Concise and informative title: evaluation of selected spices in extending shelf life of paneer

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Abstract Black pepper, cardamom, cinnamon and clove were tested for their relative efficacy in improving shelf life of paneer. All the spices were incorporated in paneer @ 0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 % by wt. of expected yield of paneer. Addition of black pepper, cardamom or clove at the rate of 0.6 % by weight or cinnamon at the rate of 0.4 % by weight was found to be acceptable. Therefore, paneer was prepared by incorporating black pepper, cardamom and clove i.e. Bp, Ca and Cl @ 0.6 % and cinnamon (Ci) @ 0.4 % by wt. of expected yield of paneer. All the paneer samples viz. Bp, Ca, Cl and Ci were subjected to sensory evaluation when fresh and after interval of 7 days during storage at 7 ± 1 °C up to 28 days. Results indicated that control remained acceptable up to 7 days, Bp up to 14 days of storage and Cl up to 21st day of storage. The overall acceptability score of Ca remained well above the acceptable level even on 28th day of the storage. Results of changes in chemical characteristics indicate that amongst all the spices studied, cardamom had maximum ability to control the rate of increase in acidity, free fatty acids content and soluble nitrogen content in paneer during storage. The order of the relative effectiveness in enhancing shelf life of paneer was cardamom > cinnamon > clove > black pepper. The effect of cardamom on microbial counts of paneer viz. standard plate counts (SPC), yeast and mould count and coliform count were evaluated. Among the spices studied, cardamom was found to be the best spice to improve shelf life of paneer up to 28 days of storage at 7 ± 1 °C.

Keywords Paneer · Spices · Cardamom · Cinnamon · Clove · Sensory

Introduction

Paneer is an important heat and acid coagulated milk product, which is used as a base material for the preparation of a large number of culinary dishes. Paneer, like other indigenous product is a highly perishable product and suffers from limited shelf life, largely because of its high moisture content (Arora and Gupta 1980). Its shelf life is reported to be only 6 days under refrigeration, though its freshness is lost within 3 days (Bhattacharya et al. 1971). The spoilage of paneer occurs mainly due to the growth of microorganisms, which bring about various physico-chemical changes leading to the development of off-flavour in the product. Over the years, various attempts have been made to increase the shelf life of paneer. There has been increasing concern of the consumers about foods free of chemical preservatives because of their possible toxic effect in human beings (Arora and Kaur 1999). Consumers are also demanding foods with long shelf life and absence of risk of causing food borne diseases. There is an increasing demand for foods containing natural ingredients. The consumers' demand has forced the food industry to exploit potential of natural alternatives for synthetic antimicrobial compounds.

Spices offer a promising alternative for food safety. Inhibitory activity of spices and derivatives on the growth of bacteria, yeasts, fungi and microbial toxins synthesis has been well reported, so they could be used in food conservation as main or as adjuvant antimicrobial compounds in order to assure the production of microbiologically stable foods. Spices have been well known for their medicinal, preservative and antioxidant properties (Souza et al. 2005). They are currently used mainly for enhancing the flavour of foods rather

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than extending shelf life (Almeida and Regitano 2000). In addition to imparting flavour, certain spices prolong the shelf life of foods due to their bacteriostatic or bacteriocidal activity, and some prevent rancidity by their antioxidant activity (Shelef 1984). Many plant essential oils of spices are active against various food borne bacteria and moulds (Aureli et al. 1992). Selected spices have been reported to have antimicrobial properties. The majority of the antimicrobial components of spices are phenol compounds with a hydroxyl group. Ahmed and Stoll (1996) reported that the main active principles in black pepper are alkaloids: methylpyroline, piperovatine, chavicine, pipieridine and piperine which control various pests including fungi. Sen and Rajorhia (1996) reported that cardamom contain several antimicrobial phenolics such as limonene, nerolidon, cineol, terpene, sabinine, pinene etc. Cardamom inhibit the growth of *S. aureus*, *B. stearothermophilus* and *B. coagulans* etc. (Zhi-He and Nakano 1996). Cinnamon and clove inhibited the growth of eight species of yeast, including *Saccharomyces cerevisiae* (Shelef 1984). In addition to compatibility, acceptability and antimicrobial properties of the spices they also have several health benefits. Many health benefit attributes of these common food adjuncts have been recognized in the past few decades by pioneering experimental research involving both animal studies and human trials. These studies documented digestive stimulant action, hypolipidemic effect, antidiabetic influence, antilithogenic property, antioxidant potential, anti-inflammatory property, antimutagenic, and anticarcinogenic potential of spices. These beneficial physiological effects also have the potential of possible therapeutic application in a variety of disease conditions (Srinivasan 2005). However, no work has been reported about use of spices in extending shelf life of paneer. Therefore, the present study was contemplated to select the best spice for application in paneer, and to evaluate its effectiveness in extending shelf life of paneer.

Materials and methods

Preparation of spices

Spices were collected from local market of Anand city from a retailer. The different spices used were: black pepper, cardamom, cinnamon and clove. The dry spices (black pepper, cardamom, cinnamon and clove) were finely ground using domestic mixer.

Preparation of paneer

The paneer was prepared in the laboratory using method described by De (1983). Mixed milk (standardized to 5.5 % fat) was heated to 95 °C for 5 min. and cooled to 70 °C. Citric acid, heated to 70 °C, was added in form of 1 % solution. The

solution was added with continuous agitation until the coagulation was complete. The coagulum was allowed to settle for 5 min. Whey was drained through a muslin cloth by gentle squeezing with hand and coagulum was collected. In the first part of study in order to select the optimum rate of addition of spices, the coagulum was divided into six equal parts and the spices were incorporated in the product at the rate of 0.0 (control), 0.2, 0.4, 0.6, 0.8 and 1.0 % by weight of expected yield of paneer. Three replications were conducted.

Based on the results obtained in the first part of the study black pepper (BP), cardamom (Ca), and clove (Cl) were incorporated in the coagulum at the rate of 0.6 % and cinnamon (Ci) was incorporated in the coagulum at the rate of 0.4 % by weight of expected yield of paneer. The sample of paneer without addition of spice was used as a control (C). Three replications were conducted for the selected spices. Each sample of coagulum was then filled in a sterilized stainless steel hoop (5 cm height; 17 cm length and 10 cm breadth) lined with muslin cloth. The coagulum was pressed for 20 min. followed by immersing in chilled water (4–6 °C) for 2 h. The samples were removed from chilled water and blocks were kept on wooden planks for allowing the water to drain off for 15 min. Composite polyethylene terephthalate (PET)/low density polyethylene (LDPE) film (50 µ thickness) having the following specifications: permeability to water vapour (g/m²/24 h: 1.8; permeability to oxygen at 20 °C: 100 g/m²/24 h) was used for packaging of samples during storage study. All the chemicals used in the study were of analytical grade.

Each block of paneer was cut in to four equal parts and packed separately in to PET/LDPE film pouches and stored at 7±1 °C for 28 days. The samples of paneer were subjected to sensory evaluation by panel of judges using 9-point hedonic scale.

Chemical analysis

The samples stored at 7±1 °C were subjected to analysis for the chemical characteristics at a regular interval of 7 days during the storage. Moisture content in paneer was determined according to BIS (1983) procedure specified for paneer under IS: 10484. Fat content of paneer was estimated by following the method described for cheese (Anon 1972). Protein content was determined by Kjeldahl method as described by Horwitz (1980). Lactose content in paneer was estimated by difference. The ash content of the paneer sample was estimated by the method of BIS (1981). The acidity of the paneer sample was estimated according to BIS (1983) procedure specified for paneer under IS: 10484. Free fatty acids content of paneer was estimated using the method described for cheese (Thomas et al. 1954). The soluble nitrogen content of the paneer sample was estimated by Kjeldahl method as described by Kosikowski (1970).

Microbiological analysis

Total viable count of paneer was determined by following the method was described by Messer et al. (1985) except that the diluents used were 2 % sodium citrate. The plating, incubation and counting for the enumeration of yeast and mold was done by method described by Frank et al. (1985). The plating, incubation and counting method to enumerate coliforms were carried out as described by Hartman and Lagrange (1985).

Statistical analysis

The values of each attribute under study were subjected to statistical analysis using Completely Randomized Design with equal number of observations using the model proposed by Steel and Torrie (1980).

Results and discussion

Chemical composition of paneer

Selection of rate of addition of spices

The data obtained for changes in sensory attributes of paneer with increasing rate of addition of cardamom, cinnamon, clove and black pepper are presented in Table 1. There was a significant ($P<0.05$) difference between flavour score of the paneer, when spices were added at different rates. The body and texture and colour and appearance scores declined gradually with increase in rate of addition of spices. There was a significant ($P<0.05$) difference in overall acceptability score of the paneer when spices were added at different rates. The overall acceptability score declined gradually on addition of

Table 1 Effect of addition of selected spices on average sensory score of paneer

Rate of addition (% of expected yield of paneer)	Flavour scores			
	Cardamom	Cinnamon	Clove	Black pepper
0.0	7.41±0.14 ^b	7.40±0.20 ^{ab}	7.86±0.16 ^a	7.34±0.20 ^{ab}
0.2	7.44±0.15 ^b	7.46±0.12 ^{ab}	7.70±0.18 ^b	7.13±0.15 ^{bc}
0.4	7.79±0.15 ^a	7.75±0.14 ^a	7.70±0.20 ^b	7.36±0.14 ^{ab}
0.6	7.83±0.12 ^a	7.21±0.15 ^{bc}	7.68±0.20 ^b	7.61±0.14 ^a
0.8	6.99±0.12 ^c	6.87±0.15 ^{cd}	6.89±0.13 ^c	6.89±0.18 ^{cd}
1.0	6.79±0.15 ^c	6.63±0.20 ^d	6.70±0.10 ^c	6.60±0.14 ^d
CD (0.05)	0.27	0.44	0.67	0.36
Body and texture scores				
0.0	8.39±0.15 ^a	8.20±0.11 ^a	8.47±0.10 ^a	8.20±0.15 ^a
0.2	8.20±0.20 ^a	7.81±0.15 ^{ab}	8.18±0.12 ^a	7.86±0.14 ^{ab}
0.4	8.20±0.11 ^a	7.79±0.14 ^{ab}	8.07±0.11 ^a	7.87±0.16 ^{ab}
0.6	8.04±0.10 ^a	7.54±0.14 ^{bc}	7.87±0.13 ^a	7.87±0.16 ^{ab}
0.8	7.64±0.12 ^b	6.98±0.13 ^c	7.28±0.15 ^b	7.56±0.14 ^{bc}
1.0	7.46±0.12 ^b	7.07±0.12 ^c	7.11±0.12 ^b	7.17±0.13 ^c
CD (0.05)	0.42	0.60	0.68	0.49
Colour and appearance scores				
0.0	8.18±0.12 ^a	8.12±0.15 ^a	8.65±0.11 ^a	8.22±0.14 ^a
0.2	8.03±0.15 ^a	7.52±0.20 ^{ab}	7.97±0.10 ^{bc}	7.72±0.14 ^b
0.4	7.89±0.15 ^{ab}	7.22±0.21 ^{bc}	7.60±0.21 ^{cd}	7.22±0.16 ^c
0.6	7.30±0.18 ^{bc}	6.45±0.20 ^{cd}	7.10±0.21 ^{de}	6.90±0.10 ^c
0.8	6.68±0.18 ^{cd}	6.12±0.15 ^{de}	6.95±0.15 ^e	6.45±0.14 ^d
1.0	6.27±0.20 ^d	5.75±0.16 ^e	6.05±0.14 ^f	6.17±0.15 ^d
CD (0.05)	0.72	0.88	0.57	0.41
Overall acceptability scores				
0.0	7.42±0.10 ^{ab}	7.84±0.11 ^a	8.22±0.15 ^a	7.75±0.20 ^a
0.2	7.47±0.15 ^{ab}	7.50±0.14 ^{ab}	7.78±0.15 ^b	7.25±0.15 ^{ab}
0.4	7.83±0.08 ^a	7.36±0.15 ^{bc}	7.64±0.14 ^b	7.31±0.14 ^b
0.6	7.81±0.14 ^a	7.00±0.15 ^{cd}	7.47±0.13 ^b	7.33±0.10 ^b
0.8	7.17±0.15 ^b	6.69±0.16 ^{de}	6.75±0.13 ^c	6.77±0.11 ^c
1.0	6.99±0.10 ^b	6.36±0.14 ^e	6.41±0.10 ^d	6.39±0.14 ^c
CD (0.05)	0.63	0.43	0.33	0.63

Each observation is mean ± SD of 3 replications

^{a–d} Superscript letters following numbers in the same column denote significant difference ($P<0.05$)

black pepper up to 0.6 %. However, further addition resulted into sharp decline in the overall acceptability score of the paneer. The overall acceptability score improved slightly on addition of cardamom up to 0.6 %. However, further addition resulted in to sharp decline in the score. The overall acceptability score declined gradually on addition of cinnamon up to 0.4 %. However, further addition resulted into sharp decline in the overall acceptability score of the paneer. The overall acceptability score declined gradually on addition of clove up to 0.6 %. However, further addition resulted into sharp decline in the overall acceptability score of the paneer. Based on results presented in Table 1 addition of black pepper, cardamom or clove at the rate of 0.6 % and cinnamon at the rate of 0.4 % by weight of expected yield of paneer was found to be most acceptable and used in the next part of the study. No data are reported in the literature for effect of spices on sensory attributes of paneer.

Based on the results obtained above, in this part of the study paneer was prepared by incorporating black pepper, cardamom and clove i.e. Bp, Ca and Cl @ 0.6 % and cinnamon (Ci) @ 0.4 % by wt. of expected yield of paneer. The average yield of control was 18.01 % whereas the yield of experimental samples varied from 17.5 % (Ci) to 17.61 (Ca) (Table 2). The calculated quantity of spices in paneer was 0.58, 0.59, 0.39 and 0.59 g/100 g in paneer samples Bp, Ca, Ci and Cl respectively.

The effect of incorporating spices on the yield and chemical composition of paneer is presented in Table 2. There was a significant ($P < 0.05$) difference in moisture, fat, FDM and protein content of samples. The lactose content of samples varied from 1.94 % (control) to 2.0 % (Bp and Ci) and the ash content varied from 1.67 (control) to 1.71 (Bp and Ci). The yield of control was maximum i.e. 18.01 % and Ci was minimum i.e. 17.50 %. Even though the difference in yield was only 0.5 %, control had significantly ($P < 0.05$) higher yield compared to all the other experimental samples. According to FSSA (2006), paneer shall not contain more than 70.0 % moisture and milk fat content shall not be less than 50.0 % of the dry matter. According to BIS (1983) moisture content in paneer shall not be more than 60.0 % by mass (max) and milk fat content shall be 50.0 % by mass on

dry matter basis (min). All the experimental samples of paneer prepared in the present study fulfilled both the FSSA and the BIS requirements. 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 % respectively (Bhattacharya et al. 1971; Pal and Yadav 1991; Singh et al. 1991; Dhole et al. 2009). Therefore, in the present study data obtained for chemical composition of paneer are well within those reported in the literature.

Effect of spices on sensory attributes of paneer The data obtained for changes in flavour score of paneer during storage at 7 ± 1 °C are presented in Table 3. The results indicated that the flavour score of fresh samples of paneer was in the order of $Ca > C > BP > Ci > Cl$. The flavour score of Ca was higher than that of C indicating that addition of cardamom improved the flavour score of paneer. The flavour score of C declined sharply from beginning of the storage. Amongst the experimental samples the highest rate of decrease in flavour score was observed in Bp and the lowest rate were observed in Ca. The flavour score of C went below acceptable level (6.0) on 7th day of the storage. It became unacceptable due to putrid odour and acidic and bitter taste. Incorporation of spices showed significant ($P < 0.05$) effect on flavour scores of paneer. The effect of storage on flavour scores and the interaction effect between spices and storage period was found to be significant ($P < 0.05$). Ca (at 0 days and 7 days storage) had significantly ($P < 0.05$) higher score compared to all the other experimental samples. The body and texture score of fresh samples of paneer was in the order of $C > BP > Ca > Ci > Cl$. The addition of all the spices used in the study were found to reduce body and texture score of paneer. This adverse effect may be attributed to interference of spice particles in development of body and texture of the paneer. Incorporation of spices had significant ($P < 0.05$) effect on body and texture scores of paneer. The effect of storage on body and texture scores and the interaction effect between spices and storage period was found to be significant ($P < 0.05$). The body and texture scores of fresh samples of control was significantly ($P < 0.05$) higher and at par ($P > 0.05$) with Bp, Ca and Ci.

Table 2 Effect of addition of selected spices on yield and composition of paneer

	Level of addition of spice (% by wt. of expected yield)	Yield (%)	Moisture (%)	FDM (%)	Fat (%)	Protein (%)
Control	0	18.01 $\pm$ 0.03 ^a	50.21 $\pm$ 0.09 ^a	58.33 $\pm$ 0.07 ^a	29.02 $\pm$ 0.05 ^c	17.19 $\pm$ 0.04 ^c
Bp	0.6	17.52 $\pm$ 0.09 ^b	49.81 $\pm$ 0.10 ^b	59.47 $\pm$ 0.07 ^a	29.85 $\pm$ 0.08 ^a	17.68 $\pm$ 0.04 ^a
Ca	0.6	17.61 $\pm$ 0.07 ^b	49.71 $\pm$ 0.11 ^b	50.01 $\pm$ 0.11 ^b	29.69 $\pm$ 0.08 ^b	17.57 $\pm$ 0.04 ^b
Ci	0.4	17.50 $\pm$ 0.05 ^b	49.52 $\pm$ 0.08 ^c	59.12 $\pm$ 0.12 ^b	29.85 $\pm$ 0.08 ^a	17.68 $\pm$ 0.07 ^a
Cl	0.6	17.60 $\pm$ 0.05 ^b	49.70 $\pm$ 0.12 ^b	59.01 $\pm$ 0.11 ^b	29.69 $\pm$ 0.07 ^b	17.59 $\pm$ 0.06 ^a
CD (0.05)	-	0.12	0.16	0.18	0.14	0.11

Each observation is a mean $\pm$ SD of 3 replications

^{a-d} Superscript letters following numbers in the same column denote significant difference ($P < 0.05$)

Table 3 Effect of incorporation of spices on sensory attributes of paneer during storage (7 ± 1 °C)

Spice added	Storage period (days)				
	0	7	14	21	28
Flavour score					
Control	7.61 $\pm$ 0.15	5.82 $\pm$ 0.14	4.52 $\pm$ 0.14	VMG ^a	-
BP	7.53 $\pm$ 0.20	6.95 $\pm$ 0.15	6.62 $\pm$ 0.15	5.50 $\pm$ 0.12	4.83 $\pm$ 0.11
Ca	8.12 $\pm$ 0.15	8.11 $\pm$ 0.16	7.68 $\pm$ 0.15	7.50 $\pm$ 0.18	7.23 $\pm$ 0.20
Ci	7.43 $\pm$ 0.14	7.53 $\pm$ 0.15	7.33 $\pm$ 0.20	7.08 $\pm$ 0.11	5.44 $\pm$ 0.11
Cl	7.07 $\pm$ 0.14	7.27 $\pm$ 0.14	7.08 $\pm$ 0.21	6.87 $\pm$ 0.14	5.71 $\pm$ 0.12
CD (0.05) $T=0.18$, $S=0.27$; $T\times S=0.15$					
Body and texture score					
Control	7.87 $\pm$ 0.10	7.16 $\pm$ 0.23	5.95 $\pm$ 0.12	VMG ^a	-
BP	7.74 $\pm$ 0.14	7.38 $\pm$ 0.21	6.86 $\pm$ 0.10	6.50 $\pm$ 0.13	5.41 $\pm$ 0.14
Ca	7.73 $\pm$ 0.20	7.69 $\pm$ 0.12	7.16 $\pm$ 0.15	7.11 $\pm$ 0.15	7.00 $\pm$ 0.20
Ci	7.72 $\pm$ 0.23	7.36 $\pm$ 0.14	7.11 $\pm$ 0.15	7.11 $\pm$ 0.16	5.80 $\pm$ 0.14
Cl	7.53 $\pm$ 0.21	7.40 $\pm$ 0.14	6.96 $\pm$ 0.19	6.64 $\pm$ 0.17	5.87 $\pm$ 0.13
CD (0.05) $T=0.18$, $S=0.28$; $T\times S=0.15$					
Colour and appearance					
Control	8.02 $\pm$ 0.12	7.59 $\pm$ 0.14	7.42 $\pm$ 0.13	VMG ^a	-
BP	7.42 $\pm$ 0.11	6.95 $\pm$ 0.16	7.03 $\pm$ 0.15	6.42 $\pm$ 0.11	6.27 $\pm$ 0.11
Ca	7.89 $\pm$ 0.13	7.47 $\pm$ 0.16	7.43 $\pm$ 0.16	7.29 $\pm$ 0.13	7.27 $\pm$ 0.13
Ci	7.37 $\pm$ 0.14	7.01 $\pm$ 0.20	6.63 $\pm$ 0.14	6.38 $\pm$ 0.14	6.30 $\pm$ 0.13
Cl	6.82 $\pm$ 0.14	6.56 $\pm$ 0.12	6.31 $\pm$ 0.11	6.12 $\pm$ 0.15	6.07 $\pm$ 0.11
CD (0.05) $T=0.13$, $S=0.20$; $T\times S=0.11$					
Overall acceptability					
Control	7.77 $\pm$ 0.14	6.78 $\pm$ 0.16	5.67 $\pm$ 0.16	VMG ^a	-
BP	7.37 $\pm$ 0.14	6.93 $\pm$ 0.10	6.58 $\pm$ 0.14	5.95 $\pm$ 0.15	5.03 $\pm$ 0.16
Ca	7.92 $\pm$ 0.14	7.78 $\pm$ 0.08	7.27 $\pm$ 0.15	7.27 $\pm$ 0.11	7.10 $\pm$ 0.18
Ci	7.37 $\pm$ 0.16	7.22 $\pm$ 0.13	6.88 $\pm$ 0.15	6.75 $\pm$ 0.14	6.17 $\pm$ 0.11
Cl	6.91 $\pm$ 0.14	6.83 $\pm$ 0.14	6.88 $\pm$ 0.15	6.62 $\pm$ 0.14	5.87 $\pm$ 0.10
CD (0.05) $T=0.13$, $S=0.21$; $T\times S=0.11$					

Each observation is mean $\pm$ SD of 3 replications, T type of spice added, S storage period

^a VMG visible mould growth

The colour and appearance score of fresh samples of paneer was in the order of $C > Ca > BP > Ci > Cl$. The colour and appearance score of C was the highest. The addition of all the spices used in the study was found to reduce colour and appearance score of paneer. This adverse effect on colour and appearance score of the paneer may be attributed to the brown to black colour imparted by respective spices to the paneer. Incorporation of spices resulted in significant ($P<0.05$) effect on colour and appearance scores of paneer. The effect of storage on colour and appearance scores was found to be significant ($P<0.05$). The interaction effect between spices and storage period was statistically significant ($P<0.05$). The colour and appearance score of all the samples of paneer containing spices remained above the acceptable level (6.0) all through out the storage period.

The overall acceptability score of fresh samples of paneer was in the order of $Ca > C > BP = Ci > Cl$. Incorporation of spices showed significant ($P<0.05$) effect on overall

acceptability scores. The effect of storage on overall acceptability scores and the interaction effect between spices and storage period was found to be significant ($P<0.05$). The overall acceptability score of C went below acceptable level (6.0) on 14th day of the storage. BP had acceptable overall acceptability score up to 14th day of the storage and it went slightly below the acceptable level on 21st day of the storage. The overall acceptability score of Cl remained acceptable up to 21st day of the storage and went slightly below acceptable level on 28th day of the storage. Similarly overall acceptability score of Ci remained acceptable up to 21st day of the storage and went below acceptable level on 28th day of the storage. However, the overall acceptability score of Ca remained well above the acceptable level even on 28th day of the storage.

Reports in literature indicate that paneer can be kept good for only 6 days although it starts losing its freshness on the 3rd day (Bhattacharya et al. 1971; Sachdeva and Singh 1990a). Arora and Gupta (1980) also reported that paneer, like other

indigenous product is a highly perishable product and suffers from limited shelf life, largely because of its high moisture content. Therefore, data obtained for effect of storage on sensory attributes for control sample of paneer is exactly in accordance with those reported in the literature. Sen and Rajorhia (1996) reported that the cardamom powder added at the rate 0.1 % enhanced the shelf life of sandesh to 90 days as against 60 days for control sample at 7 °C. Agaoglu et al. (2007) reported the antimicrobial effect of cinnamon in meat products. Sharma and Verma (2006) investigated the efficiency of four plant essential oils bay, clove, cinnamon and thyme as natural food preservatives. Ratiba et al. (2006) reported that incorporation of cardamom, thyme and clove powder in goat milk cheese resulted in improvement in flavour scores. Choudhary and Sandey (2009) used cardamom extract in preparation of whey based mango herbal (cardamom) beverage and reported that the product was highly acceptable up to 30 days at refrigerated temperature. However, no data are reported in the literature on the effect of black pepper, cardamom, cinnamon and clove on acceptability of paneer during storage.

Changes in chemical characteristics

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

and soluble nitrogen content. The data obtained for changes in these parameters during storage at 7 ± 1 °C are presented in Table 4.

Acidity

The average acidity of the fresh control sample of paneer was 0.49 % lactic acid. The acidity of fresh samples of paneer was in the order of $Ca > BP > C > Cl > Ci$. The acidity of paneer containing cardamom as well as black pepper was lower than that of the control sample of paneer. Thus addition of cardamom or black pepper decreases the acidity of paneer. The acidity of all the samples tended to rise sharply up to 7th day of the storage, except cinnamon. On further storage acidity continued to rise in case of control sample, however, in case of the samples containing spices acidity was found to decrease up to 14th day of the storage. Incorporation of spices showed significant ($P < 0.05$) effect on acidity of paneer. The effect of storage on overall acceptability scores and the interaction effect between spices and storage period was found to have significant ($P < 0.05$) effect on acidity. In literature, the minimum acidity (% lactic acid) of paneer is reported to be 0.20 (Sachdeva and Singh 1990c). However, this is the extreme range of the values, but most of the reports (Kumar and Bector 1991; Venkateswarlu et al. 2003; Yellamanda et al. 2006) suggest the normal range of 0.47–0.59 % lactic acid.

Table 4 Effect of incorporation of selected spice on some chemical properties of paneer during storage (7 ± 1 °C)

Spice added	Storage period (days)				
	0	7	14	21	28
Acidity (% lactic acid)					
Control	0.49±0.01	1.01±0.03	1.08±0.05	VMG	-
BP	0.38±0.01	0.90±0.03	0.85±0.04	0.91±0.01	0.99±0.02
Ca	0.33±0.03	0.83±0.02	0.52±0.03	0.50±0.00	0.50±0.02
Ci	0.65±0.00	0.73±0.02	0.52±0.02	0.72±0.02	0.74±0.03
Cl	0.52±0.01	0.87±0.02	0.58±0.01	0.70±0.01	0.75±0.01
CD (0.05) $T=0.03$, $S=0.03$; $T \times S=0.02$					
Free fatty acids (% oleic acid)					
Control	0.06±0.00	0.08±0.01	0.09±0.00	VMG	-
BP	0.06±0.00	0.08±0.01	0.07±0.01	0.15±0.00	0.27±0.02
Ca	0.06±0.01	0.05±0.01	0.05±0.01	0.10±0.05	0.11±0.04
Ci	0.07±0.01	0.05±0.01	0.05±0.01	0.11±0.06	0.17±0.01
Cl	0.06±0.01	0.05±0.01	0.06±0.01	0.12±0.01	0.19±0.05
CD (0.05) $T=NS$, $S=0.01$; $T \times S=0.01$					
Soluble nitrogen (%)					
Control	0.202±0.02	0.357±0.02	0.519±0.01	VMG	-
BP	0.232±0.01	0.283±0.01	0.464±0.01	0.500±0.01	0.578±0.02
Ca	0.146±0.00	0.227±0.00	0.215±0.00	0.308±0.02	0.392±0.02
Ci	0.214±0.01	0.239±0.01	0.296±0.02	0.438±0.02	0.522±0.02
Cl	0.143±0.02	0.249±0.02	0.352±0.02	0.451±0.03	0.491±0.03
CD (0.05) $T=0.02$, $S=0.03$; $T \times S=0.02$					

Each observation is mean ± SD of 3 replications, T type of spice added, S storage period

VMG visible mould growth

Therefore, acidity value of paneer in the present study is well with in the reported normal range.

It is surprising to note that addition of cardamom or black pepper to paneer decreased the acidity of paneer. On the other hand addition of cinnamon or clove increased the acidity of paneer. To substantiate such effects of the spices on acidity of paneer some trials were taken to determine acidity of the spice samples, taking distilled water as control by following the method used for the paneer. The negative values were obtained for cardamom and black pepper when compared with the value obtained for the water. Similarly, positive values were obtained for cinnamon and clove when compared with the value obtained for the water. The observations of the trails supports the results obtained for the samples of paneer. No data are reported in the literature regarding effect of addition of these spices on acidity of paneer or other food products.

Cardamom proved to be the best spice to control acidity of paneer during storage. These observations are well correlated with highest flavour score of the paneer containing cardamom during storage. Kumar and Bector (1991) reported that the acidity of control samples (0.54 %) increased slowly during storage and reached 0.9 % on day four and thereafter declined and reached a value of 0.59 % on day ten. However, Venkateswarlu et al. (2003) found that the acidity of paneer increased gradually from 0.224 to 0.472 % at the end of ninth day storage, followed by sharp increase towards end of storage. Sachdeva and Singh (1990c) suggested that 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 Kumar and Bector (1991). While studying the effect cardamom, thyme and clove powder on quality of white cheese made from goat's milk Ratiba et al. (2006) observed that the development of acidity was slower in cheese containing added spices especially that containing 0.2 % cardamom which showed the lowest development acidity. No data are reported in the literature for effect of black pepper, cardamom, cinnamon and clove on acidity of paneer during storage.

Free fatty acids (FFA)

The results indicated that the FFA content (expressed as % oleic acid) of all fresh samples of paneer was same (0.06 %), except cinnamon which has slightly higher value (0.07 %). During storage there was slight increase in FFA content of the C and BP up to 7th day of the storage, whereas, in case of the Ca, Ci and Cl there was a slight decrease the values. The FFA content in Ca remained stagnant during storage between 21 and 28 days. Cardamom proved to be the best spice to control

free fatty acids content of paneer during storage. These observations are well correlated with highest flavour score of the paneer containing cardamom during storage. Storage period had significant ($P < 0.05$) effect on FFA content of paneer whereas effect of type of spice used was non significant ($P < 0.05$). The interaction effect between type of spice used and storage period was also found to be significant ($P < 0.05$). Kumar and Bector (1991) reported FFA content of 0.28 % for paneer. Venkateswarlu et al. (2003) obtained a value of 0.11 % FFA for paneer. Gokhale and Pandya (2009) reported the FFA content of fresh sample of paneer was 0.04 %. Therefore, values obtained for free fatty acid content of paneer in present study are in agreement with those reported by Gokhale and Pandya (2009). Kumar and Bector (1991) reported that the FFA content of paneer increased from 0.28 to 0.5 % on 22 days of storage at 5 °C. The author also reported that market sample of paneer has a rise its initial value of 0.0699–0.080 % on 12 days of storage at 7 ± 1 °C. A similar trend was observed in the present study. The effect of addition of spices on FFA content in paneer during storage has not been reported.

Soluble nitrogen

The soluble nitrogen content of fresh samples of paneer was in the order of $Cl > Ca > C > Ci > BP$. The soluble nitrogen content of paneer containing cardamom as well as clove was lower and cinnamon and black pepper was higher compared to the control sample of paneer. Thus addition of cardamom or clove decreases the soluble nitrogen content, whereas, addition of black pepper or cinnamon increases the soluble nitrogen content in fresh sample of paneer. This effect may be attributed to effect of added spices on solubility of nitrogenous compounds in paneer. Incorporation of spices resulted in significant ($P < 0.05$) effect on soluble nitrogen content of paneer. The effect of storage on soluble nitrogen and the interaction effect between spices and storage period was found to have significant ($P < 0.05$) effect on soluble nitrogen content. Kumar and Bector (1991) reported differences between soluble nitrogen content of control samples of paneer and that of the samples added with BHA and TBHQ. The lower values were reported by the authors in the samples containing BHA and TBHQ. Similar effects were also observed by Ratiba et al. (2006).

At the end of the storage minimum increase in soluble nitrogen content was found in the samples of paneer containing cardamom. Thus, cardamom proved to be the best spice to control soluble nitrogen content of paneer during storage. Sachdeva and Singh (1990c) reported the soluble nitrogen content of 0.10 %. Kumar and Bector (1991) reported the soluble nitrogen content of 0.06 % in laboratory made paneer. Therefore, values obtained for soluble nitrogen content of

paneer in the present study were in general agreement with those reported in the literature.

Sachdeva and Singh (1990c) 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 % increased to 0.24 %. 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 collaborated with increased reported by Sachdeva and Singh (1990c). In the study of white cheese made from goat's milk Ratiba et al. (2006) observed that the incorporation of cardamom, thyme and clove powder had significant effect on the changes in total nitrogen (TN) during storage. Slight but not significant differences were found in cheese containing spices as compared to control. A similar increase in soluble nitrogen content during storage was also observed during the present study. No data has been reported in literature on the effect of spices on soluble nitrogen content in paneer during storage.

Microbial counts of paneer containing cardamom

The results obtained for changes in sensory attributes during storage suggested cardamom as the best spice to extend shelf life of paneer. Therefore, effect of cardamom on microbial counts of paneer was evaluated. The fresh and stored samples of paneer were analyzed for microbial counts viz. standard plate counts (SPC), yeast and mould counts and coliform counts. The data obtained for changes in SPC, yeast and mould counts and coliform counts are presented in Table 5.

Standard plate count

It is evident from the Table 5 that there was a sharp increase in SPC on 7th day of the storage, both in control as well as samples containing cardamom. On further storage the count continued to increase on 14th day of the storage in control sample, but the count decreased considerably in case of cardamom containing samples. In the Ca again count increase up

to 21st day of storage, followed by marginal increase on 28th day of the storage. Incorporation of cardamom resulted in significant ($P<0.05$) decrease in SPC of paneer. Compared to control, the samples containing cardamom had significantly ($P<0.05$) lower SPC count during storage. The interaction effect between treatment and storage period was found to be significant ($P<0.05$).

Gupta (1985) reported that standard plate count of fresh sample of market paneer ranged from 2.5×10^4 to 3.5×10^5 cfu per g (average initial count of 3.0×10^5). The standard plate count of NDRI dairy made paneer sample ranged from 5.0×10^3 to 1.8×10^5 (average 7.9×10^9), for laboratory prepared paneer contain count was as low as 1.0×10^4 cfu per g. Sachdeva and Singh (1990b) 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 – 10^3 per g which increased to the number of 10^4 – 10^6 per g regard less of treatment. Kumar and Bector (1991) reported the initial counts of control, 3.0×10^3 increased to 2.8×10^5 per g on day four and 9.0×10^6 per g on day seven during storage at 15 °C. Vaishnavi et al. (2001) reported total plate count of 3×10^2 to 9.7×10^{10} in the samples of paneer collected from Chandigarh market. Ratiba et al. (2006) studied effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat's milk. The author found that the total viable count (TVC) of control significant increased throughout the storage period while, count of different treatments decreased up to 15 days, then slightly increased up to 15 days. It then slightly increased till the end of storage period. Cardamom, thyme and clove concentration of 0.20, 0.15 and 0.20 % respectively had the highest effect on TVC especially 0.20 % cardamom.

The data obtained for SPC for control sample of paneer and changes in the count during storage are in accordance with those reported in the literature. Fresh samples of both control and cardamom containing paneer met the standards prescribed by BIS(1983) for the yeast and mould counts. In paneer containing cardamom SPC remained within BIS requirement up to 21 days of storage. The effect of

Table 5 Changes in microbial quality (counts in log cfu/g) in paneer containing cardamom during storage of paneer at 7 ± 1 °C

Storage period	SPC		Yeast and mould		Coliforms	
	Control	Ca	Control	Ca	Control	Ca
0	3.53±0.08	3.49±0.06	2.23±0.05	2.3±0.10	Absent	Absent
7	6.18±0.10	5.93±0.08	2.11±0.05	2.48±0.08	1.30±0.08	1.0±0.02
14	6.76±0.08	4.18±0.10	4.65±0.10	3.20±0.10	2.74±0.04	1.30±0.02
21	VMG*	5.89±0.04	-	3.40±0.10	-	1.0±0.01
28	-	6.18±0.10	-	4.51±0.10	-	1.48±0.02
	CD (0.05) $T=0.19$, $S=0.13$; $T \times S=0.13$		CD (0.05) $T=0.25$, $S=0.18$; $T \times S=0.18$		CD (0.05) $T=0.3$, $S=0.3$; $T \times S=0.31$	

Each observation is mean ± SD of 6 replications, T treatment (with/without cardamom), S storage period, VMG visible mould growth

addition of cardamom on SPC during storage of paneer has not been reported.

Yeast and mould count

The yeast and mould count increased sharply in case of control sample of paneer. In case of the samples containing cardamom the count increased gradually and at a slower rate all through out the storage period. Incorporation of cardamom resulted in significant ($P<0.05$) decrease in yeast and mould of paneer. Compared to control, the samples containing cardamom had significantly ($P<0.05$) lower yeast and mould count on storage. The interaction effect between treatment and storage period was found to be significant ($P<0.05$). Sachdeva and Singh (1990b) 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 35×10^1 – 52×10^1 . The yeast and mould count at the time of spoilage in the respective samples ranged from 53×10^2 to 63×10^3 . Kumar and Bector (1991) analyzed the yeast and mould counts of control paneer sample and found that initial count increased from 10 per g to 50 per g after 4 days and 250 per g after 7 days of storage at 15 °C. Venkateswarlu et al. (2003) reported that yeast and mould count of paneer 2.45 per g. Dhole et al. (2009) evaluated the 70 samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The yeast and mould counts in market samples of paneer were ranged between 1×10^2 and 99×10^2 cfu per g.

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

Coliform count

There was a sharp increase in coliform count on 7th day of the storage, both in control as well as cardamom added samples.

On further storage the count continued to increase on 14th day of the storage in control sample, but the count decreased considerably in case of cardamom containing samples. In the cardamom containing sample again count increase up to 21st day of storage, followed by marginal increase on 28th day of the storage. Incorporation of cardamom resulted in significant ($P<0.05$) decrease in coliform count of paneer. Compared to control, the samples containing cardamom had significantly ($P<0.05$) lower coliform count on storage. The interaction effect between treatment and storage period was found to be significant ($P<0.05$). 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 3–4 in the first dilution of all the paneer samples and this increased to a maximum of 30–50 over the storage period. Kumar and Bector (1991) reported the initial level of coliform counts of control increased from 90 per g to 3.5×10^3 per g after 4 days and 8.0×10^6 per g after 7 days of storage.

While evaluating the effect of cardamom, thyme and clove powder on the composition and quality of white cheese made from goat's milk Ratiba et al. (2006) found that the count of coliforms gradually increased till 30 days of storage period, then decreased at the end of storage period. Dhole et al. (2009) evaluated the 70 samples of fresh paneer from seven vendors of Ahmednagar City (M.S.) for microbiological quality. The average coliform counts in the market samples of paneer were found in the range of 12.6×10^3 to 23.2×10^3 cfu per g. The data obtained for coliform count for control sample of paneer and changes in the count during storage were corroborated well with those reported in the literature. Fresh samples of both control and cardamom containing paneer met the standards prescribed by BIS for the coliform counts. Up on storage only in cardamom containing samples coliform count remained within BIS requirement up to 21 day of storage. However, effect of addition of spices on coliform count during storage of paneer has not been reported.

Conclusion

It can be concluded from the study that addition of black pepper, cardamom and clove in paneer at the rate greater than 0.6 % and cinnamon at the rate greater than 0.4 % was found to be acceptable. Addition of black pepper, cardamom or clove at the rate of 0.6 % or cinnamon at the rate of 0.4 % 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. Among the spices studied, cardamom was found to be the best spice to improve shelf life of paneer up to 28 days as $7\pm 1^\circ\text{C}$ of storage.

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