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Effect of Traditional Spices on the Quality and Antioxidant Potential of Paneer Prepared from Buffalo Milk

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Abstract: This study aims to evaluate the effect of different spices (black pepper, cumin, clove, nigella, cardamom, and cinnamon) on the physicochemical characteristics and microbial quality, as well as antioxidant potential, of paneer during storage. Different types of spices were incorporated into the paneer at different levels (0.2 and 0.3%). In addition to paneer, the antioxidant potential of spices was also investigated. The results concerning total plate counts (TPC) or yeast and molds (Y & M) ($\log_{10}$ CFU/g) of all treatments were substantially ($p < 0.05$) increased during storage. Generally, all freshly prepared spicy paneer and control had higher sensory scores for all the sensory characteristics which declined during subsequent storage. All the paneer samples having 0.3% spices showed very slight variations (nonsignificant) in sensory score of all the attributes of their relative samples containing 0.2% spice. The incorporation of spices into the paneer matrix also showed promising results concerning all the above-mentioned attributes revealing antioxidant potential. There was significant ($p < 0.05$) effect of treatments and storage days on antioxidant potential of paneer. The freshly prepared control paneer (P_0) showed the lowest total phenol (TP), total flavonoids (TF), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), reducing power (RP), and total antioxidant capacity (TAC) values compared to all the spicy paneer (treatments). The freshly prepared control paneer (P_0) showed the lowest antioxidant potential compared to all the spicy paneer (treatments). The maximum antioxidant potential was observed in the paneer having 0.3% clove (P_6). All the spicy paneer showed increasing trend of all the attributes, showing antioxidant potential up to 6 days of storage, but afterwards, the activities were slightly decreased. It may be concluded that spicy paneer would be considered as a functional dairy product with enhanced sensory and antioxidant properties, and shelf stability.

Keywords: spices; paneer; storage; proximate; composition; sensory evaluation; antioxidant potential

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1. Introduction

Among the dairy products, paneer is considered a conventional soft cheese variety, produced by heat and acid coagulation of the milk [1]. It is used in the preparation of many sweet products such as rasogolla, rasamalai, and sandesh, and it is also used in culinary dishes and snacks, thereby deemed as a rich source of fat, minerals, vitamins, and high-quality proteins.

Spices are reported to have been used for culinary and medicinal purposes for centuries. In addition to improving the taste and color of food as well as beverages, they also offer protection from both acute and chronic diseases [2]. It is obvious that regular

use of spicy foods is associated with a minimal risk of death from ischemic heart diseases and cancer [2]. The health aspects ascribed to spices include antimicrobial, antioxidant, anti-inflammatory, anti-Type 2 diabetes, etc. [3,4].

Among the spices, black pepper is a popular spice and is locally identified as "kali mirch". Owning to its carminative property, immune enhancer ability, and antimicrobial activity, it is known as "Black Gold" or "The King of Spices" [5]. Gülçin et al. [6] found strong antioxidant and radical scavenging action of the extracts of black pepper seeds due to presence of phenolic compounds. According to the findings of [7], adding black pepper and piperine to a diet can lessen the oxidative stress that a high-fat diet causes in the cells of rats.

Clove (*Syzygium aromaticum* L.) is locally known as "long" and is reported to have strong antioxidant activity [8,9] owning to existence of tocopherol, ascorbates, and phenolic compounds. Moreover, clove oil contains eugenol followed by eugenyl acetate, β-caryophyllene, gallic acid, caryophyllene, and α-humulene [10–12], and eugenol is considered to be the main bioactive compound of clove oil [13]. Cumin (*Cuminum cyminum* L.) is locally known as "zeera" and is widely used in cooking due to its unique fragrance [14].

The *Nigella sativa* (locally called kalonji) is an annual herb whose essential oil could be used as natural antifungal agent in soft cheese [15]. Moreover, *N. sativa* oil is also reported to have marked antioxidant activity and anti-inflammatory properties due to presence of a high level of thymoquinone [16].

Cardamom (*Elettaria cardamomum*) is locally known as "ilaichi". It comes from a perennial herbaceous plant and is reported to contain predominant compounds including α-terpineol, α-terpinyl acetate, 1,8-cineole, β-linalool, and sabinene [17,18]. Owing to the existence of such compounds in crucial oil of cardamom, it is reported to have antioxidant potential [17,19,20].

The bark of cinnamon, locally known as "darchini", is typically employed as a flavoring agent and a spice. Many studies confirmed that cinnamon contains many properties including antidiabetic, antioxidant, antimicrobial, and anticancer, and inhibits cardiovascular diseases [21].

Since the extracts from spices are reported to have antioxidant activities [8,16,20,22–24], it would be of great interest for the food industries to incorporate such kinds of spices (powders or their extracts) into their products for their value addition.

Consumption of different spices for different dishes is a well-established trend in our society. Therefore, to induce the trend of paneer consumption, it would be enticing to incorporate spices in the matrix of paneer. In addition, it would be a good idea to use products having negligible quantities of fat as the people with hypertensive conditions may consume the aforementioned type of products. In the present study, skimmed milk was used for the preparation of paneer, which would be good for the people with hypertensive conditions.

It may be anticipated that the consumption of paneer would be increased after making it somewhat spicy. Moreover, owing to the presence of health aspects of spices as well as paneer, the spicy paneer would have more beneficial effects regarding health. Different previous studies focused on antioxidant activity of whey cheese [25], burfi (milk-based confection) [26], and paneer [27] through incorporation or application of black cumin (*Nigella damascena* L.), clove bud essential oil, and clove essential oil, respectively. It is also reported that spices have been incorporated in different dairy products such as cheese, butter, ghee, and ice-cream [28], but so far, no comprehensive studies have been carried out concerning monitoring of antioxidant potential of paneer through incorporation of powders from different traditionally used spices at different concentrations. Therefore, this study aimed to monitor the physicochemical and microbiological quality and antioxidant potential of spicy paneer during storage. Moreover, sensory evaluation was also performed to check the suitability of different spicy paneer.

2. Materials and Methods

2.1. Procurement of Raw Material

Buffalo milk used for paneer manufacturing was taken from a local farm at Sargodha city (Pakistan), and spices, i.e., black pepper, cumin, clove, nigella, cardamom, and cinnamon, were acquired from the city's local market. All the spices were ground separately to make powders. Fresh buffalo skimmed (9.34% solids not fat (SNF), 0.5% fat) milk was used for the manufacturing of paneer. Aluminum foil, plastic sheet, and muslin cloth were purchased from a local store in Sargodha.

2.2. Production of Spicy Paneer

Paneer was produced from fresh raw skimmed buffalo milk as suggested by Khan et al. [29], with a few variations. First of all, pH of milk was computed employing a pH meter as suggested by Ardö [30], whereas fat was explored by the Gerber–van Gulik method of Ardö and Polychroniadou [30]. For the preparation of one batch of paneer, 40 L of skimmed buffalo milk was coagulated using diluted lemon juice at 75 °C after its pasteurization (82 °C for 5 min). The whey was drained off using a muslin cloth. The coagulum acquired after whey drainage was firstly pressed for 5 min. The paneer matrix obtained was crushed and made into grains. The whole paneer matrix was separated into 13 equal parts and spices were added in different ratios (see treatment plan, Table 1).

Table 1. Treatment plan of the present study.

Treatments	Spices (% by Weight of Expected Yield) Addition into Paneer Matrix
P_0	Control, without spices
P_1	Black pepper powder (0.2%)
P_2	Black pepper powder (0.3%)
P_3	Cumin powder (0.2%)
P_4	Cumin powder (0.3%)
P_5	Clove powder (0.2%)
P_6	Clove powder (0.3%)
P_7	Nigella powder (0.2%)
P_8	Nigella powder (0.3%)
P_9	Cardamom powder (0.2%)
P_{10}	Cardamom powder (0.3%)
P_{11}	Cinnamon powder (0.2%)
P_{12}	Cinnamon powder (0.3%)

Then, the paneer of each treatment was separately pressed for 60 min. The manufacturing of paneer was carried out separately in the same manner three times. In our preliminary trials, spices were added into the paneer matrix at different ratios, but, finally, two levels were selected, i.e., 0.2% and 0.3%, based on sensory perception. By increasing the quantities of spices into paneer, irritating taste was being developed as assessed by the panel of assessors.

2.3. Sampling of Paneer

The paneer from each treatment was cut into four identical pieces and stored for 3, 6, and 9 days at refrigerated temperature. The remaining one piece was frozen instantly (0 day) till evaluated. All paneer's pieces were enveloped in polyethylene films with an outer covering of aluminum foil. In our preliminary trials, the spicy paneer samples were also stored for up to 12 days, but their outer appearance and color were not liked by the panel of assessors due to slight mold growth over the surfaces of paneer. Therefore, paneer samples were stored for only up to 9 days for all kind of analysis.

2.4. Preparation of Water Soluble Extract (WSE) of Spicy Paneer and Spices

The WSEs of paneer were prepared according to the procedure described by [31] with a few changes. Briefly, 20 g of paneer was included into 60 mL of distilled water and stirred for one hour at room temperature. The pH was adjusted at 4.6 and centrifuged at 10 kg for 10 min at 4 °C. The supernatant was designated as WSE and filtered further via Whatman filter paper. These were immediately frozen at −20 °C. The WSEs of spices were also prepared. Approximately 0.01 kg powder of each spice was added into 200 mL distilled water and mixed for 180 min at room temperature. Then, the mixture was centrifuged at 10 kg for 10 min at 4 °C. The mixture was filtered, and the filtrate was immediately frozen at −20 °C.

2.5. Physicochemical Characteristics of Spicy Paneer

The pH of paneer was examined according to the method of Ardö and Polychroniadou [30] using a pH meter, whereas the acidity of paneer samples was explored by AOAC method [32]. The IDF standard 4/ISO 5534 was employed for determining the paneer moisture content (MC) [33]. The paneer fat contents were computed by the method of Ardö and Polychroniadou [30]. Total nitrogen (%) was computed by the well-known IDF standard 20B [34]. Additionally, protein contents (%) were computed by multiplying total nitrogen TN (%) by 6.38.

2.6. Microbiological Analysis of Spicy Paneer

Total plate count (TPC, log CFU/g of paneer) and yeast and mold (Y & M, log CFU/g of paneer) counts were carried out as described by [35]. Out of each treatment, 10 g of crushed paneer sample was homogenized in a domestic blender for 5 min by inserting 90 mL of sterilized sodium citrate (2%, pH 7.5) water. Different dilutions of the above suspension were made up to10^{-4} and then 1 mL of diluted samples were plated on plate count agar media (Titan Biotech Ltd., Rajasthan, India). The TPC was computed after incubating the plates for 2 days at 37 °C. The counts of Y & M were also carried out in a similar way (after 48 h at 30 °C), employing potato dextrose agar (HiMedia, Mumbai, Maharashtra 400086, India).

2.7. Sensory Evaluation of Spicy Paneer

Sensory evaluated of paneer was performed using a 9-point hedonic scale for every attribute including appearance, flavor, texture, color, and overall acceptability [36]. A group of 15 people, including faculty and students from our institute, were selected for evaluation of the paneer.

2.8. Determination of Total Phenolic Contents (TP)

The TP of paneer were explored using the method given by Reis et al. [37]. A 1 mL aliquot of WSE of both spices and paneer was used in the assay, and gallic acid (in ethanol) was employed as a standard. One mL of Folin–Ciocalteu reagent (10%) was added into each sample. After being vortexed, 2 mL of sodium carbonate (20%) solution was included. The absorbance was evaluated at 760 nm using a spectrophotometer (Halo DB-20, UV–Vis double beam, Dynamica Scientific Ltd., Livingston, UK) after incubating the mixture for 60 min at 30 °C.

2.9. Determination of Total Flavonoid Contents (TF)

The TF were also explored via the spectrophotometric method given by Jia et al. [38]. A 1 mL aliquot of WSE of both spices and paneer was used in the assay, and the results were articulated as mg catechin equivalent (CE) per 100 g of paneer or spices. Approximately 75 µL of sodium nitrite (5%) solution was added into each sample. After vortexing for 1 min, 150 µL of aluminum chloride (10%) solution was also inserted. The absorbance was evaluated at 510 nm, employing the spectrophotometer after inserting 500 µL of 1 M NaOH into the above mixture. All calculations were performed in triplicate.

2.10. Determination of Reducing Power (RP) or Ferricyanide/Prussian Blue Assay

The RP of WSE of paneer was computed via method as given by Reis et al. [37] with slight changes. A 1 mL aliquot of WSE of both spices and paneer was used in the assay, and calculation was performed employing the standard curve of Trolox as mg of Trolox per 100 g of paneer or spices. The sample was mixed into 500 µL sodium phosphate (Na_3PO_4) buffer (0.2 M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN_6)$). After incubation of the mixture for 20 min at 50 °C, 500 µL of trichloroacetic acid (%) was included and vortexed. The mixture was centrifuged (Hermle Labortechnik GmbH Siemensstr-25 D-78564 Wehingen, Germany) at 3000× *g* for 10 min at 4 °C in order to obtain clear supernatant. Then, 150 µL of ferric chloride (0.1%) was inserted to the supernatant and the absorbance was computed employing spectrophotometer (Halo DB-20, UV–Vis double beam, Dynamica Scientific Ltd., Livingston, UK) at 700 nm.

2.11. Determination of DPPH Radical Scavenging Activity Assay

The potential for WSE of paneer to scavenge 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) was acquired via the method of Yi et al. [39] with some modifications. A 1 mL aliquot of WSE of both spices and paneer was used in the assay, and DPPH radical scavenging activity was calculated from the Trolox standard (TE). Approximately 2 mL of DPPH (60 µM in absolute ethanol) solution was added into each sample. The mixture was vortexed and then incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm, employing the spectrophotometer. All calculations were performed in triplicate.

2.12. Determination of Total Antioxidant Capacity (TAC) Assay

The TAC of paneer was determined employing a similar method to that given by Prieto et al. [40]. A 1 mL aliquot of WSE of both spices and paneer was used in the assay and TAC was calculated using the Trolox standard. Approximately 4 mL of reagent (0.6 M sulfuric acid, 28 mM Na_3PO_4, and 4 mM ammonium molybdate) solution was added into each sample. After incubating the mixture for 95 min at 90 °C, the absorbance was computed at 695 nm using a spectrophotometer. All calculations were assessed in triplicate, while experiments were performed in duplicate.

2.13. Statistical Analysis

Statistical software (Minitab 16, Minitab, LLC, State College, PA, USA) was employed for statistical analysis using two-way ANOVA and Tukey's test for pairwise assessment at a level of $p < 0.05$. The normal distribution of the data was tested by Shapiro–Wilk test, and the normality assumptions were observed to be satisfied.

3. Results

3.1. Physicochemical Characteristics of Spicy Paneer

Data regarding physicochemical characteristics of all the paneer samples (control and spicy paneer) made in the current study are presented in Table 2. The initial pH of all the samples (treatments) was in the range 5.65–5.69 and significantly ($p < 0.05$) decreased during storage. That decrease in pH was slight after subsequent storage period. Such kind of decreasing trend might be due to the activities of contaminated microorganisms resulting in the accumulation of more and more acids during storage. The most significant decreasing trend was observed in the control paneer, reaching 5.32 at the end of the storage period (9 days). The initial MCs of all the treatments were around 58%, but spicy paneer showed slightly lower MCs compared to the control treatment, which might be due to slightly less water residing in the paneer matrix. The MCs of all the paneer were significantly decreased during storage which may have been caused by moisture loss from the paneer matrix during storage. The fat contents of fresh paneer samples were less than 4%, which slightly increased during subsequent storage. There were no great differences in the fat contents among all treatments. The skimmed milk was used for the preparation of paneer

in all the treatments; therefore, the paneer showed very low quantities of fat. Similarly, all the freshly prepared paneer (control and spicy) showed around 32% of protein. Even though there was significant effect of treatments and days of protein contents of all the paneer samples, that increase was slight during successive storage period. The increased contents of protein during storage might be due to increase in dry matter during storage. A similar trend was also experienced in the ash contents of all the paneer samples during storage. Although there was significant effect of storage on the protein contents of paneer, that increasing trend was very slight. All the prepared paneer showed ash contents in the range 2.42–3.00%. The maximum fat to dry matter ratio (fat/DM) was observed in freshly prepared control paneer (8.83%) followed by freshly prepared paneer incorporated with cardamom (8.71–8.79%). The increased contents of fat/DM of the control paneer might be due to lower contents of DM compared to other paneer samples having spice powders. The MCs have an inverse relationship with dry matter. Most of the paneer samples showed increasing trend regarding fat/DM. In general, it was observed that all the paneer samples having 0.3% spices showed nonsignificant variations concerning physicochemical characteristics of their relative samples containing 0.2% spice.

3.2. Microbiological Quality of Spicy Paneer

Figure 1 represents the data depicting the microbiological quality of spicy paneer during storage. The counts of microbes in paneer may be affected due to the quality of milk, heat treatment of milk, hygienic practices during handling and manufacturing, and post-manufacture conditions. The TPC values of freshly prepared control treatment were 3.04 ($\log_{10}$ CFU/g), whereas all other freshly prepared spicy paneer depicted slightly lower values (2.78–3.00 $\log_{10}$ CFU/g). The results concerning TPC of all treatments were significantly ($p < 0.05$) increased during storage, which showed that the counts steadily increased after each successive storage. At the end of the storage period, the control treatment showed the maximum TPC values up to 5.58 ($\log_{10}$ CFU/g), whilst most of the treatments showed significant ($p < 0.05$) variations in comparison to the control. All the spicy paneer treatments showed TPC values around 5.30 ($\log_{10}$ CFU/g) at the end of the investigated storage period (9 days). All the paneer samples having 0.3% spices showed very slight variations (nonsignificant) in TPC counts of their relative samples containing 0.2% spice. A similar trend was also seen considering Y & M ($\log_{10}$) counts. The Y & M values ($\log_{10}$) of freshly prepared control treatment showed 2.60 ($\log_{10}$ CFU/g), whereas all other freshly prepared spicy paneer showed slightly lower values (nonsignificant). The values of Y & M were significantly increased during storage in control paneer, reaching up to 3.45 ($\log_{10}$ CFU/g), which was the highest among all other treatments. All the paneer samples having 0.3% spices showed very slight variations (nonsignificant) in Y & M counts of their relative samples containing 0.2% spice. Thus, microbial counts raised with the progress of storage in all treatments having spices, but to a lesser extent as compared to control paneer. It was suspected that fungus might began to grow very slightly on the paneers' surfaces that were stored for 12 days. The taste of 12-days-stored spicy paneer was slightly changed.

Table 2. Proximal characteristics (means ± SD) of paneer supplemented with different spices during storage.

Treatments	Time (days)	pH	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fat/DM
Control paneer (P_0)	0	5.67 ± 0.06 a	58.33 ± 1.65 a	3.68 ± 0.56 a	31.03 ± 0.98 f	2.53 ± 0.03 h–j	8.83 ± 0.23 a
	3	5.48 ± 0.05 a–i	56.93 ± 1.33 a–e	3.88 ± 0.43 a	32.34 ± 1.13 a–f	2.70 ± 0.04 c–j	9.02 ± 0.41 a–d
	6	5.37 ± 0.04 g–i	56.96 ± 1.84 a–e	3.88 ± 0.63 a	32.56 ± 1.19 a–f	2.66 ± 0.06 b–j	9.04 ± 0.54 a–c
	9	5.32 ± 0.06 i	55.26 ± 1.36 d–k	3.91 ± 0.71 a	32.93 ± 0.92 a–e	2.84 ± 0.04 b–g	8.75 ± 0.14 b–j
Black pepper (0.2%) paneer (P_1)	0	5.68 ± 0.06 a	56.80 ± 1.52 a–f	3.71 ± 0.59 a	32.43 ± 0.87 a–f	2.83 ± 0.07 b–h	8.58 ± 0.29 a–f
	3	5.56 ± 0.07 a–g	55.80 ± 1.47 b–k	3.80 ± 0.60 a	32.63 ± 0.95 a–f	2.98 ± 0.03 a–c	8.59 ± 0.26 a–i
	6	5.48 ± 0.09 b–i	55.06 ± 1.32 d–k	3.86 ± 0.75 a	33.40 ± 0.84 a–d	2.99 ± 0.04 a–c	8.61 ± 0.49 b–m
	9	5.45 ± 0.07 b–i	54.33 ± 1.21 h–k	3.90 ± 0.64 a	33.76 ± 0.90 ab	3.16 ± 0.05 a	8.54 ± 0.39 e–n
Black pepper (0.3%) paneer (P_2)	0	5.67 ± 0.05 a	56.73 ± 1.89 a–g	3.68 ± 0.69 a	31.96 ± 1.21 b–f	2.78 ± 0.06 b–i	8.51 ± 0.26 a–g
	3	5.47 ± 0.04 b–i	55.63 ± 1.56 c–k	3.76 ± 0.59 a	32.76 ± 0.86 a–f	2.90 ± 0.04 a–f	8.49 ± 0.24 b–k
	6	5.47 ± 0.09 b–i	54.56 ± 1.69 f–k	3.86 ± 0.62 a	33.46 ± 0.81 a–c	2.92 ± 0.05 a–f	8.50 ± 0.19 d–n
	9	5.40 ± 0.04 f–i	54.50 ± 1.59 f–k	3.92 ± 0.81 a	33.90 ± 0.69 a	2.91 ± 0.03 a–c	8.62 ± 0.14 c–n
Cumin (0.2%) paneer (P_3)	0	5.69 ± 0.03 a	56.30 ± 1.48 a–j	2.80 ± 0.66 fg	32.33 ± 0.78 a–f	2.42 ± 0.03 j	6.41 ± 0.10 i–p
	3	5.58 ± 0.07 a–f	55.93 ± 1.55 b–j	2.89 ± 0.79 e–g	32.76 ± 0.95 a–f	2.51 ± 0.05 i	6.56 ± 0.28 i–p
	6	5.51 ± 0.09 a–i	55.23 ± 1.84 d–k	2.92 ± 0.55 d–g	32.33 ± 1.17 a–f	2.55 ± 0.07 ij	6.53 ± 0.23 l–p
	9	5.47 ± 0.06 b–i	54.03 ± 1.57 j–k	2.98 ± 0.68 c–g	32.83 ± 0.85 a–f	2.65 ± 0.08 e–j	6.49 ± 0.41 o–p
Cumin (0.3%) paneer (P_4)	0	5.67 ± 0.05 a	57.06 ± 1.69 a–d	2.78 ± 0.73 fg	31.56 ± 0.88 d–f	2.52 ± 0.09 h–j	6.47 ± 0.47 f–o
	3	5.55 ± 0.09 a–h	55.46 ± 1.39 c–k	2.84 ± 0.83 fg	32.16 ± 0.96 a–f	2.53 ± 0.05 g–j	6.39 ± 0.56 l–p
	6	5.45 ± 0.04 b–i	54.16 ± 1.49 i–k	2.93 ± 0.81 d–g	33.16 ± 0.91 a–e	2.61 ± 0.04 e–j	6.40 ± 0.26 o–p
	9	5.41 ± 0.05 e–i	53.46 ± 1.44 k	2.98 ± 0.79 c–g	32.83 ± 1.16 a–f	2.81 ± 0.03 b–i	6.41 ± 0.37 p
Clove (0.2%) paneer (P_5)	0	5.67 ± 0.04 a	56.60 ± 1.77 a–h	2.77 ± 0.52 g	31.70 ± 1.17 c–f	2.77 ± 0.06 b–i	6.38 ± 0.36 h–p
	3	5.52 ± 0.08 a–h	55.63 ± 1.79 c–k	2.82 ± 0.67 f–g	32.73 ± 1.11 a–f	2.84 ± 0.05 b–g	6.38 ± 0.27 k–p
	6	5.51 ± 0.06 a–i	55.23 ± 1.68 d–k	2.89 ± 0.56 e–g	32.73 ± 0.97 a–f	2.95 ± 0.06 a–e	6.46 ± 0.38 m–p
	9	5.46 ± 0.03 b–i	54.63 ± 1.59 e–k	2.97 ± 0.62 c–g	33.46 ± 0.85 a–c	2.97 ± 0.04 a–d	6.54 ± 0.36 n–p
Clove (0.3%) paneer (P_6)	0	5.68 ± 0.09 a	56.66 ± 1.66 a–h	2.85 ± 0.69 fg	32.40 ± 0.98 a–f	2.80 ± 0.06 b–i	6.58 ± 0.22 f–o
	3	5.47 ± 0.06 b–i	56.10 ± 1.97 a–j	3.03 ± 0.76 b–f	32.46 ± 0.91 a–f	2.94 ± 0.03 a–f	6.90 ± 0.38 g–o
	6	5.52 ± 0.07 a–h	55.53 ± 1.69 c–k	3.15 ± 0.83 b–d	33.40 ± 0.93 a–d	2.96 ± 0.04 a–e	7.09 ± 0.27 h–p
	9	5.36 ± 0.05 h–i	54.53 ± 1.74 f–k	3.19 ± 0.91 bc	33.33 ± 1.19 a–e	3.12 ± 0.07 ab	7.02 ± 0.42 l–p
Nigella (0.2%) paneer (P_7)	0	5.67 ± 0.04 a	56.43 ± 1.59 a–i	2.86 ± 0.63 e–g	32.06 ± 1.15 a–f	2.94 ± 0.07 a–f	6.57 ± 0.51 h–p
	3	5.56 ± 0.09 a–g	55.60 ± 1.56 c–k	3.03 ± 0.74 b–f	32.36 ± 0.89 a–f	3.03 ± 0.08 a–c	6.83 ± 0.15 i–p
	6	5.51 ± 0.04 a–i	55.06 ± 1.48 d–k	3.10 ± 0.85 b–e	32.90 ± 0.87 a–e	3.07 ± 0.04 ab	6.90 ± 0.32 j–p
	9	5.47 ± 0.07 b–i	54.23 ± 1.46 i–k	3.22 ± 0.96 bc	33.46 ± 0.90 a–c	3.08 ± 0.06 ab	7.04 ± 0.55 m–p

Table 2. *Cont.*

Treatments	Time (days)	pH	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fat/DM
Nigella (0.3%) paneer (P_8)	0	5.66 ± 0.08 a	56.00 ± 1.38 a–j	2.97 ± 0.91 c–g	32.96 ± 1.15 a–e	2.94 ± 0.08 a–f	6.75 ± 0.23 h–p
	3	5.59 ± 0.05 a–f	55.06 ± 1.11 d–k	3.00 ± 0.59 c–g	33.06 ± 0.96 a–e	3.05 ± 0.09 ab	6.69 ± 0.27 l–p
	6	5.55 ± 0.03 a–h	54.43 ± 1.72 g–k	3.15 ± 0.66 b–d	33.43 ± 0.99 a–c	3.07 ± 0.05 ab	6.92 ± 0.16 m–p
	9	5.48 ± 0.08 b–i	54.13 ± 1.38 i–k	3.26 ± 0.78 b	33.66 ± 0.93 ab	3.17 ± 0.03 a	7.11 ± 0.37 m–p
Cardamom (0.2%) paneer (P_9)	0	5.68 ± 0.04 a	57.80 ± 1.92 a–c	3.71 ± 0.98 a	31.96 ± 1.18 b–f	2.83 ± 0.07 b–h	8.79 ± 0.27 ab
	3	5.52 ± 0.08 a–h	56.80 ± 1.68 a–f	3.80 ± 0.91 a	32.63 ± 1.18 a–f	2.98 ± 0.04 a–c	8.80 ± 0.48 a–e
	6	5.44 ± 0.03 c–i	56.06 ± 1.62 a–j	3.86 ± 0.87 a	33.40 ± 1.17 a–d	3.02 ± 0.06 a–c	8.76 ± 0.15 a–h
	9	5.41 ± 0.06 e–i	55.33 ± 1.83 d–k	3.90 ± 0.68 a	33.76 ± 1.16 ab	3.16 ± 0.08 a	8.72 ± 0.12 b–j
Cardamom (0.3%) paneer (P_{10})	0	5.67 ± 0.05 a	57.73 ± 1.59 a–c	3.68 ± 0.69 a	31.96 ± 0.98 b–f	2.78 ± 0.05 b–i	8.71 ± 0.25 ab
	3	5.43 ± 0.08 c–i	56.63 ± 1.69 a–h	3.76 ± 0.88 a	32.76 ± 0.89 a–f	2.90 ± 0.03 a–f	8.65 ± 0.28 a–f
	6	5.43 ± 0.05 c–i	55.56 ± 1.60 c–k	3.86 ± 0.69 a	33.46 ± 0.86 a–c	2.92 ± 0.06 a–f	8.68 ± 0.16 a–j
	9	5.36 ± 0.09 hi	55.50 ± 1.89 c–k	3.92 ± 0.74 a	33.90 ± 0.97 a	3.04 ± 0.08 a–c	8.82 ± 0.14 a–i
Cinnamon (0.2%) paneer (P_{11})	0	5.68 ± 0.05 a	57.30 ± 1.58 a–d	2.80 ± 0.82 fg	32.33 ± 1.17 a–f	2.42 ± 0.06 j	6.55 ± 0.29 e–n
	3	5.54 ± 0.06 a–h	56.93 ± 1.47 a–e	2.89 ± 0.85 e–g	32.76 ± 1.12 a–f	2.51 ± 0.09 ij	6.72 ± 0.51 e–n
	6	5.47 ± 0.09 b–i	56.23 ± 1.84 a–j	2.92 ± 0.77 d–g	32.33 ± 1.15 a–f	2.55 ± 0.04 ij	6.69 ± 0.31 h–p
	9	5.43 ± 0.05 d–i	55.03 ± 1.42 d–k	2.98 ± 0.71 c–g	32.83 ± 0.93 a–f	2.65 ± 0.07 e–j	6.65 ± 0.13 l–p
Cinnamon (0.3%) paneer (P_{12})	0	5.68 ± 0.08 a	58.06 ± 1.27 ab	2.78 ± 0.73 fg	31.50 ± 0.85 e–f	2.52 ± 0.05 h–j	6.64 ± 0.32 b–l
	3	5.51 ± 0.04 a–i	56.46 ± 1.28 a–i	2.84 ± 0.96 fg	32.16 ± 0.99 a–f	2.53 ± 0.07 g–j	6.53 ± 0.44 h–p
	6	5.41 ± 0.08 f–i	55.16 ± 1.85 d–k	2.93 ± 0.85 d–g	33.16 ± 0.94 a–e	2.63 ± 0.08 f–j	6.54 ± 0.41 l–p
	9	5.37 ± 0.09 g–i	54.46 ± 1.89 f–k	2.98 ± 0.74 c–g	32.83 ± 1.14 a–f	2.81 ± 0.04 b–i	6.55 ± 0.11 n–p

Means with different letters in the same column present significant ($p < 0.05$) differences among treatments and storage.

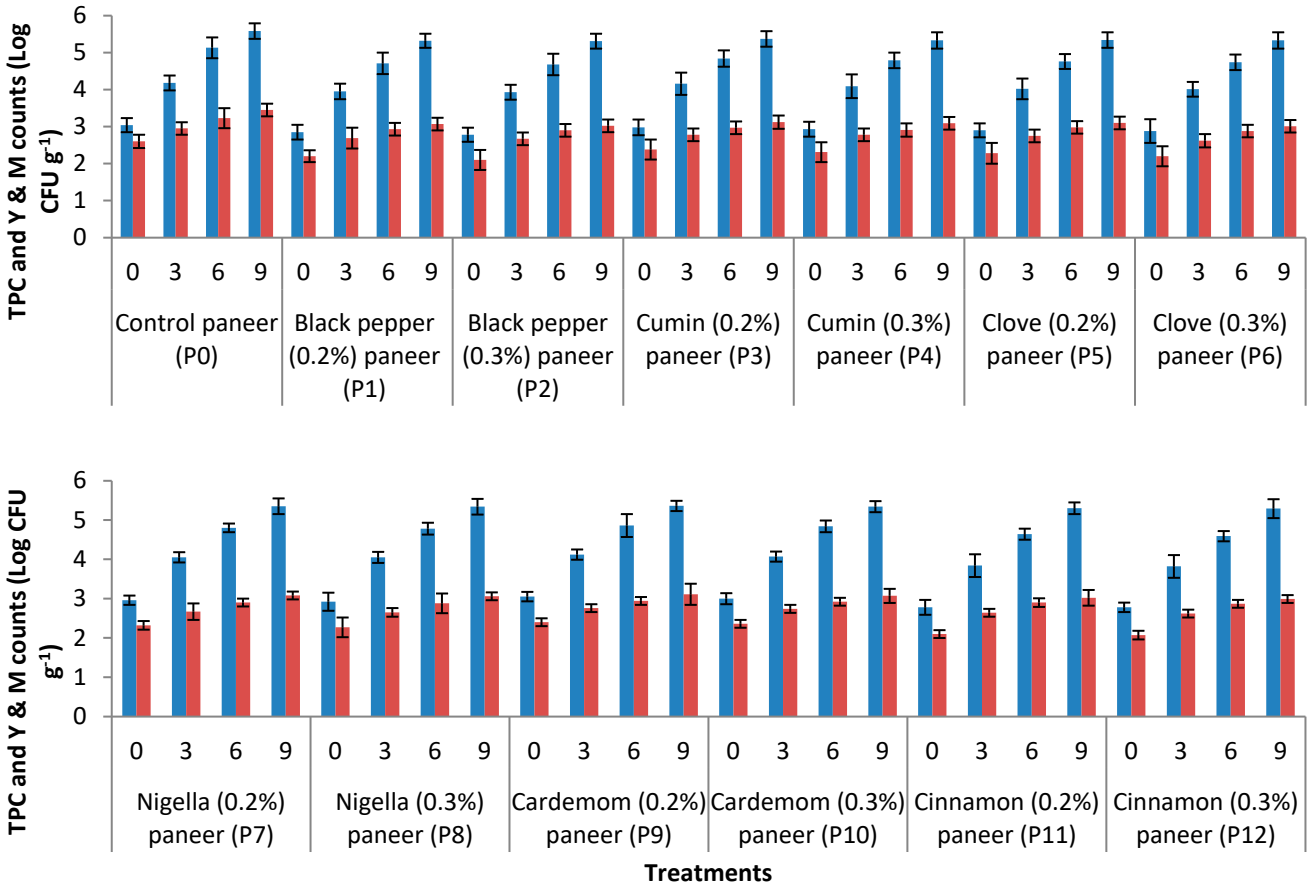

Figure 1. TPC (means ± SD, blue bars) and Y & M (means ± SD, red bars) of spicy paneer during storage at 5 °C.

3.3. Sensorial Quality of Prepared Paneer

Figure 2 shows the data regarding sensorial quality of paneer formulated in this study during storage. Different sensory characteristics, i.e., color, texture, appearance, flavor, and overall acceptability, were used for evaluating sensorial quality of experimentally prepared paneer. These sensory attributes illustrate the acceptance of the prepared paneer. Generally, all freshly prepared spicy paneer and control treatment had higher sensory scores for all the sensory characteristics, which declined during subsequent storage. Moreover, all the spicy paneer had higher sensory scores than control paneer at each storage period. All the paneer samples having 0.3% spices showed very slight variations (nonsignificant) in sensory score of all the attributes of their relative samples containing 0.2% spice. Among all the spicy paneer, paneer having black pepper (0.2%) was much liked by the people, followed by paneer with cumin (0.3%). The control paneer stored for 12 days showed visible mold growth on the surfaces. The taste of spicy paneer was also changed a little bit. Therefore, all the paneers stored for 12 days were excluded in the current study.

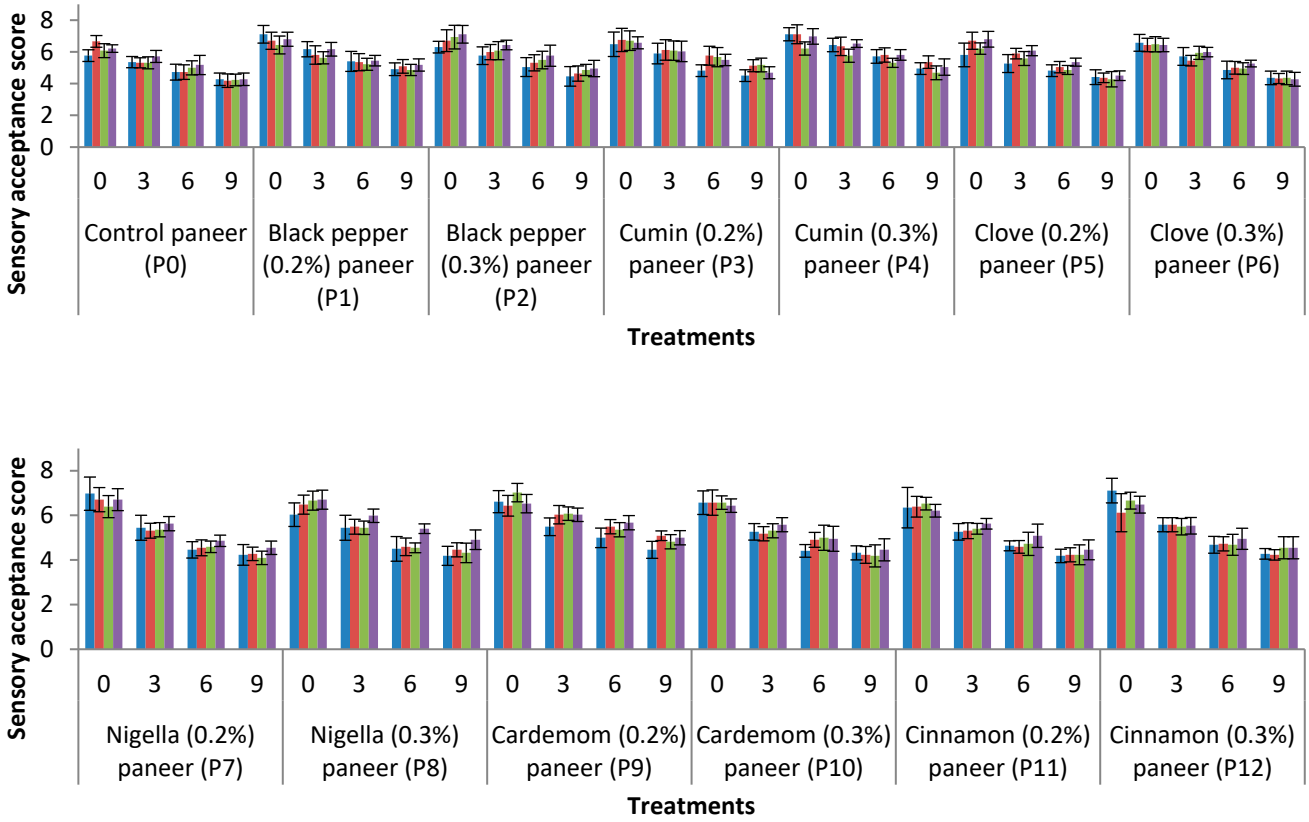

Figure 2. Sensory acceptance scores regarding appearance and color (means ± SD, blue bars), flavor (means ± SD, red bars), texture (means ± SD, green bars), and overall acceptability (means ± SD, purple bars) of spicy paneer during storage at 5 °C.

3.4. Antioxidant Potential of Spices and Spicy Paneer

Figure 3 shows the results regarding antioxidant potential of spices, whereas Figures 4 and 5 depict data regarding the antioxidant potential of paneer formulated in this study. There was a significant ($p < 0.05$) effect of spices on the values of TP, TF, RP, TAC, and DPPH radical scavenging activity. The maximum TP values were shown by cumin (354.60 mg GAE/100 g), followed by clove (142.53 mg GAE/100 g) and black pepper (109.00 mg GAE/100 g). The maximum TF values were also shown by cumin (526.40 mg CE/100 g), followed by clove (247.15 mg CE/100 g) and cinnamon (144.52 mg CE/100 g). Regarding RP, the maximum RP values were shown by cumin (512.04 mg TE/100 g), followed by cinnamon (162.66 mg TE/100 g) and clove (153.79 mg TE/100 g). The highest values of DPPH radical scavenging activity were shown by cumin (18.89 mM TE/g), followed by Nigella (16.59 mM TE/g) and cinnamon (11.18 mM TE/g). The maximum values of TAC were shown by cumin (791.45 mg TE/100 g), followed by clove (539.61 mg TE/100 g). All other spices also showed moderate values in connection to total antioxidant capacity.

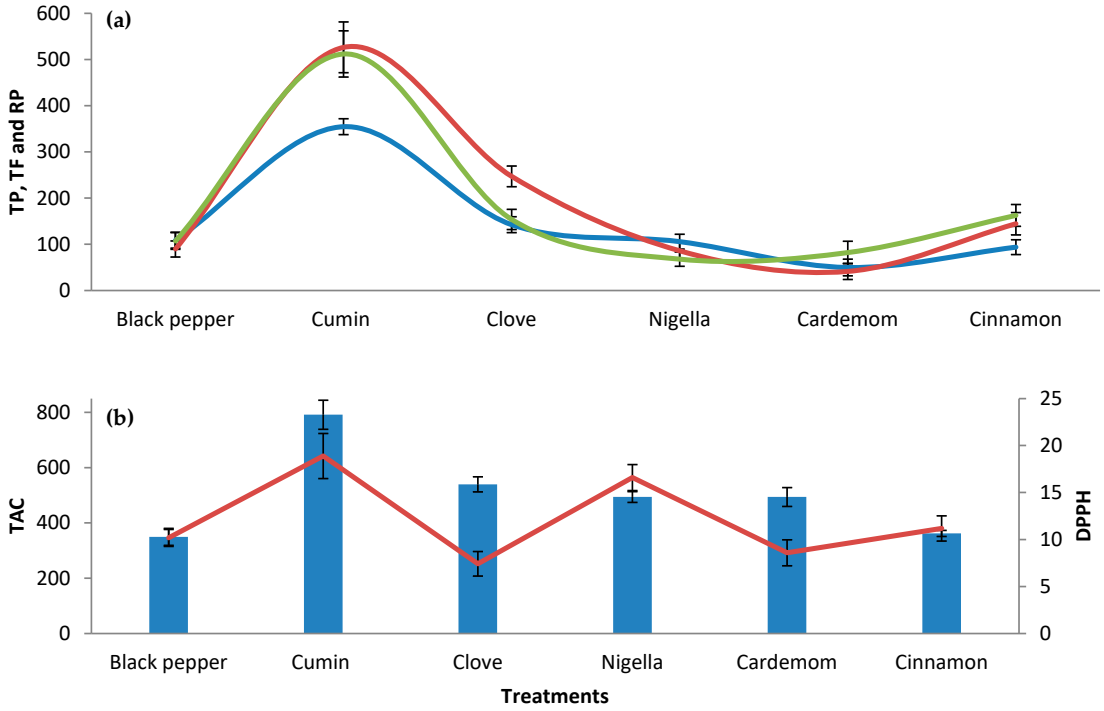

Figure 3. (**a**) Total phenolics (TP, means ± SD, mg GAE/100 g) (blue line), total flavonoids (TF, means ± SD, mg CE/100 g) (red line), reducing power (RP, means ± SD, mg TE/100 g) (green line), (**b**) total antioxidant activity (TAC, means ± SD) (blue bar, mg TE/100 g), and DPPH radical scavenging activity (means ± SD, red line, mM TE/g) of spices used for incorporation into paneer.

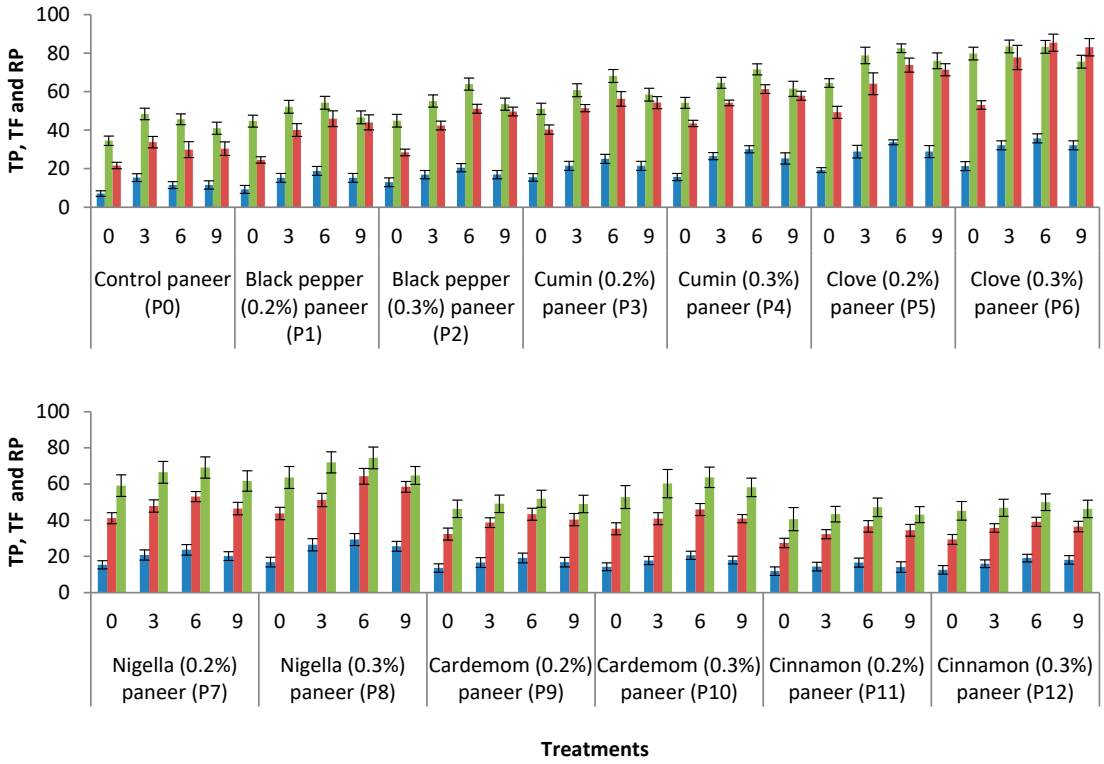

Figure 4. Total phenolics (TP, means ± SD, mg GAE/100 g) (blue bar), total flavonoids (TF, means ± SD, mg CE/100 g) (red bar), and reducing power (RP, means ± SD, mg TE/100 g) (green bar) of spicy paneer during storage at 5 °C.

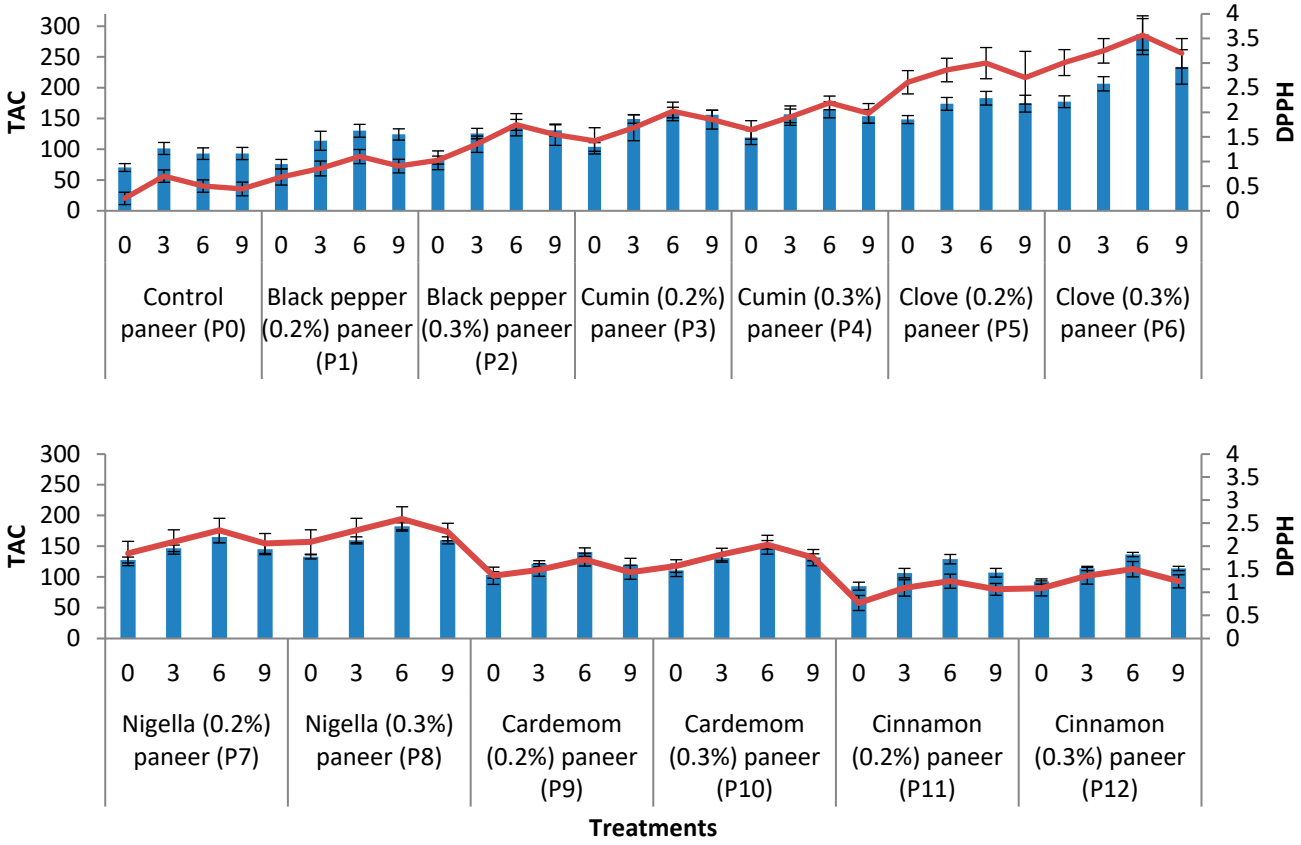

Figure 5. Total antioxidant activity (TAC, means ± SD) (blue bar, mg TE/100 g) and DPPH radical scavenging activity (means ± SD, red line, mM TE/g) of spicy paneer during storage at 5 °C.

There was a significant effect of treatments and storage days on the values of TP (Figure 4) of paneer. The freshly prepared control paneer (P_0) showed the lowest TP mean values (7.10 mg GAE/100 g) compared to the values of all other respective spicy paneer (treatments), whereas the highest value (21.36 mg GAE/100 g) was obtained by freshly prepared paneer containing 0.3% clove (P_6). All the spicy paneer showed increasing trend of TP values up to 6 days of storage, but afterwards, TP values were slightly decreased.

Regarding TF values, there was a significant effect of treatments and storage days (Figure 4). The freshly prepared control paneer (P_0) showed the lowest TF mean values (21.66 mg CE/100 g) compared to the values of all other respective spicy paneer (treatments), whereas the highest value (53.06 mg CE/100 g) was obtained by freshly prepared paneer containing 0.3% clove (P_6). All the spicy paneer showed increasing trend of TF values up to 6 days of storage, but afterwards, TF values were slightly decreased.

There was significant effect of treatments and storage days on RP of paneer (Figure 4). The freshly prepared control paneer (P_0) showed the lowest RP mean values (34.52 mg TE/100 g) compared to the values of all other respective spicy paneer (treatments), whereas the highest value (79.74 mg TE/100 g) was obtained by freshly prepared paneer having 0.3% clove (P_6). All the spicy paneer showed increasing trend of RP values up to 6 days of storage, but afterwards, RP values were slightly decreased.

There was significant effect of treatments and storage days on DPPH radical scavenging activity of paneer (Figure 5). The freshly prepared control paneer (P_0) showed the lowest DPPH mean values (0.25 mM TE/g) compared to the values of all other respective spicy paneer (treatments), whereas the highest value (3.01 mM TE/g) was obtained by freshly prepared paneer having 0.3% clove (P_6). All the spicy paneer showed increasing trend of DPPH values up to 6 days of storage, but afterwards, DPPH values were slightly decreased.

There was a significant ($p < 0.05$) effect of treatments and storage days on total antioxidant capacity of paneer (Figure 5). The freshly prepared control paneer (P_0) showed the lowest TAC mean values (70.52 mg TE/100 g) compared to the values of all other respective spicy paneer (treatments), whereas the highest value (177.27 mg TE/100 g) was obtained by freshly prepared paneer having 0.3% clove (P_6). All the spicy paneer showed increasing trend of antioxidant capacity up to 6 days of storage, but afterwards, TAC values were slightly decreased.

In general, it was observed that there was significant variation in the values of TP, TF, RP, DPPH, TAC, and RP among the paneer having different concentrations of the same spice. The control paneer (P_0) showed lower values of all these assays compared to all other treatments at all stages during the storage period.

4. Discussion

The pH at the time of milk coagulation during the manufacturing of paneer was observed to be more or less the same (around 5.65) in all the treatments, which was in agreement with the results (5.63) obtained by [26]. The more significant decreasing trend in pH of control paneer at the end of the storage period might be because of more activities of microorganisms compared to spicy paneer. Such kinds of conditions may cause more accumulation of acids during the storage period, thereby leading to more acidic conditions. The spices may cause a delay in the activities of pathogenic microorganisms, thereby keeping the freshness of paneer until 9 days of storage. The control paneer lost its freshness after 9 days. Regarding the decreasing trend of pH of paneer, the current study outcomes are in good agreement with the findings of [41,42]. The MCs (~58%) of fresh paneer satisfied the Bureau of Indian Standards [43]. A slight decrease in MCs of paneer during storage could be because of removal of MCs from paneer. Khatkar et al. [27] also observed the same kind of decreasing trend of MCs during the storage of paneer, as observed in this study. The fat contents of paneer treatments were very low because paneer was prepared from skimmed milk. On the other hand, protein contents were slightly increased during storage in the paneer matrix because of increased dry matter during storage. Moreover, protein constitutes most of the dry matter of paneer due to very low quantities of fat. The higher ash contents of all the treatments of paneer might also be due to lower quantities of fat, thereby constituting more of the dry matter. The slight increasing trend of ash contents during storage might be due to the increase in dry matter during storage.

Usually, bacteria as well as Y & M are mostly damaged during heating of milk, but these microorganisms may reinfect the paneer matrix during post-manufacture conditions, thereby leading to increased counts during storage. The rising trend of TPC and Y & M counts were inconsistent with the observations of [44,45]. Moreover, Rani et al. [46] also observed a rising trend of TPC and Y & M counts of paneer incorporated with cumin and black pepper during storage (up to 8 days). It was also observed that, due to presence of spices in the paneer matrix, there was an inhibitory effect against bacteria and yeast and molds. Therefore, microbial counts did not increase to such an extent as observed in the control paneer. In this study, it was suspected that all the spicy paneer might have slight fungus on the surface of paneer stored for 12 days due to its slight change in taste. On the other hand, the control paneer showed visible growth of fungus over its surface after 12 days of storage. Das et al. [47] also reported visible reddish brown discoloration on the surface of 12-days-stored paneer. As the paneer does not contain any starter or probiotic bacteria which cause inhibition of harmful microbes, deterioration happened in paneer with the advancement of storage period due to increase in the number of microbes.

The decreasing score of all the sensory characteristics of paneer in this study was concurrent with the results obtained by [45,47]. Our results were concurrent with the results obtained by [27], who observed decreased sensory acceptance score of clove-treated paneer during storage. The control paneer was accepted for up to 6 days on the basis of sensory acceptance score, which was in accordance with the results obtained by [27], who reported 5-day-old paneer as acceptable. The control paneer showed some signs of mold

growth after 9 days due to its flavor. The growth of fungus on the surfaces of control paneer after 12 days of storage caused exclusion of such paneer for sensory evaluation. All the spicy paneer after 12 days of storage also showed some signs of mold growth due to a slight change in taste. Khatkar et al. [27] observed the shelf life of clove-treated paneer up to 10 days. In addition, some people liked the taste of paneer having 0.2% spices, and some people liked the paneer containing 0.3% spices; therefore, paneer with both percentages of each spice were included in the study.

The results of DPPH and TAC of all the spicy paneers were very promising, which might be due to the presence of enormous quantities of TP and TF of spices. Our results regarding TP of spicy paneer that incorporated 0.3% cardamom (14.25 mg GAE/100 g) were lower in comparison with the results (47.2 mg GAE/100 g) obtained by [26], who incorporated mixture of black pepper (0.25%) and cardamom (0.50%). These discrepancies might be due to variations in raw materials, i.e., milk and quantity and source of spices. The hydroxy groups of flavonoids have the potential to donate hydrogen or electrons to DPPH free radicals, thereby leading to the termination of reactions of free radicals [48]. The RP of spices, as well as spicy paneer, may exhibit potent antioxidant activity due to the existence of antioxidants. Such compounds caused the diminution of ferricyanide complex to ferrocyanide, which subsequently reacts with ferric chloride to form ferric ferrous complex, exhibiting maximum absorption at 700 nm [49]. All the parameters showing antioxidant potential of spices (depicting the highest values) were many folds compared to spicy paneer (depicted the highest values). In this context, TP values of spices were 17 folds compared to the values of spicy paneer. Similarly, TF values of spices were 10 folds compared with the values of spicy paneer. Moreover, RP and DPPH values of spices were 6 folds compared with the values of spicy paneer. Such kinds of manyfold antioxidant potential of spices revealed that these contained abundant quantities of bioactive compounds (phenolics, flavonoids, and many other compounds) relevant to antioxidant activities. For instance, the pericarp of black pepper contained significant quantities of phenolic and flavonoid compounds [22] as well as some other compounds such as ascorbic acid, β-carotene, camphene, carvacrol, eugenol, piperine, and ubiquinone [5], which are considered to be strong antioxidants. Similarly, clove is reported to have strong antioxidant activity [8,9] due to the presence of tocopherol, ascorbates, and phenolic compounds [23]. It also contains some health-promoting components such as volatile oils, acids, cymene, pinene, cuminaldehyde, terpinene, thymol, and oleoresin [14,45]. Chaudhry et al. [50] also reported that the extracts of *Nigella sativa* contained thymol and thymoquinone which exhibit strong antioxidant and antibacterial potential. Cardamom is reported to contain predominant compounds including α-terpineol, α-terpinyl acetate, 1,8-cineole, β-linalool, and sabinene [17,18]. Owing to the existence of such compounds in essential oil of cardamom, it was reported to have antioxidant potential [17,19,20]. The bark of cinnamon contains phytoconstituents, such as flavonoids, phenolics, and carotenoids, which are considered to be antioxidants [51]. Ghosh et al. [52] evaluated the antioxidant potential of pectic polysaccharides extracted from its bark. They found that the extracted compounds responsible for the antioxidant potential include arabinogalactan, uronic acid, and glucan. It was also reported that cinnamon had antioxidant potential [53,54] due to the presence of major constituents such as (*E*)-cinnamaldehyde, linalool, *β*-caryophyllene, eucalyptol, eugenol, benzyl benzoate, α-felandrene, α-pinene, and cinnamaldehyde acetate [24,51]. Since the spices are reported to have antioxidant activities, it would be of great interest for the food industries to incorporate such kinds of spices (powders or their extracts) into their products for their value addition.

5. Conclusions

Based on the results of the present study, it may be concluded that all the treatments concerning spicy paneer (varying percentages of spices) were sensorially acceptable. Similarly, it may be concluded that spicy paneer had more antioxidant potential compared to the control paneer due to the presence of more phenolics and flavonoids and many other

compounds. Moreover, paneer having 0.3% clove (P_6) showed the maximum values regarding TP, TF, RP, DPPH, and TAC. In this way, it may be recommended that spicy paneer with very-low-fat contents should be produced by the dairy industry as it would be more nutritious with noteworthy worth due to the presence of more antioxidant compounds as mentioned above in all the spices.

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