TESTING OF THE COMMON HERBS FOR THEIR COMPATIBILITY IN PANEER AND EVALUATION OF THE SELECTED HERBS AS PRESERVATIVES FOR PANEER

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ANAND - 388 110, INDIA
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TESTING OF THE COMMON HERBS FOR THEIR COMPATIBILITY IN PANEER AND EVALUATION OF THE SELECTED HERBS AS PRESERVATIVES FOR PANEER

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ABSTRACT

Paneer is an important heat and acid coagulated indigenous dairy product. The poor keeping quality of paneer is a major obstacle in its large scale industrial production. A great deal of study has been carried out for enhancing the shelf life of paneer employing various preservatives and preservation methods. Now-a-days consumers are interested in having foods that are natural or close to natural, minimally processed and free of chemical preservatives.

Inhibitory activity of herbs and spices on the growth of bacteria, yeasts and mold synthesis has been well reported. Therefore, herbs and spices offer a promising alternative for preservation of food. However, they are currently used mainly for enhancing the flavour of foods rather than extending shelf life. There is no work reported about use of herbs in extending the shelf life of paneer. Therefore, attempt was made by conducting the present study to test the common herbs.
for their compatibility in paneer and evaluate the selected herbs as preservatives for paneer.

Study of evaluating the selected herbs for compatibility and extending shelf life of paneer was conducted. The entire study was divided into five phases viz., testing of herbs for compatibility, selection for rate of addition of the herbs, screening of the herbs for effectiveness in extending the shelf life, selection of stage for addition of the herb and final testing for effectiveness of the selected herb in extending the shelf life of paneer.

Seven herbs, commonly used with paneer during culinary preparations viz., ajwain, asafoetida, coriander, cumin, fenugreek, mint and turmeric were incorporated in paneer at the rate of 0.5 per cent. The samples containing four herbs (ajwain, coriander, cumin and turmeric) were found acceptable in organoleptic testing. Therefore, these four herbs were selected for further study.

For selecting rate of addition in paneer, each herb 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, the rate of addition for ajwain, cumin and turmeric was selected as 0.4 per cent and for coriander the rate was selected as 0.6 per cent.

In screening of the selected herbs for their effectiveness to enhance shelf life of paneer ajwain, cumin and turmeric were incorporated in the coagulum at the rate of 0.4 per cent and coriander was incorporated in paneer at the rate of 0.6 per cent. The sample of paneer without addition of herb was used as a control. The samples were packed in PET/LDPE film pouches and stored at 7°C±1°C for 7 days. The prepared samples of paneer were subjected to sensory evaluation when fresh and after 7 days of storage. On the basis of performance of various herbs in screening turmeric was selected for further study.
In the fourth phase of the study, work was carried out to select stage for addition of the selected herb (turmeric) to enhance the shelf life of paneer. The work was carried out to evaluate effect of addition of turmeric before heat treatment of milk and after heat treatment of milk. The prepared samples of paneer were subjected to sensory evaluation when fresh and after the interval of every 3 days (except first interval which was of six days) up to 12 days of storage at 7°C±1°C. The samples of stored paneer obtained in case of turmeric added before heating of milk scored higher.

In the final phase of the study work was carried out to evaluate the effectiveness of turmeric in extending the shelf life of paneer when added at the rate of 0.4 and 0.6 per cent. The prepared samples of paneer were subjected to analysis for sensory evaluation, chemical characteristics and microbial counts when fresh and after the interval of every 3 days (except first interval which was of six days) up to 12 days of storage. The samples of paneer with 0.6 per cent turmeric remain acceptable up to 12 days on storage at 7°C±1°C.

The present study entailed to conclude that addition of turmeric in paneer at the rate greater than 0.6 per cent results into sharp decline in sensory score of paneer. Addition of turmeric at the rate of 0.6 per cent extends the shelf life of paneer up to 12 days on storage at 7°C±1°C.
CERTIFICATE-I

This is to certify that Buch Shweta Arunkumar has successfully completed the comprehensive examination of Major and Minor subject held on /06/2010 as required under the Regulation for M.Sc. degree in Dairy Science.

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This is to certify that the thesis entitled “Testing of the Common Herbs for their Compatibility in Paneer and Evaluation of the Selected Herbs as Preservatives for Paneer” submitted for the degree of Master of Science in the subject of Dairy Chemistry embodies bonafide research work carried out by Buch Shweta Arunkumar under my guidance and supervision and that no part of this thesis or research work has been submitted for any other degree. The assistance, guidance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by Advisory Committee on / /.

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This is to certify that Buch Shweta Arunkumar, Dairy Chemistry Department, Sheth M.C. College of Dairy Science, Anand has made all corrections / modifications in the thesis entitled, “Testing of the Common Herbs for their Compatibility in Paneer and Evaluation of the Selected Herbs as Preservatives for Paneer” which were suggested by the External Examiner and the Advisory Committee in the oral examination held on / 06 / 2010. The final copies of the thesis duly bound and corrected were submitted on / 06 / 2010 are enclosed herewith for approval.

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# SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
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<tr>
<td>°C</td>
<td>degree Celsius</td>
</tr>
<tr>
<td>ADI</td>
<td>acceptable daily intake</td>
</tr>
<tr>
<td>Anon</td>
<td>Anonymous</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BHA</td>
<td>Butylated hydroxy anisole</td>
</tr>
<tr>
<td>BIS</td>
<td>Bureau of Indian Standards</td>
</tr>
<tr>
<td>C. D.</td>
<td>Critical difference</td>
</tr>
<tr>
<td>C. V.</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>cfu</td>
<td>colony forming unit</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene Diamine Tetra-acetic Acid</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GRAS</td>
<td>generally recognized as safe</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>IU</td>
<td>International Unit</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>LDPE</td>
<td>Low Density Polyethylene</td>
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<tr>
<td>m</td>
<td>Meter</td>
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<tr>
<td>mg</td>
<td>Milligram</td>
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<tr>
<td>min</td>
<td>Minute</td>
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<td>ml</td>
<td>Milliliters</td>
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<tr>
<td>mm</td>
<td>Millimeter</td>
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<tr>
<td>N</td>
<td>Normality</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NDRI</td>
<td>National Dairy Research Institute</td>
</tr>
<tr>
<td>PFA</td>
<td>Prevention of Food Adulteration Act, 1954</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>RU</td>
<td>Reading Unit</td>
</tr>
<tr>
<td>S. Em</td>
<td>Standard error of mean</td>
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<tr>
<td>sp. gr.</td>
<td>specific gravity</td>
</tr>
<tr>
<td>SPC</td>
<td>Standard plate count</td>
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<tr>
<td>sq. cm</td>
<td>square centimeter</td>
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<tr>
<td>TBHQ</td>
<td>Tertiary butylated hydroxy quinone</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-Violet</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>WTO</td>
<td>World trade organization</td>
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<td>μ</td>
<td>Micro</td>
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Dairy industry in India occupies a place of pride in food process industry. It is playing a significant role in our socio-economic growth. Milk production is growing at rapid rate of 4-5 per cent per annum during the last 35 years and reaching a level of about 100.9 million tonnes 2006-2007. The previous year production was 97.1 million tonnes. India has already entered in the international market in milk products, exporting 59,746 million tonnes of milk products during 2005-06. Under the WTO regime, Indian exports will have to adhere to stringent quality and food safety standards.

The pattern of milk utilization 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 tones (Aneja et al., 2002).

Paneer is an important protein rich dairy product among the heat and acid coagulated milk products. It is widely used as a base material for the preparation of various culinary dishes and snacks (Mathur et al., 1991). 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.

Like other indigenous product paneer is a highly perishable product and suffers from limited shelf life, largely of because its high
moisture content (Arora and Gupta, 1980). It is not able to find its rightful place in Indian market due to its relatively high cost of production and short shelf life. The shelf life of paneer is reported to be only six days at refrigeration temperature ($10^\circ C$) without much deterioration in quality but the freshness of the product is lost after three days (Bhattacharya et al., 1971). Its flavour remains acceptable even after 120 days when stored at -13 or -32°C. At room temperature paneer does not keep well for more than one day. It has been noticed that the spoilage of paneer occurs due to growth of microorganisms (Thakral, 1986). A greenish yellow slime formation on the surface of paneer and the discoloration is accompanied by an off-flavour (Kanawjia, 1990).

Various attempts have been made over the years to increase the shelf life of paneer. Food additives such as sorbic acid, potassium sorbate (Thakral, 1986), solutions of $H_2O_2$ and brine and delvocid (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. Hence, less use of synthetic additives is in demand (Membre et al., 2001). In recent years many attempts have put emphasis on the search of natural antimicrobial compounds that can properly serve the needs of food manufacturers and consumers. Various herbs and spices have been recognized for their antimicrobial activity and used throughout the past as an alternative approach to preserve foods. They contain antimicrobial compounds that may find use as natural preservatives.
The preservative properties of aromatic and medicinal plant essential (volatile) oils and extracts have been recognized since Biblical times, while attempts to characterize these properties in the laboratory date back to the early 1900s (Hoffman and Evans, 1911). Several studies have revealed the results on the preservative action of herbs or their essential oils (Zaika, 1988). Therefore, substitution of chemical preservatives with herbs and spices in food is becoming highly desirable in the traditional and novel foods. This approach enhances safety of the foods (Farag et al., 1990).

The consumers demand has forced the food industry to exploit potential of natural alternatives for synthetic antimicrobial compounds. Herbs offer a promising alternative for food safety. Inhibitory activity of herbs 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. Herbs and 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 et al., 2000). In addition to imparting flavour, certain herbs prolong the shelf life of foods due to their bacteriostatic or bacteriocidal activity and prevent rancidity by their antioxidant activity (Shelef et al., 1980). Many plant essential oils of herbs are active against various food borne bacteria and molds (Aureli et al., 1992). However, no work has been reported about use of herbs in extending shelf life of paneer.

Therefore the present study has been contemplated with the following objectives:
(a) To evaluate herbs for their compatibility in paneer.
(b) To select the rate for addition of compatible herbs in paneer.
(c) To screen herbs for effectiveness in preservation of paneer.
(d) To select stage for addition of herb(s) in paneer.
(e) To test the selected herb(s) as preservatives for paneer.
CHAPTER: II

REVIEW OF LITERATURE

Paneer is an important indigenous dairy product prepared by the heat and acid coagulation of milk. It is used as a 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).

The term ‘herb’ has more than one definition. The word "herb" comes from the Latin ‘herba’ meaning a medicinal plant. Thus, in the most generally accepted sense, herbs are plants valued for their medicinal and aromatic properties and are often grown and harvested for these unique properties. Some of the common herbs include ajwain, asafoetida, basil, coriander, cumin, fenugreek, mace, mint, nutmeg, rosemary, thyme, turmeric, etc., are used in day to day foods (Farag et al., 1990).

2.1 Market for paneer in India

Paneer market in Indian is estimated to be around one lakh metric tones 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 tones (Aneja et al., 2002).

2.2 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 there of (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”.

2.3 Methods for manufacture of paneer

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

2.3.1 Traditional method

Milk is first heated to boiling. 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, immersed in chilled water for 3-4 hour to make it firm and cut into suitable sizes (Singh and Kanawjia, 1988; Sachdeva and Singh, 1988; Rao et al., 1992).
2.3.2 Industrial method

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

An industrial process for the manufacture of paneer has been developed by the NDDB. The milk is heated to 85°C to obtain a co-precipitate through a plate heat exchanger, pumped to a cheese vat and cooled to 75°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 3 hours for cooling and firming the paneer (Pruthi, 1999; Rao, et al., 1992).

2.3.3 Development in paneer manufacturing

The development in paneer manufacturing 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 manufactured 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). Chhana is more commonly know as paneer in certain parts of India.
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).

2.4 Chemical Aspects

Chemical composition and chemical characteristics of paneer are summarized below.

2.4.1 Chemical composition

Gross composition of paneer, 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 usually consists of almost all the fat, casein and insoluble salts together with part of the moisture which contained lactose, whey proteins, soluble salts, vitamins and other milk components. It contains approximately 53-55 per cent moisture, 23-26 per cent fat, 17-18 per cent protein, 2-2.5 per cent carbohydrate and 1.5-2.0 per cent minerals (Kanawjia et al., 1990).

Goel (2000) reported 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 1.49 to 1.60 per cent respectively.

Dhole et al., (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar city (Maharastra) for
chemical quality. The average moisture, fat and protein contents of market samples of paneer were found in the range of 42.62 to 60.39, 21.60 to 23.50 and 15.06 to 20.33 per cent respectively. Amongst the samples collected, 21 samples (30.00%) fulfilled the minimum standard fat content prescribed by BIS.

The mineral composition of market paneer and changes occurring in their profile during storage at \( \leq 10^\circ\text{C} \) 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 and iron 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.

### 2.4.2 Chemical characteristics

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

#### 2.4.2.1 Acidity of paneer

Mistry (1988) prepared paneer from cow 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 at 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) 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 15th day of storage, respectively. During storage of paneer acidity increased in $T_0$ and $T_1$ while decreased in $T_2$, $T_3$ and $T_4$. 

Sachdeva and Singh (1990c) reported the chemical changes occurring in paneer treated with different dipping treatments 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 there was increase in titratable acidity, whereas, that in buffered water decrease in titratable acidity. A gradual rise in titratable acidity followed by a sharp increase towards the end of storage indicated the marked 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 moldy 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 was stored at 5°C, initial titratable acidity of control samples increased slowly from 0.54 per cent during storage and reached to 0.9 per cent on day 4 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. The mean acidity values were ranging from 0.45 to 0.74 per 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.

Boghra et al., (1997) studied the laboratory made paneer and simulated for approximate concentration of iron and copper based on market survey. The paneer was stored for 8 days at ≤ 10°C. The acidity of fresh control sample was 0.434 per cent which increased to 0.533 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 water and UV rays exposure) 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 reported that laboratory made paneer on storage at 7 ± 2°C showed increase in per cent lactic acidity from 0.49 to 0.524 per cent in $T_1$ and 0.556 per cent in $T_3$ on the day 12. The 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.

Venkateswarlu et al., (2003) studied the acidity of coconut milk paneer during storage at refrigeration with and without preservatives. The acidity of paneer preserved with 0.2 per cent $\text{H}_2\text{O}_{2}$, 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 respectively at the end of storage period. 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 temperature for 90 days. The titratable acidity of
the treated paneer was higher (0.29%) compared to control sample and it was due to the treatments it received.

2.4.2.2 **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. The FFA content increased during storage indicating lipolytic changes. The rate of increase in FFA was lower in samples containing TBHQ and BHA as compared to control.

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 samples of market paneer was 1.308.

Boghra *et al.*, (1997) studied the laboratory made paneer and simulated for approximate concentration of iron and copper based on market survey. The paneer was stored for 8 days at ≤ 10°C. The free fatty acid of fresh control sample was 0.28 µeq. per g, which increased to 0.59 µeq. per g.

Goel (2000) studied the laboratory made paneer and reported that the free fatty acid content of expose paneer increase from its initial value of 0.0665 to 0.1383 (T₁) and 0.209 (T₃) 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, whereas, on the day 2, with a value of 0.249 per cent pronounced rancid flavour was perceived at 22 ± 2°C.
The free fatty acid content 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$_2$O$_2$ 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 respectively at the end of the storage.

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

**2.4.2.3 Soluble nitrogen content of paneer**

Mistry (1988) prepared paneer samples from cow 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 (1990c) studied that chemical changes occurring in paneer treated with different dipping treatments 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 (1990c) also reported the soluble nitrogen content of samples treated with potassium sorbate and delvocid 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) prepared paneer by using standardized milk. The paneer cubes of approximately 1.5 cubic cm sizes were given the treatment of acidification, vacuum drying and salting. Then the cubes were packed in LDPE (90 micron) poly bags and stored at 8 ± 2°C temperature for 90 days. Soluble nitrogen content of fresh paneer was 0.02 per cent which increased to 0.12 per cent.

2.4.2.4 Oxidative deterioration of paneer

Pal et al., (1993) prepared low-fat paneer from 1:1 ratio of cow and buffalo milk, it 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, whereas, 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.

Boghra et al., (1997) studied the laboratory made paneer and simulated for approximate concentration of iron and copper based on market survey. The paneer was stored for 8 days at ≤ 10°C. The peroxide value (ml of 0.002 N Na$_2$S$_2$O$_3$/g) of fresh control sample was 0.0, which increased to 0.173.

2.5 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 100 g of which fat and protein individually contributes 218.5 to 247 and 73.1 to 77.4 kcal per 100 g respectively, i.e. around 95 per cent of total calories when values are 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.

2.6 Microbiological aspects

The microbiological quality of paneer depends mainly upon quality of milk, heat treatment, moisture content in paneer, degree of contamination, 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; Ghodekar, 1989). The type and number of microorganisms including yeast and mold and their distribution in the product, may vary depending upon the location of the 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).

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

Kohlon and Grover (1984) reported the incidences of staphylococci in paneer. 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 paneer made in Experimental Dairy Plant of NDRI, Karnal 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 (1990b) studied that total plate count of paneer treated with different dipping treatments 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 10th day and then showed a sharp increase to $165 \times 10^4$ on the 12th 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
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water was reduced markedly. Initial count of $237 \times 10^1$ decreased to $160 \times 10^1$ at the end of 8\textsuperscript{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\textsuperscript{th} day and increased thereafter reaching $95 \times 10^5$ on the 32\textsuperscript{nd} day of storage. The count further increased to $180 \times 10^5$ on the 34\textsuperscript{th} day of storage.

Kumar and Bector (1991) reported that there was progressive increase in the total plate count 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 count 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 count less than $10^3$ per g increased to $1.96 \times 10^5$ per g on day four and $2.0 \times 10^6$ per g on day seven during storage. The corresponding values for paneer sample 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 \textit{et al.}, (1993) prepared low-fat paneer from 1:1 ratio of cow and buffalo milk, it was coated with paraffin wax and stored at 10\textdegree C, together with non-waxed control paneer. Total plate count of control paneer sample showed the increase from $3.03 \pm 0.09$ to $6.27 \pm 0.07$ 15\textsuperscript{th} day storage, whereas, in paraffined sample the corresponding count were $3.12 \pm 0.06$ to $5.91 \pm 0.03 \log \text{cfu per g}$. 

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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. 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 within the prescribed BIS limit for SPC.

Goel (2000) studied the microbiological changes in market and laboratory made paneer samples during storage at 22 ± 2°C and 7 ± 2°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.

Vaishnavi et al., (2001) reported total plate count of 3 x 10^2 to 9.7 x 10^{10} cfu per g in the samples of paneer collected from Chandigarh market.

Venkateswarlu et al., (2003) studied the total count of coconut milk paneer during storage at refrigeration with and without preservatives. 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 (Maharastra) for microbiological quality. The average standard plate count of market samples of paneer were ranged from $1 \times 10^4$ to $224 \times 10^5$ cfu per g amongst the seventy samples, only four samples (5.7 per cent) met the BIS specification.

Gokhale and Pandya (2009) prepared paneer by using standardized milk. The paneer cubes of approximately 1.5 cubic cm sizes were given the treatment of acidification, vacuum drying and salting. Then the cubes were packed in LDPE (90 micron) poly bags and stored at $8 \pm 2^\circ C$ temperature for 90 days. Fresh paneer had total viable count of $12.54 \times 10^2$ per g which increased to 29-36 $\times 10^3$ per g within 90 days.

### 2.6.2 Coliform count

Gupta (1985) studied the presence of coliform in Experimental Dairy Plant of NDRI, Karnal made and laboratory prepared sample of paneer. The ranges of colifoms in paneer samples were $1.0 \times 10^1$ to $5.5 \times 10^3$ (average $2.7 \times 10^3$), count of Experimental Dairy Plant of NDRI, Karnal 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.

Visweshaaraiah and Ananthakrishnan (1985) while assessing the quality of 8 to 24 h old 54 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 Experimental Dairy Plant of NDRI,
Karnal 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 coliform 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 (1990b) 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 to 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 that the initial level of coliform count increased from 90 per g to $3.5 \times 10^3$ per g after four days and $8.0 \times 10^5$ per g after seven days of storage in control sample. 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 $4.9642 \text{ log } \text{cfu per g}$ among 18 producers. For four 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 $2.0 \leq 4.0$ and another 33.4 per cent sample had count even $\geq 4.0 \text{ log cfu per g}$.

Dhole *et al.*, (2009) evaluated the seventy samples of fresh paneer from seven vendors of Ahmednagar City (Maharastra) for microbiological quality. The average coliform count in the market
samples of paneer were found in the range of 12.6x 10^3 to 23.2 x 10^3 cfu per g. amongst the samples collected only two samples (2.8 per cent) fulfilled the BIS specification.

2.6.3 Yeast and mold count

Thakral (1986) studied the status of yeast in market, dairy and laboratory made paneer during storage at 37°C. When the product got spoiled, market paneer showed more increase in number of yeasts (3.1 x 10^4 cfu per g) as compared to dairy (6.0 x 10^3 cfu per g) and laboratory (3.0 x 10^1 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, the number increased to 1.0 x 10^1 cfu per g only after 2 day and reached a population of 3.1 x 10^3 cfu per g after 5 days of storage. After storage at a temperature of 5-7°C, no significant increase was observed in yeast and mold count of the laboratory prepared paneer.

According to Thakral (1986) the keeping quality for all the 3 types of paneer at 37°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.

Survival and growth pattern of artificially inoculated yeast culture viz., Saccharomyces cervisiae var ellipsoides-522 and Candida quilliermondii-3124 strains individually at two different levels 1.0 x 10^3 - 5.0 x 10^3 and 1.0 x 10^5 to 5.0 x 10^5 cfu per ml to paneer milk was studied by Thakral (1986). The growth and changes in number of yeast artificially inoculated showed that S. cervisia-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.

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

Kumar and Bector (1991) analyzed the yeast and mold count of control and antioxidants added paneer samples. The authors 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 mold 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 molds 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) than the molds (general mean 2.2363 log cfu per g). Yeast and mold count from 18 different producers indicated the count varying from 1.6990 to 5.1746 log cfu per g, showing highly significant difference in count. The mean of the count 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 count were significant. The general total yeast and mold 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 mold 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 with in the BIS prescribed limit for yeast and mold count.

Goel (2000) studied that the initial average yeast and mold count of laboratory made exposed paneer samples indicate lower count in samples exposed to superior to the outside environment at 22 ± 2°C. Market sample of paneer contained high initial count of 3.84 log cfu per g, which is much higher than the laboratory made paneer sample. On day 4, the yeast and mold count in market sample increased to 6.77 log cfu per g. A rapid growth in yeast and mold 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 mold 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 mold in the
market sample was very high as compared to the laboratory made samples.

Venkateswarlu et al., (2003) studied the yeast and mold count of coconut milk paneer during storage at refrigeration with and without preservatives. They found that yeast and mold count of coconut milk paneer preserved with \( \text{H}_2\text{O}_2 \), the log values of yeast and mold count 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 mold 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 (Maharastra) for microbiological quality. The yeast and mold count 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 4 (5.7 per cent) samples, met the standards prescribed by BIS for the yeast and mold count for paneer.

Gokhale and Pandya (2009) prepared paneer by using standardized milk. The paneer cubes of approximately 1.5 cubic cm sizes were given the treatment of acidification, vacuum drying and salting. Then the cubes were packed in LDPE (90 micron) poly bags and stored at \( 8 \pm 2 \degree C \) temperature for 90 days. The yeast and mold of fresh paneer samples were lower (73 cfu per g) and did not increase much (117 cfu per g).

2.7 Measures to improve shelf life of paneer

Paneer is rich in nutrients and there is also enough moisture content in the product to permit growth of variety of microorganisms.
The ambient storage conditions are generally favorable for their survival and rapid growth. Even though 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 very low 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. The author reported that the shelf life of paneer was about 6 days at 10°C storage, however, its freshness was lost in just 3 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 were tried to improve shelf life of paneer which 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.

### 2.7.1 Antimicrobial agents

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

Thakral (1986) studied the effect of 0.1 per cent potassium sorbate and 100 µg nisin on shelf life of paneer. The author found that the keeping quality of market and dairy paneer gets very much reduced due to the growth of yeasts and other organisms, depending upon the temperature of storage. It also indicates that if paneer is under clean and healthy conditions, it will be free from yeasts. If a small number of cells however, enter into the milk after heat treatment, they would grow and attain sufficient numbers in the final
product within a small period. The keeping quality of paneer can be further increased by the addition of potassium sorbate and nisin to it as these preservatives are known to inhibit several microorganisms. From the study author concluded that incorporation of 0.1 per cent potassium sorbate and nisin (100 µg) improved the shelf life of paneer to a great extent but this again depended upon the temperature of storage. The keeping quality of treated paneer was more at 5 -7°C as compared to 22°C, 30°C and 37°C.

Singh et al., (1989) added sorbic acid at the rate of 0.15 per cent to milk before preparing paneer. The paneer was subsequently wrapped with sorbic acid coated butter paper, which extended the shelf life up to 36 days on storage of paneer at 25-35°C. 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 (1990a) studied effect of dipping paneer samples in various antimicrobial agents and storage at 8-10°C. The comparison showed that control paneer 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, while 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.

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. Addition of the antioxidants in paneer markedly reduced the initial count 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. The
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study showed that the shelf-life of treated samples increased by two times as compared to control samples.

Shive Kumar and Mathur (1994) reported that paneer were manufactured from buffalo milk that had been preserved by the lactoperoxidase system (70:30 SCN-: H$_2$O$_2$) 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.

Nayak and Bector (1999) found that addition of H$_2$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 H$_2$O$_2$ added to milk appeared in paneer which decreased as storage period increased.

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.

Pashupati et al., (2007) reported that 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.

Krishna Kumari (2009) evaluated seven different spices viz., black pepper, cardamom, cinnamon, clove, garlic, ginger and onion for extending the shelf life of paneer. 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 the
relative effectiveness in enhancing shelf life of paneer is cardamom >
cinnamon > clove > black pepper.

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.

2.7.2 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.

2.7.3 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 autoclaving at a steam pressure of 15 psi for 15 min.
The paneer kept well over a period of 50 days under ambient temperature thereafter, the perception of a moldy character rendered the paneer organoleptically unacceptable.

2.7.4 Deep fat frying

Sachdeva (1983) 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.

2.7.5 Dipping of paneer in treated water

Sachdeva (1983) studied different materials known for their antimicrobial activity was 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 (35 ppm), (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 H₂O₂ and delvocid. It was found that 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 microorganisms 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 useful in hence the shelf life of paneer. Use of 5 per cent brine and 0.2 per cent \( \text{H}_2\text{O}_2 \) 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 8 h of refrigerated storage. However when delvocid was used in combination with germicide (\( \text{H}_2\text{O}_2 \)), excellent results was obtained as the paneer sample thus treated kept good for a period of about 32 days.

Sachdeva and Singh (1990a) 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 solution. 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 increased its shelf life but adversely affected the sensory attributes.

### 2.7.6 Packaging

Sweta et al., (2008) reported that packaging of paneer samples in high barrier bags (LLD/BA/Nylon-6/BA/LDPE) had significant influence on moisture, titratable acidity, \( \text{pH} \), free fatty acids and tyrosine content of the paneer during storage.

### 2.7.7 Low temperature

Arora and Gupta (1980) observed that the storage of paneer at sub zero temperature (-13 and -32°C) for 120 days the flavour and
appearance was not affected but its body and texture deteriorated. The product becomes crumbly and fluffy on thawing.

Visweshwaraiah (1987) had tried to extend 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.

Kanawjia, et al., (1990) reported that heat sterilization of paneer enhances its keeping quality to 4 months at room temperature. However, 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.

2.7.8 Other treatments

Mini et al., (1995) studied 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 that 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) had tried pickling of low fat paneer by using tomato and tamarind base with spices and oils at a ratio of 1:3
Oven dried pickled paneer samples scored significantly higher overall acceptability scores compared to oil fried pickled paneer samples.

### 2.7.9 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 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-irradiatiated 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) studied the application of hurdle technology for manufacture of paneer and found that paneer was stable for ≥14 days at 30°C. 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) prepared paneer by using standardized milk. The paneer cubes of approximately 1.5 cubic cm sizes were given the treatment of acidification, vacuum drying and salting. Then the cubes were packed in LDPE (90 micron) poly bags and stored at 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 mold 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 4th 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.

2.7.10 Trends toward use of natural ingredients

There has been increasing concern of consumers about food free of chemical preservatives because of their possible toxic effects in 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 required to ensure that manufactured foods remain safe and unspoiled (Rasooli, 2007).

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

2.8 Herbs

Herbs and spices 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 herbs and spices 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 herbs and spices are desirable food ingredients to create and explore new tasty products. Lewis (1984) reported that herbs and 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 herbs and 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 herbs and 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.

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

Table 2.1 Main constituents and botanical name of herbs

<table>
<thead>
<tr>
<th>Name</th>
<th>Botanical name</th>
<th>Main constituents of essential oil/oleoresin</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Herb/Spice</th>
<th>Scientific Name</th>
<th>Chemical Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajwain (Seed)</td>
<td><em>Carum Copticum</em></td>
<td>Thymol, γ-terpenene</td>
</tr>
<tr>
<td>Asafoetida (dried latex)</td>
<td><em>Ferula assafoetida</em></td>
<td>Ferulic acid, umbelliferone</td>
</tr>
<tr>
<td>Coriander (Fruit)</td>
<td><em>Coriandrum sativum</em></td>
<td>D-linalool, D-α-pinene, β-pinene</td>
</tr>
<tr>
<td>Cumin (Fruit)</td>
<td><em>Cuminum cyminum</em></td>
<td>Cuminaldehyde, β-pinene, cis-β-farnesene</td>
</tr>
<tr>
<td>Fenugreek (Fruit)</td>
<td><em>Trigonella Foenum-Graecum</em></td>
<td>Diosgenin</td>
</tr>
<tr>
<td>Mint (Leaf/terminal shoot)</td>
<td><em>Mentha piperita</em></td>
<td>α, β-pinene, 1,8-cineole, menthol, limonene</td>
</tr>
<tr>
<td>Turmeric (Rhizome)</td>
<td><em>Curcuma Longa</em></td>
<td>ar-Turmerone, turmeron, curcumin, demethoxycurcumin, bis-demethoxycurcumin</td>
</tr>
</tbody>
</table>

(Parthasarathy et al., 2008)

2.8.2 Antioxidant activity of herbs and spices

Antioxidants are an increasingly important ingredient in food processing, as they inhibit the development of oxidative rancidity in fat-based foods, particularly meat, dairy products and fried foods.

Usually synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate deterioration of food. Yet, these antioxidants suffer from the drawback that they are volatile and easily decompose at high temperatures. Additionally, it is still unclear whether chronic consumption can lead to health risks (Martinez-Tome et al., 2001). Many herbs and spices, usually used to flavour dishes, are an excellent source of phenolic compounds which have been reported to show good antioxidant activity (Rice-Evans, et al., 1996; Zheng and Wang, 2001). Therefore, they may serve as natural antioxidants for food in addition to their preservative effect.

Sage, rosemary, oregano, thyme, cilantro and marjoram are found to have stronger antioxidant properties than other spices. Rosemary and sage are currently used as natural antioxidants in foods,
while other spices such as cilantro are being explored. Rosmanol, caffeic acid, myristphenone, curcurmin, eugenol, thymol and sesaminol in rosemary, clove, thyme, oregano, ginger, turmeric, nutmeg, sage and sesame seed are found to be strong antioxidants with meat, lard and soybean oil (Raghavan, 2007).

Ramanadhan and Das (1993) have reported that the ginger and turmeric juices were more effective than the commonly used garlic and onion juices (at 10% concentration); in inhibiting lipid oxidation in NaCl pretreated cooked ground fish.

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.

Bandyopadhyay et al., (2008) observed that the use of natural sources of antioxidant viz., beet (Beta vulgaris), mint (Mentha spicata L.) and ginger (Zingiber officinale L.) to fortify sandesh. They were done three sets of experiments viz., antioxidant activity, peroxide value and ultra-violet absorbance to evaluate the effectiveness of natural antioxidants in reducing lipid oxidation in sandesh as compared to synthetic antioxidants like tertiary butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) under thermal treatment. They found that among the natural sources, ginger has the highest antioxidant activity and it was similar to TBHQ and BHA-BHT combined. Control sandesh (without any antioxidants) showed the highest peroxide value and ultra-violet absorption. All the natural sources and their combinations significantly improved the
oxidative stability of sandesh and their effectiveness was comparable with synthetic antioxidant TBHQ, a combination of BHA and BHT. Regarding antioxidant activity and lipid oxidation, combination of mint or ginger with beet showed better result as compared to beet alone. Besides, sensory evaluation of freshly prepared natural source fortified sandesh samples was done as compared to control sandesh in order to commercialize the herbal sandesh in market. Moreover, Sandesh containing beet, ginger, combination of beet with ginger or mint, or combination of mint with ginger were found more acceptable than control sandesh.

2.8.3 Antibacterial activity of herbs and spices

There have been relatively few studies of the antimicrobial action of essential oils in model food systems and in real foods. The efficacy of essential oils in vitro is often much greater than in vivo or in situ, i.e. in foods (Nychas and Tassou, 2000; Davidson, 1997). For example, the essential oil of mint (Mentha piperita) has been shown to inhibit the growth of Salmonella enteritidis and Listeria monocytogenes in culture media for 2 days at 30ºC. However, the effect of mint essential oil in the traditional Greek appetizers tzatziki (pH 4.5) and taramasalata (pH 5.0) and in paté (pH 6.8) at 4ºC and 10ºC was variable. Salmonella enteritidis died off in the appetizers under all conditions examined but not when inoculated in paté and maintained at 10ºC. Similarly, L. monocytogenes numbers declined in the appetizers but increased in paté (Tassou et al., 1995a, b, 2000).

In general, the mode of action of essential oils is concentration dependent (Prindle and Wright, 1977). Low concentrations inhibit enzymes associated with energy production while higher amounts may precipitate proteins. However, it is uncertain whether membrane damage is quantitatively related to the amount of active antimicrobial compound to which the cell is exposed, or the effect is such that, once
small injuries are caused, the breakdown of the cell follows (Judis, 1963).

Billing and Sherman (1998) compared the antimicrobial properties of 30 different herbs and summarized the antibacterial spectrum of each herb. All of the herbs evaluated inhibited the growth of some bacteria, 80% of herbs inhibited more than 50% of bacteria tested, 50% of herbs inhibited more than 75% of bacteria tested and 13% of herbs inhibited all of bacteria tested.

MICs of basil, clove, garlic, horseradish, marjoram, oregano, rosemary, thyme and turmeric were determined under several conditions by Yano et al., (2006). In the nutrient rich medium (Na-Hi broth), the lowest MIC at 30°C was 0.125% in clove and marjoram and at 5°C was 0.063% in marjoram and turmeric. In other spices and herbs, the MICs ranged from 0.5% to >2.0% at 30°C and 0.25% to >2.0% at 5°C. Reducing the incubation temperature produced little effect on the MICs of the spice and herbs except for turmeric, but the MIC of turmeric decreased from >2% at 30°C to 0.063% at 5°C.

The MICs of other spices and herbs were increased with the decreasing of temperature except for marjoram and turmeric and the increase may be due to the lower solubility of antibacterial substances such as terpenes at low temperature (Yano et al., 2006).

In the broth added turmeric, the growth was delayed at concentrations of 1% and 0.25% when incubated at 30°C but not stopped. On the other hand, the bacterial count decreased just after addition at concentrations of 1 and 0.25% and 3 h at a concentration of 0.063% when incubated at 5°C. In the present study also, the MIC of turmeric was more than 2% at 30°C (Yano et al., 2006).

2.8.3.1 Ajwain

The ajwain seed has been popular from ancient time for its use in folk medicines. In addition it has many uses like flavouring, culinary,
household and cosmetic. The entire plant has its herbal value in medicinal industry but commercially it is valued for its seed. Ajwain seeds have an aromatic smell and a warm pungent taste. They are used both as spices and condiment in many countries. They are used in India as a traditional spice in many foods, including curries. The major processed products are ajwain oil, oleoresin, thymol, thymol crystals, dethymolized oil (thymene) and fatty oils (Peter, 2004).

The antibacterial activity of homogenized seed oil and residues indicates that the powdered seeds of *C. copticum* exhibit antibacterial activity against *Staphylococcus aureus* only and not *E. coli*. The oil extracted with *n*-hexane exhibited antibacterial activity against both organisms, while the oil extracted with ethanol and *n*-hexane did not. The residue left after ethanol extraction exhibits antibacterial activity against *E. coli* only (Qasim and Khan, 2001).

The antimicrobial activity of ajwain oils against *A. niger, S. cerevisiae, Mycoderma* sp., *Lactobacillus acidophilus* and *B. cereus*, as estimated by the paper disc agar diffusion method, has been reported by Meena and Sethi (1994).

Among six fractions derived from ajwain had the strongest antifungal activity and inhibited the growth of *A. niger* and *C. albicans* at less than 20 µg/mL. Ajwain treated *A. niger* had several morphological changes including disappearance of surface ornaments, thinning of cell wall, detachment of cell membrane and cell wall, surface depression of hypha and destruction of cell organella. (Yoshida et al., 1987)

The antimicrobial activities of the essential oil distilled from ajwain seed was tested against a range of microorganisms such as *Lactobacillus acidophilus*, *Bacillus cereus*, *Saccharomyces cerevisiae*, *Mycoderma* sp. and *Aspergillus niger* by Meena and Sethi (1994). The authors found various degrees of inhibition against test organisms.
Mycoderma sp. was the most susceptible and Bacillus cereus was the most resistant. The susceptibility followed the order of B. cereus, L. acidophilus, S. cerevisiae, A. niger and Mycoderma sp., the greater antimicrobial activity was observed in oil of ajowan both at ambient temperature and 37°C. Alcoholic extracts of ajowan also exhibited potent antimicrobial effects inhibiting the growth of B. subtilis, Escherichia coli and S. cerevisiae.

2.8.3.2 Asafoetida

Asafoetida is the dried latex (oleogum) obtained from the rootstocks (or taproots) of certain species of Ferula such as F. asafoetida L., F. foetida Regel, F. alliacea Boiss, F. rubricaulis Boiss, Linn. and F. narthex Boiss. Ferula belongs to the family Apiaceae. It is also known as Devil’s dung, food of gods, asafetida, etc. Early records state that Alexander the Great carried this ‘stink finger’ to the West in 4 BC. It is also used as a flavouring agent in the kitchens of ancient Rome. This pungent, resinous gum is used widely in Indian vegetarian cooking (Morris and Mackley, 1999).

The whole plant exudes a strong characteristic smell. Several species of Ferula yield asafoetida. The bulk of the product comes from the official plant, F. asafoetida, which grows from 600 to 1200 m (2000–4000 feet) above sea level in Iran and Afghanistan. These high plains are arid in winter but are thickly covered in summer with a luxuriant growth of these plants. The cabbage-like folded heads are eaten raw by the local people (Peter, 2004).

Asafoetida has antibiotic and antimicrobial properties. Thyagaraja and Hosono (1996) studied the inhibition effect of asafoetida on Rhizopus sporus, Mucor dimorphosphorous, Penicillium commune and Fusarium solani.

Sitara et al., (2008) reported antifungal activity of asafoetida (Ferula asafoetida), black cumin seed (Nigella sativa), neem
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(Azadirachta indica) and mustard (Brassica campestris) oils against eight fungi viz., Aspergillus niger, A.flavus, Fusarium moniliforme, F. oxysporum, F. nivale, F. semitectum, Alternaria alternata, Drechslera hawiensis. They have compared results with a commercial fungicide (Ridomyl Gold MZ 68%WP). These essential oils were tested by agar diffusion plate method caused significant reduction in the growth of above mentioned fungi. Asafoetida and Nigella sativa oils possess a remarkable antifungal activity against all tested fungi. Asafoetida oil completely inhibited the growth of all tested fungi except A. flavus at 0.15% whereas A. flavus and A. niger at 0.1% showed moderate antifungal activity. Antifungal activity of asafoetida oil against Aspergillus niger and A. flavus have been also reported by Siddiqui et al., (1996).

Thyagaraja and Hosono (1996) assayed the ability of chilli, coriander, pepper, cumin and asafoetida to inhibit food spoilage molds (Rhizopus azygosporus, Mucor dimorphosphorous Penicillium commune, Fusarium solani). Asafoetida showed promising results in inhibiting the fungal growth.

2.8.3.3 Coriander

Coriander Coriandrum sativum L. is an important spice crop and occupies a prime position in flavouring substances. It was one of the first spice to be used as a common flavouring substance. The stem leaves and fruits all have a pleasant aromatic odour (Peter, 2004).

The inhibitory effects of the essential oils of coriander on the mycelial growth and ochratoxint production by A. ochraceus NRRL 3174 were studied by Basilico and Basilico (1999). Sage and coriander showed no important effect at any of the concentrations studied. Meena and Sethi (1994) also studied the potential of coriander oil in the control of A. niger, Saccharomyces cerevisiae, Mycoderma sp., L. acidophilus and Bacillus cereus.
Tolkunova (2002) investigated the influence of essential oils on microbiological indicators of meat products. The formulation of horsemint-fennel-coriander was found effective against Gram-positive microorganisms. Introducing protein to the incubation environment was found to have little effect on the antibacterial activity of the essential oil formulations. His study showed that the essential oil formulations exerted a bacteriostatic action on the development of mesophilic aerobic and facultative anaerobic bacteria in cooked sausages. Significant increases in the storage life of sausages in both natural and artificial casings were associated with the use of the essential oil formulations.

Delaquis et al., (2002) reported antimicrobial properties of distillates prepared from crude essential oils of dill, coriander, cilantro and eucalyptus. Their effectiveness against a range of microorganisms was verified alone and in combination. They found that distilled fractions of coriander essential oil were considerably more potent than the crude oil. Fraction 1, a mixture of α-pinene (89.4%) and amphene (8.5%), strongly inhibited all the test microorganisms such as, *Pseudomonas fragi, Escherichia coli O157:H7, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus* particularly the yeast (*Saccharomyces cerevisiae*). Fraction 2, a mixture of linalool (92.9), camphor (0.2) and β-myrcene (2.8) was less potent but effective against all strains.

Elgayyar et al., (2001) examined the effectiveness of cardamom, anise, basil, coriander, rosemary, parsley, dill and angelica essential oil for controlling the growth and survival of pathogenic and saprophytic microorganisms. The inhibitory property was observed incase of oregano, basil and coriander essential oil, which presented minimum lethal concentration (v/v) ranging between 8 and 50 ppm for
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*Pseudomonas aeruginosa, Staphylococcus aureus* and *Yersinia enterocolitica*.

### 2.8.3.4 Cumin

Cumin (*Cuminum cyminum*) (or *Jeerah*) is of the family Apiaceae, has been used as a spice since ancient times and is native to the eastern Mediterranean, extending to East India. Cumin seeds are used for their unique aroma (Weiss, 2002).

The essential oil of cumin exhibits strong antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*. Complete death time on exposure to cumin oil was 20, 180 and 90 min for *E. coli*, *S. aureus* and *L. monocytogenes*, respectively (Gachkar *et al*., 2007).

Singh and Upadhyay (1991) found that the essential oil of cumin seeds inhibited mycelial growth of *A. flavus* and *A. niger* completely at 3000 ppm, inhibition at 1000 ppm being 85–89%. The aldehyde fraction, separated using NaHSO₃ and HCl, contained only cuminaldehyde. This gave 100% inhibition of both fungi at 1000 ppm.

Farag *et al.*, (1989) found that the essential oils of cumin and other spices inhibited the total aflatoxin production of *A. parasiticus* at relatively low concentrations, although not as effectively as thyme oil.

Lawrence (1992) reported that cumin oil showed fungitoxic, fungicidal, antibacterial and larvicidal activity due to the cuminaldehyde content. The undiluted oil also has a distinct phytotoxic effect on mammals, but not due to the cuminaldehyde content. Marked antifungal activity is seen against the following fungi: *Penicillium notatum*, *Aspergillus niger*, *A. fumigatus*, *Microsporum canis* (Afifi *et al*., 1994), *Pseudallescheria boydii* and *A. flavus* (Atta-ur-Rahman *et al*., 1999). Cumin seed and/or callus extracts and essential oils inhibit bacteria (particularly *S. aureus*) and fungi (*Fusarium moniliforme*), as well as polio and Coxsackie viruses (Jain *et al*., 1992).
Ouattara et al., (1997) examined the inhibitory properties of cinnamon, clove, cumin, garlic, oregano, black pepper, pimento and rosemary essential oils against meat spoilage bacteria, *Carnobacterium piscicola*, *L. curvatus*, *L. sake*, *Brochothrix thermonphacta*, *P. fluorescens* and *Serratia liquefaciens*.

Among the 60 constituents of the cumin oil identified by GC, GC-MS and olfactometry as essential volatiles, cuminaldehyde (36%), β-pinene (19.3%), *p*-cymene (18.4%) and γ-terpinene (15.3%) are the principal components showing high antimicrobial activity against the mold *A. niger* the Gram-positive bacteria, *Bacillus subtilis* and *S. epidermidis*, as well as the yeasts, *Saccharomyces cerevisiae* and *Candida albicans* (Jirovetz et al., 2005).

Kivanç et al., (1991) studied the effect of herbs and spices on starter cultures (*Lactobacillus plantarum* and *Leuconostoc mesenteroides*) considering that lactic acid bacteria are relatively resistant to toxic effect of spices and derivatives (Jansen et al., 1987). Some spices have exerted stimulatory effect on these microorganisms resulting in enhanced acid production (Tiwari and Pandey, 1981). Cumin (0.5, 1.0 and 1.0% w/w) and its essential oil (150, 300 and 600ppm) stimulated the growth of *L. plantarum* and *L. mesenteroides* and acid production. Oregano was able to stimulate the growth of *L. plantarum* and acid production, however this behavior was not observed in *L. mesenteroides*.

Cinnamon, cloves and cumin showed the strongest antimicrobial effects against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Micrococcus luteus* and *Candida albicans* as test strains, with inhibition zones between <10 and >30 mm by the disc-diffusion method (Agaoglu et al., 2007).
Iacobellis et al., (2005) reported antibacterial activity of *C. cyminum* *L.* (Cumin) against Gram-positive and Gram-negative bacterial species. Singh et al., (2002) reported that the *C. cyminum* oil to be equally good or more effective when compared with standard antibiotics, at a very low concentration. Generally, *C. cyminum* oil exhibited stronger activity than did *R. officinalis* (Rosemary) oil. The essential oil from *R. officinalis* has been reported to be weakly inhibitory against *E. coli*, *S. aureus* and *L. monocytogenes* as compared to other oils (Lopez et al., 2005).

### 2.8.3.5 Fenugreek

Fenugreek, or methi (*Trigonella foenumgraecum* L.), belongs to the subfamily Papilionacae of the family Leguminosae (bean family, Fabaceae). The plant is an aromatic herbaceous annual, widely cultivated in Mediterranean countries and Asia (Leela and Shafeekh, 2008). It appears from the literature that antibacterial activity of fenugreek has not studied.

### 2.8.3.6 Mint

Many kinds of mints, Mentha species, grow wild in different parts of India and continue to be used widely as the traditional items of culinary or salad and herbal medicine. The commercial cultivation of mints as a source of essential oils is a relatively recent development. Because of the suitability of Indian agroclimates and soils for mint cultivation as an annual crop, India has become the major supplier of certain mint oil(s) to the international market (Kumar et al., 2007).

The genus Mentha clearly has marked antimicrobial characteristics across the spectrum from fungi and parasites, through bacteria, to viruses. The marked preservative action of numerous herbs
and spices has recently received increased attention. Included in this interest are peppermint and several research papers report on the ability of peppermint oil, oil constituents and other extracts to inhibit the growth of food poisoning bacteria. A study by Aktug and Karapinar (1986) demonstrated this effect with Salmonella typhi, Staph. aureus and Vibrio parahaemolyticus.

Mint has antibacterial properties (Raghavan, 2007). Mint oil was also effective at inhibiting the growth of the pathogenic fungi Pythium sp. and Fusarium sulphureum and in greenhouse experiments, the oil (at concentrations of 0.5% and 1.0%) proved efficient at limiting the spread of cucumber damping off (Klimach and Wieczorek, 1996).

An earlier study into the antimycotic properties of peppermint oil (Conner and Beuchat, 1984) demonstrated activity against a number of food spoilage yeasts. Inhibition zones around a 5-cm disk were quoted for Geotrichum candidum (so called “dairy yeast” due to its high incidence in dairy plants).

Tassou et al., (1995) studied synergism of mint essential oil (0.5, 1.0, 1.5 and 2.0% w/w) and pH against L. monocytogenes and S. enteritidis in food model system at two different storage temperatures (4 and 10ºC). Mint essential oil exhibited the most antibacterial effect in low pH system (tzatziki pH 4.5) followed by relatively less acidic food (taramosalata pH 5.0). However, no antimicrobial activity was observed in a food system with neutral pH (pate pH 6.8) (Tassou et al., 1995). In general, S. enteritidis was more susceptible than L. monocytogenes and the difference was higher at 10ºC than 4ºC. Antimicrobial activity of mint essential oil increased with the increase in the amount of essential oil.

Basilico and Basilico (1999) reported that at 1000 ppm essential oils of oregano and mint inhibited the growth of A. ochraceus and ochratoxin a production in YES broth up to 21 days.
Mint (Mentha piperita) EO was more effective against *S. enteritidis* compared to *L. monocytogenes* when added to the Greek appetizers taramosalata and tzatziki. In another study, using freshly distilled EOs showed more susceptibility to Gram-positive bacteria compared to Gram-negative (Burt, 2004).

### 2.8.3.7 Turmeric

Turmeric, *Curcuma longa* L. (Zingiberaceae), has been attributed a number of medicinal properties in the traditional system of medicine for treating several common ailments (Raghunath and Mitra, 1982). It belongs to the genus *Curcuma*, which consists of several plant species with underground rhizomes and roots. About 40 species of the genus are indigenous to India, indicating the Indian origin (Velayudhan et al., 1999). Originally, it had been used as a food additive to improve the palatability, storage and preservation of food.

Curcuminoids have also been shown to exhibit antimicrobial properties. The antibacterial effects of alcoholic extract of turmeric, curcumin and oil from turmeric have been studied by Banerjee and Nigam (1978) and Bhavanishankar and Srinivasamurthy (1979). Extracts from turmeric, as well as the active principles, the curcuminoids, were found to inhibit the growth of numerous Gram-positive and Gram-negative bacteria, fungi and the intestinal parasite, *Entamoeba histolytica*. The ethanol extract of turmeric has been reported to have anti-amoebic activity against *E. histolytica in vitro*. Curcumin at concentrations of 2.5–50.0 mg/100 ml inhibited *in vitro* growth of *Staphylococcus aureus* (Bhavani Shankar and Srinivasamurthy, 1979). Interestingly, the antibacterial and antiviral activities of curcumin were enhanced significantly by illumination with visible light (Tonnesen et al., 1987; Dahl et al., 1989). Curcumin also inhibits *in vitro* production of aflatoxins – toxins produced by the mold *Aspergillus parasiticus*, which may grow and contaminate poorly
preserved foods and is a potent biological agent causing injury to the liver, often resulting in liver cancer (Madhyastha and Bhat, 1985; Jayaprakasha et al., 2001).

Turmeric (Curcuma longa), Ginger (Zingiber officinale), galangal (Alpinia galanga) and fingerroot (Boesenbergia pandurata) extracts effective against Gram-positive and Gram-negative pathogenic bacteria at 0.2–0.4% (v/v) for fingerroot and 8–10% (v/v) for all of the spices (Pattaratanawadee et al., 2006).

Curcumin also inhibits in vitro production of aflatoxins – toxins produced by the mold Aspergillus parasiticus, which may grow and contaminate poorly preserved foods and is a potent biological agent causing injury to the liver, often resulting in liver cancer (Madhyastha and Bhat, 1985; Polasa et al., 1992; Jayaprakasha et al., 2001).

Weerasekera et al., (2006) the bactericidal activity of 21 plant extracts on Helicobacter pylori was investigated. Plants were boiled in water to produce aqueous extracts. Bactericidal activity of the extracts was assessed by a standard kill-curve using five strains of H. pylori isolated from Sri Lanka and the NCTC 11637 strain. Among the plants that showed bactericidal activity for H. pylori, turmeric and ginger were the most efficient followed by chilli and black tea. Nutmeg, liquorice, cinnamon, colombo weed, yellow-berried nightshade, threadstem carpetweed, sage, parsley, long pepper and cumin also showed bactericidal activity against H. pylori. These could serve as potent alternative therapies for H. pylori infection, avoiding the problem of resistance associated with current antibiotic treatment.

2.8.4 Herbs for preservation of food products

Antimicrobials are used in food for two main reasons: (1) to control natural spoilage processes (food preservation) and (2) to prevent/control growth of micro-organisms, including pathogenic micro-organisms (food safety) (Tajkarimi et al., 2010).
Herbs and 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). They also stimulate appetite by increasing salivation and carminative action. However, they also preserve the food by their antimicrobial and antioxidant properties (Lewis 1984). Many plant-derived antimicrobial compounds have a wide spectrum of activity against bacteria, fungi and mycobacteria and this has led to suggestions that they could be used as natural preservatives in foods (Farag et al., 1989; Conner and Beuchat, 1984). Although more than 1300 plants have been reported as potential sources of antimicrobial agents (Wilkins and Board, 1989), such alternative compounds have not been sufficiently exploited in foods to date. More than 400 herbs and spices are used in the different countries in the world. Herbs and spices have been used for preventing food spoilage and deterioration and for extending shelf-life of food, as well (Nakatani, 1994).

Since ancient times, herbs and spices have not been consciously added to foods as preservatives but mainly as seasoning additives due to their aromatic properties. Although the majority of essential oils from herbs and spices are classified as Generally Recognized As Safe (GRAS). However, their use in foods as preservatives is limited because of flavour considerations, since effective antimicrobial doses may exceed organoleptically acceptable levels (Kabara and Eklund, 1991).

Akgul and Kivanç (1988) studied antifungal activity of selected Turkish spices (black cumin, coriander, cumin, dill, laurel, oregano, parsley, spearmint, white mustard) on some food borne fungi and found that oregano ground (1.0, 1.5, 2.0 % w/v) and its essential oil (0.05%, 0.025%) showed inhibitory effect on Aspergillus flavus, A. niger, Geotrichum candidum, Mucor spp., Penicillium roqueforti and oregano essential oil exhibited higher inhibitory effect than sorbic acid.
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. Lipolytic activity and total microbial and lipolytic bacterial count were also measured. During butter storage, there was very little change in the peroxide and TBA values but a gradual increase in the acid value. The addition of cumin and thyme oils at 200 mg/kg to butter caused very little increase in the acid value. The data for lipolytic bacterial count were in general agreement with the acid values. Thyme and cumin essential oils showed a greater anti-hydrolytic effect and were better preservatives than butylated hydroxytoluene.

Tassou et al., (1995) reported that mint oil at 5–20 μl g⁻¹ was effective against S. enteritidis in low fat yoghurt and cucumber salad. In butter cakes containing 13.1% moisture and 38% crude fat, turmeric has shown an important antimycotic activity (Lean and Mohamed, 1999).

Al-Jedah et al., (2000) carried out the study evaluate action of combined spices, including cumin (Cuminum cyminum), coriander (Coriandrum sativum), mustard (Brassica juncea), black pepper (Pipper nigrum) and lemon (Citrus aurantifolia) on V. parahaemolyticus, S. aureus, S. typhi and E. coli count in fish sauce. The study showed that the spices mixtures were able to exert static effect on all assayed bacteria when in interaction with initial inoculums of 1.0 x 10⁴ CFU/mL, except on S. typhi.

Kavita Shukla (2001) invented the preservation of perishable substances such as fruits and vegetable, meat products, dairy products, edible substances, non-edible substances and other perishable substances by coating of fenugreek treated paper. One strawberry as wrapped in fenugreek treated paper and kept in an
incubator at 25°C for 120 hours. A different strawberry was wrapped in soft paper soaked in distilled water and dried overnight. This sample was also placed in an incubator at 25°C for 120 hours. Observations every 12 hours showed that the strawberry wrapped in the water soaked paper showed significant signs of spoilage after 24 hours while the strawberry soaked in the fenugreek coated paper showed no signs of spoilage until after 120 hours or a longer period of time. Signs of spoilage included growth of bacteria and fungi on the surface of the fruit and loss of natural, fresh colour of the fruits.

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 unbalanced 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.

Burt (2004) reported that essential oils of coriander, clove, oregano and thyme showed high effect against L. monocytogenes,
Aeromonas hydrophila and autochthonous spoilage flora in meat products. A 5–20 μl g\(^{-1}\) level of coriander, clove, oregano and thyme oil inhibits growth of L. monocytogenes, Aeromonas hydrophila and autochthonous spoilage flora in meat products. However, mustard, cilantro, mint and sage oils were less effective or ineffective.

Study carried out by Paramasivam et al., (2007) dealt with the hampering of the growth of histamine producing bacteria (HPB), by using NaCl and spices. Four strains of HPB viz., Vibrio parahaemolyticus, Bacillus cereus, Pseudomonas aeruginosa and Proteus mirabilis were tested against 1 to 10% concentrations of NaCl and 1 to 5% concentrations of natural preservatives (turmeric, ginger and garlic) in a basal medium. HPB showed different growth rates at different concentrations of NaCl and natural preservatives. V. parahaemolyticus, B. cereus and Ps. aeruginosa showed no growth at 10% concentration. When the HPB growth was tested with garlic, turmeric and ginger extracts, growth of all the bacteria was inhibited by garlic and turmeric extracts at 5% concentration.

Khusniati et al., (2008) investigated antibacterial effects of aromatic materials on the preservation of stored milk produced in Indonesia. Organoleptic evaluation, bacterial growth, protease activities, lipase activities, protein degradation and acidities of milk with addition of 10% aromatic materials were assessed by panelists, total count, azocasein method, modified dole extraction, formol and base-acid titrations, respectively. They found that at 5 days, after the expiry date, 19 of the 28 aromatic milk were better than control and 10 of these were better than the others, while the aromatic whole milk were shown to be better than the skimmed milk. These 10 were the ones with the additions of honey, cinnamon, ginger, turmeric, zingiber, wild ginger, nutmeg, pepper, garlic and galangale.
CHAPTER: III
MATERIALS AND METHODS

This chapter covers details on the manufacturing of paneer, selection of best herbs and rate of addition of herbs 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 is given.

3.1 Collection of raw materials

For preparation of paneer milk, citric acid and herbs 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 Herbs

The below listed seven herbs were collected of Agmark standard company from local market of Anand city from a retailer.

1. Ajwain
2. Asafoetid
3. Coriande
4. Cumin
5 Fenugreek
6 Mint
7 Turmeric

3.1.3 Packaging material
Composite polyethylene terephthalate (PET)/low density polyethylene (LDPE) film (50 μ thickness) was used for packaging of samples during storage study.

3.1.4 Chemicals
All the chemicals used in the study were of analytical grade.

3.2 Collection of herbs for paneer
The dry Ajwain, Coriander, Cumin, Fenugreek and Mint were obtained from the local market, grinded using grinder mixture (Maharaja Make) and fine powder was obtained by using sieve. Asafoetida and Turmeric of AGMARK Grade from Ramdev brand were obtained in powder form from the local market.

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

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 subsequently 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 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 herbs in paneer

The herbs were incorporated in the product at the stage after removal of whey and before pressing the curd. The prepared sample of herb 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 herbs

For selecting herbs, the seven different herbs 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 compatible herbs

For selecting rate of addition of each herb the curd was divided in to six equal parts and the herb 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 herb. The prepared samples of
paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale.

3.8 Screening of the herbs

In screening of herbs in paneer the curd was divided in to eight equal parts and the selected four herbs were incorporated in the product at the selected rate (Ajwain 0.4%, Coriander 0.6%, Cumin 0.4%, Turmeric 0.4%). In one of the sample of paneer none of the herb 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 one month. The prepared samples of paneer were subjected to sensory evaluation by panel of judges when fresh and after interval of every 3 days (except first interval which was of six days) of storage until not spoiled.

3.9 Stage of addition

For selection of stage of addition of turmeric two different stages were selected for the study, viz. before heat treatment of milk and after heat treatment of milk. The require quantity of turmeric was added in the milk at appropriate stage. Rest of the operation remains same as describe under 3.4. Total seven replications were conducted.

3.10 Final testing of the selected herb (Turmeric)

In final testing of turmeric for their suitability in paneer turmeric was added at two different rates (0.4 and 0.6%). The samples of paneer without addition of turmeric were prepared as control samples. Total six 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 15 days. The prepared samples of paneer were subjected
to sensory evaluation by panel of judges when fresh and at regular interval of 3 days (except first interval which was of six days) during storage. The prepared samples of paneer were also analyzed for acidity, free fatty acid, soluble nitrogen and peroxide value using the methods as described under 3.13.1, 3.13.2, 3.13.3 and 3.13.4 respectively.

3.11 Evaluation of turmeric for its effect on microbial counts in paneer
The prepared samples of fresh and stored paneer containing turmeric were also analyzed for microbial counts viz. standard plate count, coliform count and yeast and mold count at regular interval of 6 days.

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).

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

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

Where,
- \( W \) = Weight in g of the empty dish
- \( W_1 \) = Weight in g of the dish with paneer sample before drying
- \( W_2 \) = Weight in g of the dish with paneer sample after drying.

3.13.2 Determination of fat

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

Exactly 3 g of paneer was weighed and 10 ml of \( \text{H}_2\text{SO}_4 \) (sp. gr. 1.820 – 1.825 at 20°C) and 1 ml of iso amyl alcohol (Sp.gr.0.815 – 0.825 at 20°C) were added in cheese butyrometer. 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°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 g) was transferred into 800 ml digestion flask. To this 25 ml of concentrated \( \text{H}_2\text{SO}_4 \) and 10 g 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 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:

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

Where,

$V = \text{Volume of 0.1 N H}_2\text{SO}_4 \text{ required for titration}$

$W = \text{Weight in g of the sample}$

$\text{Protein (w/w)} = \text{Total nitrogen (w/w)} \times 6.38$

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 5 g 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 550ºC 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:
Materials & methods

Total ash (%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 as described below.

3.14.1 Determination of acidity

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

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

Calculation of acidity:

\[\text{Acidity (\% lactic acid by weight) = } \frac{9 AN}{W}\]

Where,

\(A\) = Volume in ml of standard acid used in titration
\(N\) = Normality of standard NaOH
\(W\) = Weight in g of sample taken for the test

3.14.2 Determination of free fatty acids
Materials & methods

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

Calculation of free fatty acids:

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

Where,

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

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.

About 3 g of paneer was ground into a smooth paste warm (50°C) with 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 \(\text{H}_2\text{SO}_4\)

### 3.14.4 Determination of peroxide value

The peroxide value of fresh and stored paneer sample was determined by the method (iodometric method) as described by AOAC (1990).

Three gram of paneer (paste) sample was soaked in 30 ml chloroform for 12 h in a stoppered flask. The chloroform extract was filtered through Whatman filter paper no.1 to a 150 X 25 mm test tube and 1 g of potassium iodide and 10 ml glacial acetic acid were added. The tube was heated to boil for not more than 30 sec in a boiling water bath. The test tube was transferred to a 250 ml conical flask containing 25 ml of freshly prepared 5 per cent potassium iodide solution. The test tube was rinsed well with about 25 ml of distilled water and all washings were transferred to the above flask. The contents were titrated against 0.002 N sodium thiosulphate solution using 1 per cent 1 ml of starch indicator, near to end point. A blank was also performed.

The peroxide value of paneer was calculated as milliequivalents of peroxide per kg of paneer.

Calculation of peroxide value:

\[
\text{Peroxide value \,(milli\,equivalents\,of\,peroxide/kg\,of\,sample) = \frac{2T}{W}}
\]

Where,

- \(T\) = Volume in milliliters of 0.002 N sodium thiosulphate

and
3.15 Analysis of paneer for microbial counts

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

3.15.1 Preparation of sample

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

Approximately 11 g of the paneer sample was weighed aseptically in a sterile 100 ml 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) 99 ml of 2% sterile diluents and the contents transferred to the same conical flask, to obtain first dilution (1:10).

Further dilutions were prepared using 9 ml quantity of citrate buffer 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 diluent 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% 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 coliform 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.
Paneer is a heat-acid coagulated milk product. It is extensively used for preparation of a large number of culinary dishes. About 4–5% of the total milk produced in India is converted into paneer. It is an intermediate moisture food, a highly favorable medium for the growth of various microorganisms. Paneer shows signs of deteriorations after one day of storage under ordinary condition. 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.

The shelf life of any product can be extended by use of an appropriate food additive.

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. Herbs and 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). They also stimulate appetite by increasing salivation and carminative action. However, they also preserve the food by their antimicrobial and antioxidant properties (Lewis, 1984).

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 ajwain, asafoetida, coriander, cumin, fenugreek, mint and turmeric for this purpose. This study is a step in this direction.
and is aimed at evaluating suitability of selected herbs to extend the shelf life of paneer. Recently work was carried out by Krishna (2009) to evaluate suitability of various spices in preservation of paneer. In this study highly encouraging results were obtained for some of the spices. Since some herbs are also invariably used along with paneer for culinary purpose and these herbs are also reported to have preservative effect. It was felt appropriate to complete the line of work for comprehensive suggestion in use of these natural preservatives for use in paneer. Therefore, present study is suggested to complete the goal in this direction.

The study was divided into five phases. In the first phase compatibility of herbs in the paneer was tested. In the second phase, selection for rate of addition of the herbs was studied. In third phase screening of the herbs was carried out. In fourth phase stage of addition of the herb was studied. In final phase effectiveness of the selected herb(s) was investigated.

4.1 Evaluation of herbs for their compatibility in paneer

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 herbs and spices are available. These are used in small amounts and their contribution to nutrient intake is very limited. Some of the herbs and spices are rich in iron, trace metals and potassium.

In selection of herbs and spices for enhancing the shelf life of paneer the most important point for consideration was the compatibility of the herbs/spices for use in the paneer. Therefore, from an array of available herb, seven different herbs viz., ajwain (seed), asafoetida (dried latex), coriander (fruit), cumin (fruit), fenugreek (fruit), mint (dried leaves), turmeric (rhizomes) were selected for the study. All the selected herbs are commonly used in our day to day
Results and discussion

food that we commonly consumed. These herbs are also especially added during various culinary preparations made from paneer.

In the present study these herbs were to be incorporated in paneer itself. Therefore, compatibility of the added herbs in the paneer necessitated some preliminary work for their selection. For evaluation of compatibility in paneer, the seven different herbs were incorporated at the rate of 0.5 per cent. For incorporation of the herbs paneer was prepared and divided into 8 parts. The required quantity of individual herb in powder form was added to fresh sample of paneer and mixed thoroughly with the help of sterile spatula. The paneer without addition of any herb was also given the similar treatment of mixing used as a control sample. The samples of paneer (Plate I) were subjected to sensory evaluation by panel of judges for acceptability. Three replications were conducted for evaluating compatibility of herbs in paneer. The average result of the acceptability is presented in the Table 4.1.

<table>
<thead>
<tr>
<th>Herb added</th>
<th>Judge No.</th>
<th>Average acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>Ajwain</td>
<td>+ - + + + + + - +</td>
<td>07</td>
</tr>
<tr>
<td>Asafoetida</td>
<td>- + - - - + + - -</td>
<td>03</td>
</tr>
<tr>
<td>Coriander</td>
<td>+ + + + + + + + +</td>
<td>10</td>
</tr>
<tr>
<td>Cumin</td>
<td>+ + + + + + + + +</td>
<td>10</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>- + + + - - - - -</td>
<td>03</td>
</tr>
<tr>
<td>Mint</td>
<td>+ - + - - - - -</td>
<td>03</td>
</tr>
<tr>
<td>Turmeric</td>
<td>+ + + + + + + + +</td>
<td>10</td>
</tr>
</tbody>
</table>

+ = Acceptable, - = Unacceptable
Results and discussion

Figure 4.1: Effect of addition of herbs on acceptability of paneer in sensory evaluation

The results indicated that the samples of paneer containing ajwain, coriander, cumin and turmeric were acceptable in organoleptic evaluation, whereas, the samples of paneer containing asafoetida, fenugreek and mint were found unacceptable in organoleptic evaluation. Thus, ajwain, coriander, cumin and turmeric were found compatible in paneer and selected for further study.

Ajwain, coriander, cumin and turmeric are reported to have antimicrobial properties. The majority of these antimicrobial components are phenol compounds with hydroxyl group(s).

Meena and Sethi (1994) reported the antimicrobial activities of the essential oil distilled from ajwain seed against a range of microorganisms. The susceptibility followed the order of *B. cereus*, *L. acidophilus*, *S. cerevisiae*, *A. niger* and *Mycoderma* sp. Elgayyar et al., (2001) reported that coriander essential oils showed inhibition against *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O:157:H7, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*,
Results and discussion

Lactobacillus plantarum, A. niger, Geotrichum and Rhodotorula. Coriander and basil were also highly inhibitory (MLC, 25 to 50 ppm) to E. coli O: 157: H7 and to the other bacteria and fungi. Iacobellis et al., (2005) reported the antibacterial activity of C. cyminum L. (Cumin) against Gram-positive and Gram-negative bacterial species. The essential oil of cumin exhibits strong antimicrobial activity against Escherichia coli, Staphylococcus aureus and Listeria monocytogenes (Gachkar et al., 2007). Bele et al., (2009) reported that crude extract of Punica granatum L. (pomegranate) and Curcuma longa (turmeric) showed antibacterial activity against different strains of Gram-positive such as Staphylococcus aureus, Bacillus subtilis and Gram-negative microorganisms such as Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris and Enterobacter aerogens.

In addition to compatibility, acceptability and antimicrobial properties of the herbs and spices also have several health benefits. Many health benefits 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 herbs and spices. Among these, the hypocholesterolemic and antioxidant properties of a few specific herbs have far-reaching nutraceutical value.

These beneficial physiological effects also have the potential of possible therapeutic application in a variety of disease conditions. Wide range of non-nutrient bioactive and phytochemicals such as flavonoids and other phenolics are also found in herbs and spices (Srinivasan 2005).
4.2 Selection of rate for addition of compatible herbs in paneer

The second phase of the study was carried out to decide rate of addition of four selected herbs viz., ajwain, coriander, cumin and turmeric in paneer.

For selecting rate of addition of each herb the coagulum obtained by removal of 90 per cent whey, was divided into 6 equal parts and the herb 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 herb was used as a control. Each block of paneer was cut into rectangular pieces of approximately 1cm x 2cm.

The prepared samples of paneer were subjected to sensory evaluation by panel of seven judges using 9 point hedonic scale. Three replications were conducted for each selected herb. The results obtained for sensory evaluation of samples of paneer added with the selected herbs at different rates are presented below.

4.2.1 Ajwain

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

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 ajwain was added at different rates.
The changes in flavour score presented in Figure 4.2 revealed that the score declined slightly on addition of ajwain up to 0.4 per cent. However, further addition resulted into sharp decline in the flavour score.

### Table 4.2: Effect of rate of addition of ajwain on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Sensory attribute</th>
<th>Flavour</th>
<th>Colour &amp; appearance</th>
<th>Body &amp; texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td>7.75</td>
<td>8.29</td>
<td>7.96</td>
<td>7.84</td>
</tr>
<tr>
<td>0.2</td>
<td></td>
<td>7.74</td>
<td>8.08</td>
<td>7.69</td>
<td>7.70</td>
</tr>
<tr>
<td>0.4</td>
<td></td>
<td>7.63</td>
<td>7.79</td>
<td>7.51</td>
<td>7.56</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>7.33</td>
<td>7.30</td>
<td>7.11</td>
<td>7.26</td>
</tr>
<tr>
<td>0.8</td>
<td></td>
<td>6.55</td>
<td>6.69</td>
<td>6.55</td>
<td>6.78</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>6.00</td>
<td>6.48</td>
<td>6.31</td>
<td>6.26</td>
</tr>
</tbody>
</table>

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Test</th>
<th>S. Em</th>
<th>C. D</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.167</td>
<td>0.516</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>0.142</td>
<td>0.437</td>
<td>3.30</td>
</tr>
<tr>
<td></td>
<td>0.187</td>
<td>0.576</td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td>0.140</td>
<td>0.431</td>
<td>3.35</td>
</tr>
</tbody>
</table>
Results and discussion

Figure 4.2: Effect of rate of addition of ajwain on sensory score of paneer

4.2.1.2 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 ajwain was added at different rates. The changes in colour and appearance score presented in Figure 4.2 revealed that there was a slight decline in the colour and appearance score of paneer on addition of ajwain up to 0.4 per cent. However, further addition resulted into sharp decline in the colour and appearance score.

4.2.1.3 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 ajwain was added at different rates. The changes in body and texture score presented in Figure 4.2 revealed that the
score declined slightly on addition of ajwain up to 0.4 per cent. However, further addition resulted into sharp decline in the body and texture score.

### 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 ajwain was added at different rates. The changes in overall acceptability score presented in Figure 4.2 revealed that the score declined gradually on addition of ajwain up to 0.4 per cent. However, further addition resulted into sharp decline in the overall acceptability score of the paneer. However, no data are reported in the literature for effect of added ajwain on sensory attributes of paneer.

### 4.2.2 Coriander

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of coriander 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 coriander on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Flavour</th>
<th>Colour &amp; appearance</th>
<th>Body &amp; texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.69</td>
<td>8.12</td>
<td>7.79</td>
<td>8.01</td>
</tr>
<tr>
<td>0.2</td>
<td>7.57</td>
<td>7.88</td>
<td>7.49</td>
<td>7.71</td>
</tr>
<tr>
<td>0.4</td>
<td>7.65</td>
<td>7.70</td>
<td>7.36</td>
<td>7.68</td>
</tr>
<tr>
<td>0.6</td>
<td>7.66</td>
<td>7.19</td>
<td>7.17</td>
<td>7.67</td>
</tr>
<tr>
<td>0.8</td>
<td>7.02</td>
<td>6.61</td>
<td>6.77</td>
<td>6.75</td>
</tr>
<tr>
<td>1.0</td>
<td>6.69</td>
<td>6.32</td>
<td>6.49</td>
<td>6.37</td>
</tr>
</tbody>
</table>

ANOVA TABLE

| S. Em | 0.141 | 0.141 | 0.204 | 0.167 |

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Results and discussion

<table>
<thead>
<tr>
<th>C. D</th>
<th>0.435</th>
<th>0.435</th>
<th>0.628</th>
<th>0.514</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test *</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>C V %</td>
<td>3.35</td>
<td>3.35</td>
<td>4.92</td>
<td>3.92</td>
</tr>
</tbody>
</table>

Figure 4.3: Effect of rate of addition of coriander on 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 coriander was added at different rates. The changes in flavour score presented in Figure 4.3 revealed that the score declined slightly on addition of coriander up to 0.6 per cent. However, further addition resulted into sharp decline in the flavour score.

4.2.2.2 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 coriander was added at different rates. The changes in
Results and discussion

colour and appearance score presented in Figure 4.3 revealed that the score declined slightly on addition of coriander up to 0.4 per cent. However, further addition resulted into sharp decline in the colour and appearance score.

4.2.2.3 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 coriander 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 coriander up to 0.6 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 coriander 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 coriander 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 coriander seed powder on sensory attributes of paneer.

4.2.3 Cumin

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

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 cumin was added at different rates. The changes in flavour score presented in Figure 4.4 revealed that the score declined slightly on addition of cumin at 0.2 per cent and improved on addition of 0.4 per cent. However, further addition resulted into sharp decline in the flavour score.

### 4.2.3.2 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 cumin was added at different rates. The changes in colour and appearance score presented in Figure 4.4 revealed that the score declined gradually on addition of cumin up to 0.4 per cent. However, further addition resulted into sharp decline in the colour and appearance score.

#### Table 4.4: Effect of rate of addition of cumin on sensory score of paneer

<table>
<thead>
<tr>
<th>Rate of addition (%)</th>
<th>Flavour</th>
<th>Colour &amp; Appearance</th>
<th>Body &amp; Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.36</td>
<td>8.21</td>
<td>7.67</td>
<td>7.69</td>
</tr>
<tr>
<td>0.2</td>
<td>7.54</td>
<td>7.76</td>
<td>7.46</td>
<td>7.45</td>
</tr>
<tr>
<td>0.4</td>
<td>7.84</td>
<td>7.67</td>
<td>7.39</td>
<td>7.74</td>
</tr>
<tr>
<td>0.6</td>
<td>7.62</td>
<td>7.17</td>
<td>7.21</td>
<td>6.94</td>
</tr>
</tbody>
</table>
4.2.3.3 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 cumin was added at different rates. The changes in body and texture score presented in Figure 4.4 revealed that the score declined slightly on addition of cumin at 0.4 per cent. However, further addition resulted into sharp decline in the body and texture score.

4.2.3.4 Overall Acceptability
Results and discussion

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 cumin was added at different rates. The changes in overall acceptability score presented in Figure 4.4 revealed that the score declined slightly on addition of cumin at 0.2 per cent and improved on addition of 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 cumin on sensory attributes of paneer.

4.2.4 Turmeric

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

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 turmeric was added at different rates. The changes in flavour score presented in Figure 4.5 revealed that the score declined slightly on addition of turmeric at 0.2 per cent and improved on addition of 0.4 per cent. However, further addition resulted into sharp decline in the flavour score.

Table 4.5: Effect of rate of addition of turmeric 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>Colour &amp; Appearance</td>
</tr>
<tr>
<td>79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results and discussion

<table>
<thead>
<tr>
<th>(%)</th>
<th>7.93</th>
<th>8.17</th>
<th>7.91</th>
<th>8.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>7.80</td>
<td>8.00</td>
<td>7.71</td>
<td>7.94</td>
</tr>
<tr>
<td>0.2</td>
<td>7.91</td>
<td>7.72</td>
<td>7.43</td>
<td>7.76</td>
</tr>
<tr>
<td>0.4</td>
<td>7.22</td>
<td>6.79</td>
<td>6.95</td>
<td>6.99</td>
</tr>
<tr>
<td>0.6</td>
<td>6.29</td>
<td>6.21</td>
<td>6.64</td>
<td>6.24</td>
</tr>
<tr>
<td>0.8</td>
<td>5.90</td>
<td>6.05</td>
<td>6.45</td>
<td>5.95</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th></th>
<th>0.150</th>
<th>0.238</th>
<th>0.180</th>
<th>0.140</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.462</td>
<td>0.732</td>
<td>0.554</td>
<td>0.433</td>
</tr>
<tr>
<td>C. D</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C V %</td>
<td>3.62</td>
<td>5.75</td>
<td>4.33</td>
<td>3.40</td>
</tr>
</tbody>
</table>

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

4.2.4.2 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 turmeric was added at different rates. The changes in colour and appearance score presented in Figure 4.5 revealed that the
score declined gradually on addition of turmeric up to 0.4 per cent. However, further addition resulted into sharp decline in the colour and appearance score.

4.2.4.3 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 turmeric was added at different rates. The changes in body and texture score presented in Figure 4.5 revealed that the score declined slightly on addition of turmeric 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 turmeric was added at different rates. The changes in overall acceptability score presented in Figure 4.5 revealed that the score declined slightly on addition of turmeric 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 turmeric on sensory attributes of paneer.

4.2.5 Selected rate of herbs for addition in paneer

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

It is evident from the careful observations of data that in case of ajwain, cumin and turmeric the score for the sensory attributes
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sharply decline when their rate of addition increased above 0.4 per cent. However, in case of coriander the score for the sensory attributes sharply decline when its rate of addition increased above 0.6 per cent.

Therefore, it was decided use ajwain, cumin and turmeric in paneer at the rate of 0.4 per cent and coriander at the rate of 0.6 per cent for further study.

4.3 Screening of selected herbs for effectiveness in preservation of paneer

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

For screening of the selected herbs 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 ajwain, cumin and turmeric were incorporated in the coagulum at the rate of 0.4 per cent and coriander at the rate of 0.6 per cent by weight of expected yield of paneer. The sample of paneer without addition of herb was used as a control. Three replications were conducted for the selected concentration of each herb.

Each block of paneer was cut in to two equal parts and packed separately in to PET/LDPE film pouches and stored at 7°C±1°C for 7 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after 7 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 4.6 and the trend is depicted in Figure 4.6. As indicated earlier the acceptability of any food product is influenced predominantly by its flavour score. The changes in flavour score revealed that both herb and storage period had significant effect on flavour score of the paneer. The interaction between herb and storage period was non significant.

**Table 4.6: Effect of herbs on flavour of paneer during storage (7°C) at selected concentration**

<table>
<thead>
<tr>
<th>Herbs added</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.48</td>
<td>6.88</td>
<td></td>
</tr>
<tr>
<td>Ajwain</td>
<td>7.64</td>
<td>6.95</td>
<td></td>
</tr>
<tr>
<td>Coriander</td>
<td>7.93</td>
<td>7.36</td>
<td></td>
</tr>
<tr>
<td>Cumin</td>
<td>8.07</td>
<td>7.25</td>
<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td>7.88</td>
<td>7.62</td>
<td></td>
</tr>
</tbody>
</table>

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Herb</th>
<th>Storage period</th>
<th>Storage period × Herb</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.125</td>
<td>0.079</td>
<td>0.177</td>
</tr>
<tr>
<td>C. D</td>
<td>0.369</td>
<td>0.234</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>4.08</td>
</tr>
</tbody>
</table>
Figure 4.6: Effect of herbs on flavour of paneer during storage (7°C) at selected concentration

The changes in flavour score presented in Figure 4.6 revealed that the score of all the samples of paneer decline slightly on 7th day of the storage but remained above acceptable level (6.0). The lowest score was found in the control sample closely followed by ajwain. The maximum score was found in case of turmeric. The score of coriander and cumin was intermediate to that of the score of ajwain and turmeric.

4.3.1.2 Colour and Appearance

The data obtained for changes in colour and appearance score of paneer during storage at 7°C are presented in Table 4.7 and the trend is depicted in Figure 4.7.

The changes in colour and appearance score presented in Table 4.7 revealed that herb has no significant effect on colour and appearance score of the paneer. However, storage period has significant effect on colour and appearance score of the paneer. The interaction between herb and storage period was also non significant.

Table 4.7: Effect of herbs on colour and appearance of paneer during storage (7°C) at selected concentration

<table>
<thead>
<tr>
<th>Herbs added</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.93</td>
<td>7.72</td>
<td></td>
</tr>
<tr>
<td>Ajwain</td>
<td>7.45</td>
<td>7.27</td>
<td></td>
</tr>
<tr>
<td>Coriander</td>
<td>7.60</td>
<td>7.41</td>
<td></td>
</tr>
<tr>
<td>Cumin</td>
<td>7.62</td>
<td>7.41</td>
<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td>7.71</td>
<td>7.46</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Herb</th>
<th>Storage period</th>
<th>Storage period × Herb</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.108</td>
<td>0.068</td>
<td>0.153</td>
</tr>
</tbody>
</table>
Results and discussion

<table>
<thead>
<tr>
<th>C. D</th>
<th>-</th>
<th>0.201</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td>3.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.7: Effect of herbs on colour and appearance of paneer during storage (7°C) at selected concentration

The lowest score was found in case of ajwain. The maximum score was found in case of control. The score of coriander, cumin and turmeric was intermediate to that of the score of ajwain and control.

Table 4.8: Effect of herbs on body and texture of paneer during storage (7°C) at selected concentration

<table>
<thead>
<tr>
<th>Herbs added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>7.76</td>
</tr>
<tr>
<td>Ajwain</td>
<td>7.29</td>
</tr>
<tr>
<td>Coriander</td>
<td>7.48</td>
</tr>
<tr>
<td>Cumin</td>
<td>7.41</td>
</tr>
<tr>
<td>Turmeric</td>
<td>7.48</td>
</tr>
</tbody>
</table>

ANOVÁ TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Herb</th>
<th>Storage period</th>
<th>Storage period × Herb</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.072</td>
<td>0.045</td>
<td>0.102</td>
</tr>
<tr>
<td>C. D</td>
<td>0.212</td>
<td>0.134</td>
<td>-</td>
</tr>
</tbody>
</table>
Results and discussion

<table>
<thead>
<tr>
<th>Test</th>
<th>*</th>
<th>*</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV %</td>
<td>2.39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 4.8: Effect of herbs on body and texture of paneer during storage (7°C) at selected concentration

4.3.1.3 Body and Texture

The data obtained for changes in body and texture score of paneer during storage at 7°C are presented in Table 4.8 and the trend is depicted in Figure 4.8. The changes in body and texture score revealed that both herb and storage period had significant effect on body and texture score of the paneer. However, the interaction between spice and storage period was non significant.

The lowest score was found in case of ajwain and cumin. The maximum score was found in case of control closely followed by turmeric. The score of coriander was intermediate to that of the score of turmeric and control.

4.3.1.4 Overall Acceptability
The data obtained for changes in overall acceptability score of paneer during storage at 7°C are presented in Table 4.9 and the trend is depicted in Figure 4.9.

**Table 4.9: Effect of herbs on overall acceptability of paneer during storage (7°C) at selected concentration**

<table>
<thead>
<tr>
<th>Herbs added</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>7.57</td>
</tr>
<tr>
<td>Ajwain</td>
<td>7.67</td>
</tr>
<tr>
<td>Coriander</td>
<td>7.91</td>
</tr>
<tr>
<td>Cumin</td>
<td>8.10</td>
</tr>
<tr>
<td>Turmeric</td>
<td>7.88</td>
</tr>
</tbody>
</table>

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Herb</th>
<th>Storage period</th>
<th>Storage period × Herb</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.115</td>
<td>0.073</td>
<td>0.162</td>
</tr>
<tr>
<td>C. D</td>
<td>0.338</td>
<td>0.214</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>3.72</td>
<td></td>
</tr>
</tbody>
</table>

Results and discussion
Results and discussion

Figure 4.9: Effect of herbs on overall acceptability of paneer during storage (7°C) at selected concentration

The changes in overall acceptability score presented in Table 4.9 revealed that both herb and storage period had significant effect on overall acceptability score of the paneer. However, the interaction between herb and storage period was non significant.

The lowest score was found in case of ajwain and control. The maximum score was found in case of cumin and turmeric. The score of coriander was intermediate to that of the score of ajwain and turmeric. No data are reported in the literature for effect of ajwain, coriander, cumin and turmeric on sensory attributes of paneer during storage.

On the basis of performance of ajwain, coriander, cumin and turmeric in screening for effectiveness in preservation of paneer, turmeric was selected for further study.

4.3.2 Final selection of the herb

On the basis of performance of various herbs in screening turmeric was selected for further study. Ajwain, coriander and cumin were dropped for the further study due to their relatively lower effectiveness in preservation of paneer. Especially the score for body and texture of paneer was significantly affected in case of ajwain, coriander and cumin up on storage.

4.4 Selection for stage of addition of the finally selected herb in paneer

In fourth phase of the study, work was carried out to select stage for addition of the selected herb (turmeric) to enhance the shelf life of paneer. From examination of manufacturing process for paneer
it can be envisaged that there are four possible ways to add turmeric into paneer, as listed below.

2. After heat treatment of milk but before addition of coagulant.
3. Directly into the coagulum when about 90 per cent of whey is removed.
4. Dipping of paneer blocks in turmeric containing chilled water instead of plain chilled water.

In the preliminary trials during investigation of these four stages, it was observed that addition of turmeric directly into the coagulum after draining about 90 per cent of whey resulted into uneven mixing and adverse effect on body and texture of the product. Similarly, dipping of paneer blocks in turmeric containing chilled water instead of plain chilled water, gave colouration on the surface only with little absorption inside. Therefore, these two stages for addition of turmeric were dropped from the further study. The work was carried out to evaluate effect of addition of turmeric before heat treatment of milk and after heat treatment of milk.

In each set of experiments turmeric was added at the rate of 0.4 per cent on the basis of expected yield of paneer. When second phase of the study was carried out to decide rate of addition of four selected herbs in paneer, it was found that addition of turmeric at the rate greater than 0.4 per cent resulted into sharp decline in acceptability of paneer in sensory evaluation. Therefore, in this phase of investigation concentration of 0.4 per cent was selected. Seven replications were conducted.

To find out the effect of heat treatment given to milk for paneer preparation on ability of turmeric to extend shelf life of paneer turmeric was added at two different stages. In one set of experiment, turmeric directly added into milk before initiating heat treatment and
in the other set, turmeric was added at 70°C during cooling of milk after the heat treatment at 95°C. Each block of paneer was cut in to four equal parts and packed separately in to PET/LDPE film pouches and stored at 7°C±1°C for 12 days. The prepared samples of paneer (Plate II) were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after the interval of every 3 days (except first interval which was of six days) up to 12 days of storage.

4.4.1 Changes in sensory attributes

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

Table 4.10: Effect of stage of addition on flavour of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Stage of addition</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Before heating</td>
<td>7.95</td>
<td>7.20</td>
<td>6.90</td>
<td>VMG*</td>
</tr>
<tr>
<td>After heating</td>
<td>8.07</td>
<td>6.82</td>
<td>6.19</td>
<td>VMG*</td>
</tr>
</tbody>
</table>

* VMG = Visible mold growth

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Stage of addition</th>
<th>Storage period</th>
<th>Storage period × Stage of addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.132</td>
<td>0.161</td>
<td>0.228</td>
</tr>
<tr>
<td>C. D</td>
<td>-</td>
<td>0.463</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>8.39</td>
<td></td>
</tr>
</tbody>
</table>
Results and discussion

Figure 4.10: Effect of stage of addition on flavour of paneer during storage at 7°C

4.4.1.1 Flavour

The data obtained for changes in flavour 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 flavour score presented in Table 4.10 revealed that storage period had significant effect on flavour score of the paneer, whereas, effect of stage of addition was non significant. The interaction between stage of addition and storage period was also non significant.

4.4.1.2 Colour and Appearance

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

The changes in colour and appearance score presented in Table 4.11 revealed that storage period had significant effect on colour and appearance score of the paneer, whereas, effect of stage of addition
was non significant. The interaction between stage of addition and storage period was non significant.

Table 4.11: Effect of stage of addition on colour and appearance of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Stage of addition</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Before heating</td>
<td>8.02</td>
<td>7.40</td>
<td>7.11</td>
<td>VMG*</td>
</tr>
<tr>
<td>After heating</td>
<td>8.15</td>
<td>7.16</td>
<td>7.00</td>
<td>VMG*</td>
</tr>
</tbody>
</table>

* VMG = Visible mold growth

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Stage of addition</th>
<th>Storage period</th>
<th>Storage period × Stage of addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.080</td>
<td>0.098</td>
<td>0.138</td>
</tr>
<tr>
<td>C. D</td>
<td>-</td>
<td>0.281</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>4.89</td>
</tr>
</tbody>
</table>

Figure 4.11: Effect of stage of addition on colour and appearance of paneer during storage at 7°C
4.4.1.3 Body and Texture

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

The changes in body and texture score presented in Table 4.12 revealed that storage period had significant effect on body and texture score of the paneer, whereas, effect of stage of addition was non significant. The interaction between stage of addition and storage period was non significant.
Table 4.12: Effect of stage of addition on body and texture of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Stage of addition</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Before heating</td>
<td>7.83</td>
</tr>
<tr>
<td>After heating</td>
<td>7.99</td>
</tr>
</tbody>
</table>

* VMG = Visible mold growth

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Stage of addition</th>
<th>Storage period</th>
<th>Storage period × Stage of addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.129</td>
<td>0.0158</td>
<td>0.224</td>
</tr>
<tr>
<td>C. D</td>
<td>-</td>
<td>0.454</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>8.28</td>
</tr>
</tbody>
</table>

Figure 4.12: Effect of stage of addition on body and texture of paneer during storage at 7°C

4.4.1.4 Overall Acceptability

The data obtained for changes in overall acceptability score of paneer during storage at 7°C are presented in Table 4.13 and the trend is depicted in Figure 4.13.

The changes in overall acceptability score presented in Table 4.13 revealed that storage period had significant effect on overall
acceptability score of the paneer, whereas, effect of stage of addition was non significant. The interaction between stage of addition and storage period was non significant.

**Table 4.13: Effect of stage of addition on overall acceptability of paneer during storage at 7°C**

<table>
<thead>
<tr>
<th>Stage of addition</th>
<th>Storage period (days)</th>
<th>0</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before heating</td>
<td>7.92</td>
<td>7.27</td>
<td>6.85</td>
<td>VMG*</td>
<td></td>
</tr>
<tr>
<td>After heating</td>
<td>8.07</td>
<td>6.83</td>
<td>6.30</td>
<td>VMG*</td>
<td></td>
</tr>
</tbody>
</table>

* VMG = Visible mold growth

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Stage of addition</th>
<th>Storage period</th>
<th>Storage period × Stage of addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.116</td>
<td>0.142</td>
<td>0.200</td>
</tr>
<tr>
<td>C. D</td>
<td>-</td>
<td>0.406</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>7.35</td>
</tr>
</tbody>
</table>

**Figure 4.13: Effect of Stage of addition on overall acceptability of Paneer during storage at 7°C**

### 4.4.2 Selected stage of addition

The careful examination of the data on effect of changes in sensory attributes of paneer during storage revealed that the samples
of fresh paneer obtained in case of turmeric added after heating of milk scored slightly higher than the samples of paneer obtained in case of turmeric added before heating of milk. However, trend of the score was reversed upon storage of the samples. The samples of stored paneer obtained in case of turmeric added before heating of milk scored higher than the samples of paneer obtained in case of turmeric added after heating of milk. Therefore, in further course of study it was decided to add turmeric in milk before heat treatment.

There could be two possible reasons for better performance of turmeric in enhancing the shelf life of paneer, when added before heat treatment of milk during preparation of paneer. Heating milk in presence of turmeric either enhances the bactericidal action of turmeric or increases the susceptibility of the bacteria to turmeric. No data are reported in the literature for effect of stage of addition of turmeric on extending the shelf life of paneer. However, similar observations were made by Hurst (1981) and Maisnier-Patin et al., (1995) in case of nisin, wherein better action of nisin is reported in reducing the growth of bacteria when it was used in combination with heat treatment. According to Maisnier-Patin et al., (1995) nisin enhances the effect of moderate heat and had a greater effect. Taking into account that the mode of action of nisin involves pore formation in the cell membrane the dramatic increase in thermal destruction of bacteria in the presence of nisin can be attributed to a synergistic effect of heat and nisin on the membrane damage, leading to rapid efflux of cytoplasmic constituents viz., ATP, amino acids, potassium, etc. However, according to Hurst (1981) it could be merely due to an increase in adsorption of nisin on the cell wall, as a result of modification of its surface properties brought about by heat which increases hydrophobicity by structural changes or loss of some cell
wall components. In turn, this increased adsorption would result in better activity of nisin.

4.5 Testing turmeric as preservatives for paneer

In the final phase of the study work was carried out to evaluate the effectiveness of turmeric in extending the shelf life of paneer.

In previous phase of study for selecting the stage for addition of turmeric, it was observed that the addition of turmeric before heat treatment of milk markedly reduced taste of raw turmeric in resultant samples of paneer. Therefore, it was decided to try one higher (0.6%) concentration of turmeric along with 0.4 per cent.

Each block of paneer was cut in to four equal parts and packed separately in to PET/LDPE film pouches and stored at 7°C±1°C for 12 days. The prepared samples of paneer (Plate III) were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after the interval of every 3 days (except first interval which was of six days) up to 12 days of storage.

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 acid, soluble nitrogen and peroxide value. The samples stored at 7°C ± 1°C were subjected to the sensory evaluation and analysis for the chemical characteristics at a regular interval of 6 days during the storage.

4.5.1 Chemical composition of paneer

The data obtained for chemical composition of fresh paneer sample with 0.4 per cent turmeric contained 50.40 per cent moisture, 27.77 per cent fat, 18.41 per cent protein, 1.67 per cent ash and 1.75 per cent lactose and of fresh paneer sample with 0.6 per cent turmeric contained 50.02 per cent moisture, 27.73 per cent fat, 19.15 per cent
protein, 1.60 per cent ash and 1.40 per cent lactose. The per cent fat on dry matter basis in fresh paneer sample with 0.4 per cent turmeric was 55.98 and in fresh paneer with 0.6 per cent turmeric was 55.48.

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 fulfilled both the PFA and the BIS requirements for the chemical composition.

4.5.2 Changes in sensory attributes

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

Table 4.14: Effect of concentration of turmeric on flavour of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.4</td>
<td>8.19</td>
</tr>
<tr>
<td>0.6</td>
<td>8.03</td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Concentration</th>
<th>Storage period</th>
<th>Storage period × Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.126</td>
<td>0.154</td>
<td>0.218</td>
</tr>
<tr>
<td>C. D</td>
<td>0.363</td>
<td>0.445</td>
<td>0.629</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>7.12</td>
</tr>
</tbody>
</table>
Results and discussion

4.5.1.1 Flavour

The data obtained for changes in flavour score of paneer during storage at 7°C are presented in Table 4.14 and the trend is depicted in Figure 4.14. The changes in flavour score presented in Table 4.14 revealed that concentration and storage period had significant effect on flavour score of the paneer. However, the interaction between concentration and storage period was also significant.

4.5.1.2 Colour and Appearance

The changes in colour and appearance score presented in Table 4.15 and the trend is depicted in Figure 4.15. The changes in colour and appearance score presented in Table 4.15 revealed that storage period had significant effect on colour and appearance score of the paneer, whereas, effect of concentration was non significant. The interaction between concentration and storage period was also non significant.
Table 4.15: Effect of concentration of turmeric on colour and appearance of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Storage period (days)</th>
<th>0</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>8.22</td>
<td>7.53</td>
<td>7.65</td>
<td>Slime formation</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>8.18</td>
<td>7.56</td>
<td>7.76</td>
<td>7.43</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA TABLE

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Concentration</th>
<th>Storage period</th>
<th>Storage period x Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.068</td>
<td>0.083</td>
<td>0.117</td>
</tr>
<tr>
<td>C. D</td>
<td>-</td>
<td>0.239</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>3.66</td>
</tr>
</tbody>
</table>

Figure 4.15: Effect of concentration of turmeric on colour and appearance of paneer during storage at 7°C

4.5.1.3 Body and Texture

The changes in body and texture score presented in Table 4.16 and the trend is depicted in Figure 4.16. The changes in body and
Results and discussion

texture score presented in Table 4.16 revealed that storage period had significant effect on body and texture score of the paneer, whereas, effect of concentration was non significant. The interaction between concentration and storage period was also non significant.

**Table 4.16: Effect of concentration of turmeric on body and texture of paneer during storage at 7°C**

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Storage period (days)</th>
<th>0</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td></td>
<td>8.10</td>
<td>7.22</td>
<td>7.75</td>
<td>Slime formation</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>8.00</td>
<td>7.43</td>
<td>7.80</td>
<td>7.20</td>
</tr>
</tbody>
</table>

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Concentration</th>
<th>Storage period</th>
<th>Storage period × Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.073</td>
<td>0.089</td>
<td>0.126</td>
</tr>
<tr>
<td>C. D</td>
<td>-</td>
<td>0.257</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td></td>
<td>4.00</td>
</tr>
</tbody>
</table>

**Figure 4.16: Effect of concentration of turmeric on body and texture of paneer during storage at 7°C**

4.5.1.4 Overall Acceptability
The data obtained for changes in overall acceptability score of paneer during storage at 7°C are presented in Table 4.17 and the trend is depicted in Figure 4.17. The changes in overall acceptability score presented in Table 4.17 revealed that concentration and storage period had significant effect on overall acceptability score of the paneer. However, the interaction between concentration and storage period was also significant.

**Table 4.17: Effect of concentration of turmeric on overall acceptability of paneer during storage at 7°C**

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Storage period (days)</th>
<th>0</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td></td>
<td>8.26</td>
<td>7.67</td>
<td>6.79</td>
<td>Slime formation</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>8.00</td>
<td>7.96</td>
<td>7.70</td>
<td>6.61</td>
</tr>
</tbody>
</table>

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Concentration</th>
<th>Storage period</th>
<th>Storage period x Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Em</td>
<td>0.122</td>
<td>0.149</td>
<td>0.211</td>
</tr>
<tr>
<td>C. D</td>
<td>0.352</td>
<td>0.432</td>
<td>0.610</td>
</tr>
<tr>
<td>Test</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>C V %</td>
<td></td>
<td></td>
<td>6.82</td>
</tr>
</tbody>
</table>
Results and discussion

Figure 4.17: Effect of concentration of turmeric on overall acceptability of paneer during storage at 7°C

In sensory evaluation fresh samples of paneer with 0.4 per cent turmeric score slightly better for all the sensory attributes compare to samples of paneer with 0.6 per cent turmeric. However, during storage, samples of paneer with 0.6 per cent turmeric scores slightly better for all the sensory attributes compare to samples of paneer with 0.4 per cent turmeric. On 12th day of storage development of off flavour and/or slime formation was noticed in the samples of paneer with 0.4 per cent of turmeric. Sensory score of paneer with 0.6 per cent turmeric remain acceptable on 12th day of storage, however, these samples were found spoiled on 15th day of storage.

4.5.3 Changes in chemical characteristics

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

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

The results indicated that the acidity of fresh samples of paneer was in the order of samples of paneer with 0.6 per cent turmeric > with 0.4 per cent turmeric > control. The acidity of paneer with 0.4 and 0.6 per cent of turmeric was slightly lower than that of the control samples of paneer. Thus, addition of turmeric slightly decreased the acidity of paneer.

The average acidity of the fresh control samples of paneer was 0.55 per cent. The acidity of paneer was reported to vary from 0.20 (Sachdeva and Singh, 1990) to 1.17 per cent (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 per cent. Krishna (2009) found that the average acidity of the fresh laboratory made paneer was 0.49. Therefore, acidity value of paneer in the present study was well within the reported normal range.

Table 4.18: Effect of turmeric on acidity (% lactic acid) of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>0.0</td>
<td></td>
<td>0.55</td>
<td>0.61</td>
</tr>
<tr>
<td>0.4</td>
<td></td>
<td>0.52</td>
<td>0.58</td>
</tr>
<tr>
<td>0.6</td>
<td></td>
<td>0.50</td>
<td>0.57</td>
</tr>
</tbody>
</table>

VMG*= Visible mold growth
Results and discussion

Figure 4.18: Effect of turmeric on acidity (% lactic acid) of paneer during storage at 7°C

It is surprising to notice that addition of turmeric to paneer decreased the acidity of paneer. This effect may be attributed to possible alkaline nature of turmeric. To test the nature of turmeric, solution of 0.012 g turmeric (equivalent to the amount present in 2 g of paneer taken for acidity determination) was prepared in distilled water and titrated against standard 0.1 N H₂SO₄ using methyl orange as an indicator. The titer value of 0.4 ml was obtained which indicates the alkaline nature of turmeric.

The results depicted in Figure 4.18 shows trend of changes in acidity of paneer samples during storage. The acidity of all the samples tended to rise slightly on 6th day of the storage. On 12th day of storage acidity of samples of paneer with 0.6 per cent turmeric was 0.58 per cent. Whereas, visible mold growth observed on the control samples of paneer and development of off flavour and/or slime formation was noticed in the samples of paneer with 0.4 per cent of turmeric. However, samples of paneer with 0.6 per cent turmeric was found spoiled on 15th day of storage.
Mistry (1988) observed that acidity of paneer increased from 0.475 to 0.483 per cent on 7th day of storage at 7 to 10°C and it decreased to 0.459 per cent on 15th 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 et al., (2003) found that acidity of paneer increased gradually from 0.224 to 0.472 per cent at the end of ninth day, 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 et al., (2004) prepared paneer samples by incorporating 10% coriander (Coriandrum sativum) or mint (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. Krishna (2009) reported that the acidity of laboratory made paneer increased from 0.49 to 1.08 per cent on storage at 7°C for 14 days.

However, no data are reported in the literature for effect of turmeric on acidity of paneer during storage.

4.5.3.2 Free fatty acids
The data obtained for changes in free fatty acids content of paneer during storage at 7°C are presented in Table 4.19 and trend is depicted in Figure 4.19.

The results indicated that the free fatty acids content of control samples of paneer was 0.03 per cent and that of the samples with 0.4 and 0.6 per cent turmeric containing samples were 0.02 per cent when the fresh samples were analysed. Therefore, it is evident that addition of turmeric slightly reduced the free fatty acids content of paneer. Such effect may be attributed to alkaline nature of turmeric.

Kumar and Bector (1991) found difference in free fatty acids content of control samples of paneer and those added with BHA and TBHQ. Krishna (2009) found that the average free fatty acids content of fresh laboratory made paneer was 0.06 per cent.

However, no data are reported in the literature for effect of addition of turmeric on free fatty acids content of paneer.

Table 4.19: Effect of turmeric on free fatty acid (% oleic acid) of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Storage period (days)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.03</td>
<td>0.05</td>
<td>VMG*</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>0.02</td>
<td>0.03</td>
<td>Slime formation</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

VMG* = Visible mold growth
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. Gohian (1996) analyzed the market samples of paneer collected from 18 different producers and reported that the acid degree value ranged from 0.583 to 2.250 per cent. The general mean obtained of acid degree value for overall 30 samples of market paneer was 1.308. Goel (2000) studied the laboratory made paneer and reported that the free fatty acids 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 acids content of paneer was 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 (90 micron) 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 acids 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 turmeric on free fatty acid content of paneer has not been reported.

The results depicted in Figure 4.19 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 all the samples of paneer. The free fatty acids content of all the samples tended to raise slightly on 6th day of the storage, however, the rate of increase was lower in case of turmeric containing samples. On 12th day of storage free fatty acids content of samples of paneer with 0.6 per cent turmeric declined slightly. Whereas, visible mold growth observed in the control samples of paneer and development of off flavour and/or slime formation was noticed in the samples of paneer with 0.4 per cent of turmeric. However, samples of paneer with 0.6 per cent turmeric was found spoiled on 15th day of 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. Krishna (2009) reported that the free fatty acids content of laboratory made paneer increased from 0.06 to 0.09 per cent on storage at 7°C for 14 days. The similar increase was observed in the present study.

However, no data are reported in the literature for effect of turmeric on free fatty acid content of paneer during storage.
4.5.3.3 Soluble nitrogen

The data obtained for changes in soluble nitrogen content of paneer during storage at 7°C are presented in Table 4.20 and the trend is depicted in Figure 4.20.

**Table 4.20: Effect of turmeric on soluble nitrogen (%) of paneer during storage at 7°C**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Storage period (days)</th>
<th>0</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.11</td>
<td>0.20</td>
<td>VMG*</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>0.11</td>
<td>0.12</td>
<td>Slime formation</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>0.10</td>
<td>0.11</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

VMG* = Visible mold growth

**Figure 4.20: Effect of turmeric on soluble nitrogen (%) of paneer during storage at 7°C**

The results indicated that the soluble nitrogen content of fresh control samples of paneer and the samples with 0.4 and 0.6 per cent turmeric was almost similar. Thus addition of turmeric had no effect on soluble nitrogen content in fresh samples of 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. Krishna (2009) found that the average soluble nitrogen content of the fresh laboratory made paneer was 0.2 per cent. However, effect of addition of turmeric on soluble nitrogen content of paneer has not been 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 0.510 per cent soluble nitrogen. Sachdeva and Singh (1990) reported the soluble nitrogen content of paneer sample dipped in brine was 0.10 per cent. Kumar and Bector (1991) found that the soluble nitrogen content was 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 4.20 shows trend of changes in soluble nitrogen content of paneer samples during storage. The sharp and highest increase in soluble nitrogen content was observed in control samples of paneer on 6\textsuperscript{th} day of storage (0.2\%). In the samples of paneer with 0.4 and 0.6 per cent turmeric soluble nitrogen content remained almost constant on 6\textsuperscript{th} day of the storage. On 12\textsuperscript{th} day of storage soluble nitrogen content of samples of paneer with 0.6 per cent turmeric increased slightly. Whereas, visible mold growth observed in the control samples of paneer and development of off flavour and/or slime formation was noticed in the samples of paneer.
with 0.4 per cent of turmeric. The samples of paneer with 0.6 per cent turmeric was found spoiled on 15\textsuperscript{th} day of storage.

Mistry (1988) reported that soluble protein content of paneer increased from 2.55 to 3.13 and 4.40 on 7\textsuperscript{th} and 15\textsuperscript{th} 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, 7\textsuperscript{th} and 15\textsuperscript{th} 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°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°C. Increase in soluble nitrogen content of control sample of paneer was in close agreement with increased reported by Kumar and Bector (1991). Krishna (2009) reported that the soluble nitrogen content of laboratory made paneer increased from 0.20 to 0.51 per cent on storage at 7°C for 14 days.

However, no data are reported in the literature for effect of turmeric on soluble nitrogen content of paneer during storage.

4.5.3.4 Peroxide Value

The data obtained for changes in peroxide value of paneer during storage at 7°C are presented in Table 4.21 and the trend is depicted in Figure 4.21.

The results indicated that the peroxide value of all the fresh samples of paneer was 0.0. Only a few reports are available in the literature on oxidative changes in paneer. Boghra et al., (1997) reported that the peroxide value of fresh paneer sample was 0.0. Thus, the results obtained in present study are in accordance with the value reported by this author.
Table 4.21: Effect of turmeric on peroxide value of paneer during storage at 7°C

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Storage period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>0.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

VMG* = Visible mold growth

Figure 4.21: Effect of turmeric on peroxide value of paneer during storage at 7°C

The results depicted in Figure 4.21 show the trend of changes in peroxide value of paneer samples during storage. The peroxide value of control samples was 0.06 per cent on 6th day of storage. Whereas, the peroxide value of the samples of paneer with 0.4 and 0.6 per cent turmeric was lower than that of the control samples. On 12th day of storage peroxide value of samples of paneer with 0.6 per cent turmeric was 0.05 per cent. Whereas, visible mold growth observed in the control samples of paneer and development of off flavour and/or slime formation was noticed in the samples of paneer with 0.4 per
Results and discussion

cent of turmeric. However, samples of paneer with 0.6 per cent turmeric was found spoiled on 15th day of storage.

Pal et al., (1993) prepared low-fat paneer from 1:1 ratio of cow and buffalo milk, it 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/100 g) at 0 day in the control sample increased to 0.72 ± 0.01 (mg mal/100g) on 15th day storage, whereas, 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. The values for changes in oxidative deterioration in present study can not be directly compared with these reported values due to difference in method of determination.

Boghra et al., (1997) studied the laboratory made paneer and simulated for approximate concentration of iron and copper based on market survey. The paneer was stored for 8 days at ≤ 10°C. The peroxide value (ml of 0.002 N Na₂S₂O₃/g) of fresh control sample was 0.0, which increased to 0.173. Thus, values for increase in peroxide value of paneer on storage obtained in present study are in general agreement with those reported by this author.

However, no data are reported in the literature for effect of turmeric on peroxide value of paneer during storage.

4.5.4 Microbial count of paneer containing turmeric

Effect of turmeric on microbial count of paneer was studied. The fresh and stored samples of paneer were analyzed for microbial count viz., standard plate count (SPC), yeast and mold count and coliform count. The data obtained for changes in SPC, yeast and mold count and coliform count are presented in Table 4.22.
**Table 4.22: Changes in microbial count during storage of paneer at 7°C**

<table>
<thead>
<tr>
<th>Storage period</th>
<th>SPC</th>
<th>Yeast and Mold</th>
<th>Coliform</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Turmeric</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>13.7×10³</td>
<td>13.5×10³</td>
<td>2×10²</td>
</tr>
<tr>
<td></td>
<td>(4.14)</td>
<td>(4.13)</td>
<td>(2.30)</td>
</tr>
<tr>
<td>6</td>
<td>46×10³</td>
<td>6.1×10³</td>
<td>12×10²</td>
</tr>
<tr>
<td></td>
<td>(4.66)</td>
<td>(3.79)</td>
<td>(3.07)</td>
</tr>
<tr>
<td>12</td>
<td>* VMG</td>
<td>19.2×10³</td>
<td>* VMG</td>
</tr>
<tr>
<td></td>
<td>(4.28)</td>
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<td></td>
</tr>
</tbody>
</table>

* VMG = Visible mold growth

The values in parenthesis indicates respective Log cfu/g

**Figure 4.22: Changes in SPC during storage of paneer at 7°C**
Results and discussion

Figure 4.23: Changes in yeast and mold count during storage of paneer at 7°C

4.5.5.1 Standard plate count

It is evident from the trend depicted in Figure 4.22 that there was a sharp increase in SPC on 6th day of the storage, in control samples. However, the count was declined in case of turmeric containing sample. On further storage up to 12th day visible mold growth was found in control samples and in case of turmeric containing samples also the count were increased.

Gupta (1985) studied the presence of the total bacterial load of paneer. The standard plat count of 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 $7.9 \times 10^5$), 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 $10^1$ 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 count 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. Krishna (2009) reported that standard plat count of laboratory made paneer increased from $3.4 \times 10^3$ to $57 \times 10^5$ per cent on storage at $7\,^\circ\text{C}$ for 14 days.

The data obtained for SPC for control sample of paneer and changes in the count during storage were in accordance with those reported by Gohian (1996). Fresh samples of both control and turmeric containing paneer met the standards prescribed by BIS for the SPC. On 12th day of storage, SPC of turmeric containing samples remained within BIS requirement. However, effect of addition of turmeric on SPC during storage of paneer has not been reported.

4.5.5.2 Yeast and mold count

It is evident from the trend depicted in Figure 4.23 yeast and mold count increases in case of control sample of paneer. In case of the samples of paneer with turmeric the count was nil, which increased gradually up to 6th day on storage but sharp increase was observed on 12th day of storage.

Thakral (1986) reported the yeast and mold 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 mold 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 mold count 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 mold 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 mold for all the 30 market paneer sample was 3.1101 log cfu per g. Venkateswarlu et al., (2003) reported that yeast and mold 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 mold count in market samples of paneer were ranged between $1 \times 10^2$ to $99 \times 10^2$ cfu per g. Krishna (2009) reported that yeast and mold count of laboratory made paneer increased from $1.7 \times 10^2$ to $45 \times 10^3$ per cent on storage at 7°C for 14 days.

The data obtained for yeast and mold count for control samples of paneer and changes in the count during storage were in accordance with those reported by Thakral (1986) and Gohian (1996). Fresh samples of both control and turmeric containing paneer met the standards prescribed by BIS for the yeast and mold count. The samples containing turmeric, yeast and mold count remained within BIS requirement only up to 6th day of storage. However, effect of addition of turmeric on yeast and mold count during storage of paneer has not been reported.

4.5.5.3 Coliform count

It is evident from the data presented in the Table 4.22 that no coliform count was found in control and turmeric containing samples when fresh and even during the storage.
Gupta (1985) studied the presence of coliform in Karnal, NDRI dairy made and laboratory prepared sample of paneer. The ranges of coliform 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 coliform 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 count 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.

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 count 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. Krishna (2009) reported that coliform count of laboratory made paneer increased $5.5 \times 10^3$ per cent on storage at 7°C for 14 days.

Thus, the results obtained in present study are in accordance with those reported by Gupta (1985). However, effect of addition of turmeric on yeast and mold count during storage of paneer has not been reported.
CHAPTER: V
SUMMARY AND CONCLUSION

The present study on the enhancement of the shelf life of paneer was undertaken in five phases. First phase involved testing of common herbs for compatibility in paneer. In second phase rate for addition of selected herbs in paneer was determined. In third phase screening for effectiveness of the herbs in enhancing shelf life of paneer was performed. In fourth phase stage for addition of selected herb (turmeric) was tested. Finally, in the fifth phase turmeric was tested for their relative efficiency in improving shelf life of paneer.

In first phase seven different herbs, commonly used with paneer during culinary preparations viz., ajwain, asafoetida, coriander, cumin, fenugreek, mint and turmeric were incorporated in paneer at the rate of 0.5 per cent and the samples were subjected to sensory evaluation. The samples containing four herbs (ajwain, coriander, cumin, and turmeric) were found acceptable in organoleptic testing. Therefore, these four herbs were selected for further study.

In second phase, work was carried out to select rate for addition of the selected herbs in paneer. In first phase of the study work was carried out to decide rate of addition of the four selected herbs viz., ajwain, coriander, cumin and turmeric in paneer. For selecting rate of addition of each herb the coagulum obtained by removal of 90 per cent whey, was divided in to 6 equal parts and the herb 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 herb was used as a control. The prepared samples of paneer were subjected to sensory evaluation by panel of seven judges using 9 point hedonic scale. In case of ajwain, cumin and turmeric the score for the sensory attributes sharply decline when their
rate of addition increased above 0.4 per cent. However, in case of coriander the score for the sensory attributes sharply decline when its rate of addition increased above 0.6 per cent. Therefore, it was decided to use ajwain, cumin and turmeric in paneer at the rate of 0.4 per cent and coriander at the rate of 0.6 per cent for further study.

In third phase work was carried out for screening of the selected herbs for their effectiveness to enhance shelf life of paneer. The coagulum obtained by removal of 90 per cent whey, was divided in to 5 equal parts and ajwain, cumin, and turmeric were incorporated in the coagulum at the rate of 0.4 per cent and coriander at the rate of 0.6 per cent by weight of expected yield of paneer. The sample of paneer without addition of herb was used as a control. Each block of paneer was cut in to two equal parts and packed separately in to PET/LDPE film pouches and stored at 7°C±1°C for 7 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after 7 days of storage. On the basis of performance of various herbs in screening turmeric was selected for further study. Ajwain, coriander and cumin were dropped due to their relatively lower effectiveness in preservation of paneer. Especially the score for body and texture was significantly affected in case of ajwain, coriander and cumin up on storage.

In fourth phase of the study, work was carried out to select stage for addition of the selected herb (turmeric) to enhance the shelf life of paneer. In the preliminary trials during investigaiton, it was observed that addition of turmeric directly in to the coagulum after draining about 90 per cent of whey resulted into uneven mixing and adverse effect on body and texture of the product. Similarly, dipping of paneer blocks in turmeric containing chilled water instead of plain chilled water, gave colouration on the surface only with little absorption inside. Therefore, these two stages for addiion of turmeric were
dropped from the further study. The work was carried out to evaluate effect of addition of turmeric before heat treatment of milk and after heat treatment of milk. In one set of experiment, turmeric directly added at the selected rate into milk before initiating heat treatment and in the other set, turmeric was added at 70°C during cooling of milk after the heat treatment at 95°C. Each block of paneer was cut in to four equal parts and packed separately in to PET/LDPE film pouches and stored at 7°C±1°C for 12 days. The prepared samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale when fresh and after the interval of every 3 days (except first interval which was of six days) up to 12 days of storage. The samples of fresh paneer obtained in case of turmeric added after heating of milk scored slightly higher than the samples of paneer obtained in case of turmeric added before heating of milk. However, trend of the score was reversed upon storage of the samples. The samples of stored paneer obtained in case of turmeric added before heating of milk scored higher than the samples of paneer obtained in case of turmeric added after heating of milk. Therefore, in further course of study it was decided to add turmeric in milk before heat treatment.

In the final phase of the study work was carried out to evaluate the effectiveness of turmeric in extending the shelf life of paneer. In previous phase of study for selecting the stage for addition of turmeric, it was observed that the addition of turmeric before heat treatment of milk markedly reduced taste of raw turmeric in resultant samples of paneer. Therefore, it was decided to try one higher (0.6 %) concentration of turmeric along with 0.4 per cent. Each block of paneer was cut in to four equal parts and packed separately in to PET/LDPE film pouches and stored at 7°C±1°C for 12 days. The prepared samples of paneer were subjected to sensory evaluation by
panel of judges using 9 point hedonic scale when fresh and after the interval of every 3 days (except first interval which was of six days) up to 12 days of storage. The fresh samples of paneer were subjected to sensory evaluation by panel of judges using 9 point hedonic scale. The fresh samples of paneer were analyzed for chemical characteristics viz., acidity, free fatty acid, soluble nitrogen and peroxide value. The samples stored at 7°C ± 1°C were subjected to the sensory evaluation and analysis for the chemical characteristics at a regular interval of 6 days during the storage.

In sensory evaluation fresh samples of paneer with 0.4 per cent turmeric score slightly better for all the sensory attributes compare to samples of paneer with 0.6 per cent turmeric. However, during storage, samples of paneer with 0.6 per cent turmeric scores slightly better for all the sensory attributes compare to samples of paneer with 0.4 per cent turmeric. On 12th day of storage development of off flavour and/or slime formation was noticed in the samples of paneer with 0.4 per cent of turmeric. Sensory score of paneer with 0.6 per cent turmeric remain acceptable on 12th day of storage, however, these samples were found spoiled on 15th day of storage.

The changes in chemical characteristics viz., acidity, free fatty acids content, soluble nitrogen and peroxide value are very well corroborated with changes in sensory attributes of paneer during storage. Therefore, effect of turmeric on microbial count of paneer was evaluated. The fresh and stored samples were analyzed for microbial count viz. standard plate count (SPC), yeast and mold count and coliform count. The changes in microbial count were also well corroborated with changes in sensory attributes of paneer during storage.

Results of the present study suggested that ajwain, coriander, cumin and turmeric are compatible for addition in paneer. Addition of
asafoetida, fenugreek and mint in paneer develops strong undesirable flavour in paneer. Therefore, asafoetida, fenugreek and mint are not compatible for addition in paneer. Amongst the four compatible herbs turmeric is highly convenient for incorporation in paneer and also relatively more efficient in extending the shelf life of paneer. The addition of turmeric in paneer at the rate of greater than 0.6 per cent significantly decreases the sensory score of paneer. Addition of turmeric at the rate of 0.6 per cent extends the shelf life of paneer up to 12 days on storage at 7°C.
REFERENCES


References


spices compared with common food additives. *J. Food Prot.*, **64**: 1412-1419.


References


References


Score guidelines:

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<tr>
<th>Hedonic Rating</th>
<th>Score</th>
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<td>Like extremely</td>
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</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>
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Evaluate the given sample of Paneer using above guidelines

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<td>7</td>
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<td>Colour &amp; Appearance</td>
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<td>Body &amp; Texture</td>
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<tr>
<td>Overall Acceptability</td>
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</table>

Comments (if any):

Name: ________________________

Date: _________________________

Signature: ___________________
APPENDIX - II
PFA AND BIS STANDARDS FOR PANEER

1. Standards for Paneer as per Prevention of Food Adulteration Act, 1954 (PFA, 2008):

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<th>Sr. No.</th>
<th>Requirement</th>
<th>Count</th>
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<tbody>
<tr>
<td>1.</td>
<td>Moisture</td>
<td>Not more than 70%</td>
</tr>
<tr>
<td>2.</td>
<td>Fat (dry matter basis)</td>
<td>Not less than 50%</td>
</tr>
<tr>
<td>3.</td>
<td>Total plate count</td>
<td>Not more than 50,000/g</td>
</tr>
<tr>
<td>4.</td>
<td>Coliform</td>
<td>Not more than 90/g</td>
</tr>
<tr>
<td>5.</td>
<td>E. coli</td>
<td>Absent in 1 g</td>
</tr>
<tr>
<td>6.</td>
<td>Salmonella</td>
<td>Absent in 25 g</td>
</tr>
<tr>
<td>7.</td>
<td>Shigella</td>
<td>Absent in 25 g</td>
</tr>
<tr>
<td>8.</td>
<td>Staphylococcus aureus</td>
<td>Not more than 100/g</td>
</tr>
<tr>
<td>9.</td>
<td>Yeast and Mold count</td>
<td>Not more than 250/g</td>
</tr>
<tr>
<td>10.</td>
<td>Anaerobic spore count</td>
<td>Absent in 1 g</td>
</tr>
<tr>
<td>11.</td>
<td>Listeria monocytogenes</td>
<td>Absent in 1 g</td>
</tr>
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2. BIS (1981) standards for paneer

<table>
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<th>Requirements</th>
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<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>
</tr>
<tr>
<td>Titratable acidity % lactic acid (max.)</td>
<td>0.50</td>
</tr>
<tr>
<td>Standard plate count per g (max.)</td>
<td>$5 \times 10^5$</td>
</tr>
<tr>
<td>Coliform per g (max.)</td>
<td>90</td>
</tr>
<tr>
<td>Yeast and Mold per g (max.)</td>
<td>250</td>
</tr>
</tbody>
</table>
Plate I: Control and Herbs added Paneer Samples
Plate II: Paneer Samples: Control and Turmeric added at two Different Stages
Plate III: Paneer Samples: Control and Turmeric added at two different concentrations.