition of spice on yeast and mould count during storage of paneer has not been reported.

4.4.4.3 Coliform count

It is evident from the trend depicted in Figure 19 that there was a sharp increase in coliform count on 7th day of the storage, both in control as well as cardamom added samples. On further storage the count continued to increase on 14th day of the storage in control sample, but the count decreased considerably in case of cardamom containing samples. In the cardamom containing sample again count increase up to 21st day of storage, followed by marginal increase on 28th day of the storage.

Gupta (1985) studied the presence of coliform in karnal, NDRI dairy made and laboratory prepared sample of paneer. The ranges of coliforms from karnal paneer samples were $1.0 \times 10^1$ to $5.5 \times 10^3$ (average $2.7 \times 10^3$), count of NDRI dairy made paneer sample ranged from $<1.0 \times 10^1$ to $1.5 \times 10^2$ (average $1.5 \times 10^3$) cfu per g, for laboratory prepared paneer coiform could not be detected in the laboratory prepared sample. Sachdeva and Singh (1990) determined coliform count of paneer treated with different dipping materials and reported that the initial coliform count was not more than 3 to 4 in the first dilution of all the paneer samples and this increased to a maximum of 30 – 50 over the storage period. Kumar and Bector (1991) reported the initial level of coliform counts of control increased from 90 per g to $3.5 \times 10^3$ per g after four days and $8.0 \times 10^6$ per g after seven days of storage. Gohian (1996) studied that the coliform count in market
samples of paneer and found that the count vary from 2.0 to 4.9642 log cfu per g among 18 producers. The general mean for 30 samples was 3.3398 log cfu per g.

While evaluating the effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat’s milk Ratiba et al. (2006) found that the count of coliforms gradually increased till 30 days of storage period, then decreased at the end of storage period.

Dhole et al., (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The average coliform counts in the market samples of paneer were found in the range of $12.6 \times 10^3$ to $23.2 \times 10^3$ cfu per g.

The data obtained for coliform count for control sample of paneer and changes in the count during storage were corroborated well with those reported in the literature. Fresh samples of both control and cardamom containing paneer met the standards prescribed by BIS for the coliform counts. Up on storage only in cardamom containing samples coliform count remained within BIS requirement up to 21 day of storage. However, effect of addition of spice on coliform count during storage of paneer has not be reported.

Results of the present study suggested that addition of black pepper, cardamom, clove, garlic, ginger or onion in paneer at the rate greater than 0.6 per cent and cinnamon at the rate greater than 0.4 per cent result in to sharp decline in sensory score of paneer. Amongst black pepper, cardamom, cinnamon, clove, garlic, ginger and onion; addition of garlic, ginger or onion in paneer develops strong undesirable flavour in paneer up on storage at $7^\circ$C. Addition of black pepper, cardamom or clove at the rate of 0.6 per cent or cinnamon at the rate of 0.4 per cent improves shelf life of paneer on storage at $7^\circ$C. The order of the relative effectiveness in enhancing shelf life of paneer is cardamom > cinnamon > clove > black pepper. Thus, cardamom is the best spice to improve shelf life of paneer during storage.
The present study on the enhancement of the shelf life of paneer was undertaken in four phases. First phase involve selection of suitable spices for incorporation in paneer. In second phase rate for addition of spices in paneer was selected. In third phase preliminary screening for effectiveness of the spices in enhancing shelf life of paneer was performed. Finally in the fourth phase the screened spices were tested for their relative efficiency in improving shelf life of paneer.

In first phase seven different spices, commonly used with paneer during culinary preparations viz black pepper (BP), cardamom (Ca), cinnamon (Ci), clove (Cl), garlic (Ga), ginger (Gi) and onion (On) were incorporated in paneer at the rate of 0.5 per cent and the samples were subjected to sensory evaluation. All the samples containing these spices were found acceptable in organoleptic testing. Therefore, all the seven spices were selected for further study.

In second phase, work was carried out to select rate for addition of the selected spices in paneer. In first phase of the study work was carried out to decide rate of addition of the 7 selected spices viz. black pepper, cardamom, cinnamon, clove, garlic, ginger and onion in paneer. For selecting rate of addition of each spice the coagulum obtained by removal of 90 per cent whey, was divided in to 6 equal parts and the spice was incorporated in the product at the rate of 0.0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 per cent by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control. The prepared samples of paneer were subjected to sensory evaluation by panel of six judges using 9 point hedonic scale. The overall acceptability score of paneer declined gradually, remained constant or improved slightly on addition of black pepper, cardamom, clove, garlic, ginger and onion up to 0.6 per cent and their further addition resulted in to sharp decline in the score. However, in case of cinnamon the gradual decline in overall acceptability score was up to 0.4 per cent
addition and its further addition resulted in to sharp decline in the score. During preliminary study it was found that flavour of the added spices was slightly intensified during storage of the paneer. Hence, for black pepper, cardamom, clove, garlic, ginger and onion 0.6 per cent rate of addition in paneer was selected, whereas, in case of cinnamon 0.4 per cent rate of addition in paneer was selected for further study.

In third phase work was carried out for preliminary screening of the selected spices for their effectiveness to enhance shelf life of paneer. The coagulum obtained by removal of 90 per cent whey, was divided into 8 equal parts and black pepper, cardamom, cinnamon, clove, garlic, ginger and onion were incorporated in the coagulum at the rate of 0.6 per cent and cinnamon was incorporated in the coagulum at the rate of 0.4 per cent by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control. Each block of paneer was cut into four equal parts and packed separately into PET/LDPE film pouches and stored at 7±1°C for 14 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after 14 days of storage. The overall acceptability score of black pepper, cardamom, cinnamon and clove decline slightly on 14th day of the storage but remained above acceptable level (6.0). On the other hand the overall acceptability score of garlic, ginger, onion and that of the control decline sharply and went below acceptable level due to development of strong objectionable flavour. Therefore, black pepper, cardamom, cinnamon and clove were selected, whereas, garlic, ginger and onion were dropped from the further study due to their deleterious effect on acceptability of paneer, especially up on storage.

Finally in the fourth phase the screened spices were tested for their relative efficiency in improving shelf life of paneer. For final screening of the selected spices (black pepper, cardamom, cinnamon and clove) for their effectiveness to
Summary & conclusion

enhance shelf life of paneer the coagulum obtained by removal of 90 per cent whey, was divided in to 5 equal parts and black pepper, cardamom, and clove were incorporated in the coagulum at the rate of 0.6 per cent and cinnamon was incorporated in the coagulum at the rate of 0.4 per cent by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control. Each block of paneer was cut in to four equal parts and packed separately in to PET/LDPE film pouches and stored at 7⁰C ± 1⁰C for 28 days. The samples of paneer were also stored at 32⁰C ± 1⁰C. The fresh samples of paneer were analyzed for the chemical composition, sensory attributes and chemical characteristics. The analysis for sensory attributes and chemical characteristics was also carried out at an interval of 7 days during storage at 7⁰C. Visible mould growth was observed in control samples of paneer on 21st day of storage. Therefore, sensory and chemical analysis of these samples was not carried out and their further storage study was terminated. The samples of paneer stored at 32⁰C ± 1⁰C were to be analyzed at an interval of one day. However, in the samples stored at 32⁰C ± 1⁰C very strong off flavour was developed and whey separation took place with in one day of storage. Therefore, the sensory evaluation and analysis for the chemical characteristics of the samples stored at 32⁰C ± 1⁰C were not carried out and their further storage study was terminated.

The overall acceptability score of control sample of paneer declined sharply from beginning of the storage. Amongst the samples of paneer containing spices the highest rate of decrease in overall acceptability score was observed in case of black pepper and the lowest rate was observed in case of cardamom. The overall acceptability score of control samples of paneer went below acceptable level (6.0) on 14th day of the storage. The samples of paneer containing black pepper went slightly below the acceptable level on 21st day of the storage. The overall acceptability score of samples containing cinnamon or clove went slightly below acceptable level on 28th day of the storage. However, the overall acceptability
Summary & conclusion

score of samples containing cardamom remained well above the acceptable level even on 28th day of the storage.

The acidity of all the samples tended to rise sharply up to 7th day of the storage, except cinnamon. On further storage acidity value continued to rise in case of control sample, however, in case of the samples containing spices acidity values were found to decreased up to 14th day of the storage. The decrease was marginal in case of black pepper, it was substantial in case of cardamom, cinnamon and clove. On further progress of the storage acidity values increased slowly in case of black pepper, cinnamon and clove, but acidity remained constant in case of cardamom.

There was a slight rise up to 7th day of the storage, the free fatty acids content of the control and black pepper containing samples whereas, in case of the samples containing cardamom, cinnamon and clove there was a slight decrease the values. On further storage free fatty acids content in case of control sample further increased at a slow rate, however, in case of the samples containing spices free fatty acids content tended to remain all most unchanged up to 14th day of the storage. On further progress in storage free fatty acids content increased at greater rate. The maximum rate of increase was observed in case of black pepper and the rate was minimum in case of cardamom. The free fatty acids content in samples containing cardamom remained stagnant during storage between 21 to 28 days.

Trend of changes in soluble nitrogen content of paneer samples during storage indicated sharp and continuous increase. The highest increase in soluble nitrogen content was in control samples of paneer. Amongst, the samples containing spices, maximum increase in soluble nitrogen content was observed in black pepper and the minimum increase was found the samples containing cardamom at the end of the storage. The values of soluble nitrogen content in cinnamon and clove was intermediate to that of the black pepper and cardamom.
Summary & conclusion

Thus, cardamom proved to be the best spice to control acidity, free fatty acids content and soluble nitrogen content of paneer during storage. The changes in these chemical characteristics are very well corroborated with changes in sensory attributes of paneer during storage. Therefore, effect of cardamom on microbial counts of paneer was evaluated. The fresh and store samples of paneer were analyzed for microbial counts viz. standard plate counts (SPC), yeast and mould counts and coliform counts.

There was a sharp increase in SPC on 7th day of the storage, both in control as well as in cardamom added samples. On further storage the count continued to increase on 14th day of the storage in control sample, but the count decreased considerably in case of cardamom containing samples. In the cardamom containing sample again count increase up to 21st day of storage, followed by marginal increase on 28th day of the storage. The yeast and mould count increased sharply in case of control sample of paneer. In case of the samples containing cardamom the count increased gradually and at a slower rate all through out the storage period. In coliform count there was a sharp increase on 7th day of the storage, both in control as well as cardamom added samples. On further storage the count continued to increase on 14th day of the storage in control sample, but the count decreased considerably in case of cardamom containing samples. In the cardamom containing sample again count increased up to 21st day of storage, followed by marginal increase on 28th day of the storage.

Results of the present study suggested that addition of black pepper, cardamom, clove, garlic, ginger or onion in paneer at the rate greater than 0.6 per cent and cinnamon at the rate greater than 0.4 per cent decreases the sensory score of paneer. Addition of garlic, ginger or onion in paneer develops strong undesirable flavour in paneer up on storage at 7°C. Addition of black pepper, cardamom or clove at the rate of 0.6 per cent or cinnamon at the rate of 0.4 per cent improves shelf life of paneer on storage at 7°C. The order of their relative
Summary & conclusion

effectiveness in enhancing shelf life of paneer is cardamom > cinnamon > clove > black pepper. Thus, cardamom is the best spice to improve shelf life of paneer during storage.
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