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Assessment of compatibility of spices as an additive in cottage cheese

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Abstract
The present study was carried out to evaluate the compatibility of different spices as an additive in Cottage cheese. Nine different spices namely mace, nutmeg, cumin, coriander, mustard, pepper, cinnamon, clove and fenugreek were added to Cottage cheese at the rate of 0.5%. The Cottage cheese samples with added spices at the rate of 0.5% each of cumin, pepper, coriander and cinnamon were acceptable with high flavour and overall acceptability sensory scores. Total phenolic content (TPC) and antioxidant capacity of cheese samples were evaluated using the Folin–Ciocalteu method and DPPH assay. The total phenolic content and DPPH radical scavenging activity was found higher in Cottage cheese samples containing spices than the control cheese sample.

Keywords: cottage cheese, spices, sensory characteristics, TPC, DPPH

Introduction
By 2024, the global cheese market is estimated to reach US$ 99.4 billion, with a CAGR of 8.4% from 2019 to 2024. From 2018 to 2023, the Indian cheese market is predicted to increase at a rate of roughly 18 to 19% per year (Market Research Reports, 2020) [10]. The Indian cheese market has considerable growth potential being the world's largest producer of milk. Cheese is defined as a product made from milk by coagulating the casein with rennet or similar enzymes in the presence of lactic acid produced by added or adventitious microorganisms, from which part of the moisture has been removed by cutting, cooking and/or pressing, which has been shaped in a mould, and then ripened at suitable temperature and humidity (Mijan *et al.*, 2010) [12].

Cottage cheese is a fresh product that is ingested. The sensory features of skim milk and cream dressing ingredients, as well as the properties of the lactic cultures used in the manufacturing process, all contribute to the product's flavour (Bodyfelt *et al.*, 2008) [2]. At refrigerated conditions, it has low shelf life of 7-8 days due to its high moisture content. Cheeses flavoured with herbs, spices, and other condiments are becoming increasingly popular among consumers looking for diversity and bold flavours. Using spices and spice extract blends to improve cheese flavour, extend shelf life by reducing microbiological content, create a healthier product, and minimise structural flaws by lowering salt level are just a few of the goals. Spices have been used in foods and beverages for hundreds of years for flavour, colour, aroma and preservation. Because they contain potent antioxidant molecules, they have antioxidant action (flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides) (Murcia *et al.*, 2004) [13].

Materials and Methods
Raw materials
Cottage cheese was prepared by the method given by Dekula (2016) [5] with some modifications. Skim milk was pasteurized and then cooled to 31 °C followed by addition of calcium chloride at the rate of 0.01%. DVS cultures were added at 31 °C and incubated for 45 minutes. After a 0.01% increase in acidity, rennet was added to the cheese milk at the rate of 0.3%. Cutting was done when the acidity of whey reached 0.4% lactic acid, followed by cooking in such a way that temperature increased up to 56 °C, with each 1 °C rise in every 5 min. The cheese curd washing was done with pasteurized chilled water (pH 5.2-5.4). First, second and third washing was carried out with the pasteurized chilled water at the temperature of 25 °C, 15 °C and 8 °C, respectively.

After draining, the cheese curd was dressed using pasteurized homogenized cream (20% fat). Salt was added at the rate of 1% followed by spice powders (control Cottage cheese without spice powder).

The yield of Cottage cheese curd was 13.28%. Cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters.

Preparation of spice extract

Each spice powders (0.1 g) were treated with 10 ml of methanol at 35°C for 24 h. The mixture was then cooled to room temperature and centrifuged at 4,000 rpm for 10 min. The supernatant was recovered for the determination of the total phenolic content and radical scavenging activity by DPPH assay.

Preparation of cheese sample

Cheese samples (2.5 g) were treated with 50 ml of methanol at 30°C for 24 h in a shaking water bath. The mixture was then cooled to room temperature and centrifuged at 4,000 rpm for 10 min. The supernatant was recovered for the determination of the total phenolic content and radical scavenging activity by DPPH assay.

Determination of proximate composition

Cheese samples was analyzed for its proximate composition (moisture, fat, protein, ash, lactose, acidity and pH) by the methods given by BIS (1981) [3].

Total phenolic content (TPC)

Total phenolic content of cheese samples was analyzed by Folin Ciocalteu method (Singleton and Rossi, 1965) [16].

Determination of TPC content of Cottage cheese

To 1 ml of methanolic extract in a test tube, 0.5 ml of Folin-Ciocalteu (1N FC) reagent and 10 ml of 7.5% Na_2CO_3 were added, mixed and incubated at room temperature for 30 min. Then the absorbance was measured at 750 nm against blank. Standard curve was prepared by using different aliquots of gallic acid concentration (10-100 µg). Using the standard curve, TPC of cheese samples were calculated and expressed as gallic acid equivalent (GAE) (mg GAE/g cheese).

DPPH radical scavenging activity

The radical-scavenging activity of cheese samples was evaluated according to the procedure of McCune and Johns (2002) [11].

DPPH radical scavenging activity of Cottage cheese

The free radical scavenging activity was measured using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH). Exactly 0.75 ml of methanolic extract was added to 0.75 ml of DPPH (0.2 mM). The contents were mixed for 5 s, incubated for 30 min at 37 °C away from direct light and absorbance was measured at 517 nm. Methanol was used as blank. Standard curve was prepared using (10-80 µM) of trolox solutions in methanol.

Based on the % inhibition of sample, trolox equivalent was determined from standard curve. The results were expressed as trolox equivalent antioxidant capacity (TEAC) values i.e. mM trolox equivalence per g of cheese.

$$\text{Inhibition (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A_0 is the absorbance of control
A_1 is the absorbance of sample

Sensory evaluation of Cottage cheese

All the samples of Cottage cheese were evaluated for their sensory characteristics (flavour, colour and appearance, body and texture, overall acceptability) on a 9-point hedonic scale (ranging from 9 = like extremely to 1 = dislike extremely) by a trained panel of 9 judges. The panelists included scientists, technical officers/assistants and students of the institute. All the judges were familiar with the quality characteristics of cheese. The judges received a sample of 20 g.

Results and Discussion

Total phenolic content of spices

TPC measures the reduction of the reagent by phenolic compounds through the formation of a blue complex, which was measured at 760 nm against gallic acid as standard. TPC content of nine spices used in this study was determined and result of analysis is shown in Table 1.

Table 1: Total phenolic content of spice

Types of spice	TPC (mg GAE/g)
Nutmeg	1.54 ±0.01
Mace	1.79±0.02
Cumin	0.88±0.01
Coriander	0.34±0.02
Mustard	0.56 ±0.01
Pepper	0.69 ±0.01
Cinnamon	3.60±0.02
Clove	6.36 ±0.02
Fenugreek	0.75 ±0.02
* Mean± SD; n=6	

Among the nine spices evaluated, clove (6.36 mg GAE/g) had the higher TPC followed by cinnamon (3.60 mg GAE/g), mace (1.79 mg GAE/g), nutmeg (1.54 mg GAE/g), cumin (0.88 mg GAE/g), fenugreek (0.75 mg GAE/g), pepper (0.69 mg GAE/g), mustard (0.56 mg GAE/g) and coriander (0.34 mg GAE/g).

Phenolic compounds have an impact on commonly accepted quality attributes such as bitterness, colour, and flavour (Laura *et al.*, 2019) [9]. The result of TPC analysis showed that TPC content varied widely among the spices depending on their botanical family, plant species and part of plant used as spice.

DPPH radical scavenging activity of spice

DPPH radical scavenging activity is a promising method to determine the antiradical power of an antioxidant by measuring decrease in the absorbance of DPPH at 517 nm. The DPPH radical scavenging activity of different spices used in the present study is shown in Table 2.

Table 2: DPPH radical scavenging activity of spice

Types of spice	DPPH radical scavenging activity (mM of TEAC /g)
Nutmeg	14.31±0.01
Mace	10.87±0.01
Cumin	10.17±0.01
Coriander	3.49±0.01
Mustard	7.72±0.01
Pepper	4.38 ±0.01
Cinnamon	17.89±0.01
Clove	17.49±0.01
Fenugreek	5.83±0.01

* Mean± SD; n=6

Among all the nine spices cinnamon (17.89 mM of TEAC /g spice) showed higher DPPH radical scavenging activity and lowest was for coriander (3.49 mM of TEAC /g spice).

Due to high antioxidant activity, spices suppress harmful effects of carcinogenic pollutants that may be present in foods and beverages (Yashin *et al.*, 2017) [17].

Sensory characteristics of Cottage cheese

Predominant chemical components in every spice or flavoring, produce acceptable sensory properties in the food products. Spices chemical components can provide a range of flavours from mild to intense. A spice flavour profile is determined by the balance of various chemical components (Raghavan, 2006) [14].

From an array of available spices nine different common culinary spices *viz*. mace, nutmeg, cumin, coriander, mustard, pepper, cinnamon, clove and fenugreek were selected for the study. Each of the above spices in the powder form were added at the rate of 0.5% (w/w) into the Cottage cheese. All the nine samples were subjected to sensory evaluation for their attributes *viz*., flavour, colour and appearance, body and texture and overall acceptability using 9 points hedonic scale. The Cottage cheese without spice was served as control. Results along with their statistical analysis are presented in Table 3.

Table 3: Sensory characteristic of Cottage cheese with different spices

Types of spice*	Sensory score**			
	Flavour	Colour and appearance	Body and texture	Overall acceptability
Nutmeg	6.41^{e}	7.22^{d}	7.25^{e}	6.34^{e}
Mace	6.69^{de}	7.44^{cd}	7.42^{de}	6.61^{d}
Cumin	8.23^{a}	8.13^{a}	8.03^{ab}	8.15^{a}
Coriander	8.05^{ab}	7.96^{ab}	7.95^{ab}	8.00^{a}
Mustard	7.55^{bc}	7.72^{bc}	7.78^{bc}	7.49^{b}
Pepper	8.20^{a}	8.00^{ab}	8.08^{a}	8.09^{a}
Cinnamon	7.98^{ab}	7.97^{ab}	7.88^{abc}	7.94^{a}
Clove	5.86^{f}	6.55^{e}	7.22e	5.81^{f}
Fenugreek	7.13^{cd}	7.75^{b}	7.66^{cd}	7.05^{c}
Control	8.12^{a}	8.23^{a}	8.10^{a}	8.10^{a}
SEm	0.18	0.09	0.08	0.07
CD (0.05)	0.53	0.29	0.25	0.22
CV%	4.33	2.29	1.96	1.66

*All spice powders were added at the rate of 0.5%.
Control is the Cottage cheese without spice.
**The values are means of three observations. Values within a column with same superscript did not differ significantly (p>0.05) from each other.

The flavour of Cottage cheese indicated that the flavor score for Cottage cheese samples containing nutmeg, mace, cumin, coriander, mustard, pepper, cinnamon, clove, fenugreek and control were 6.41, 6.69, 8.23, 8.05, 7.55, 8.20, 7.98, 5.86, 7.13 and 8.12, respectively.

The colour and appearance score for Cottage cheese samples containing nutmeg, mace, cumin, coriander, mustard, pepper, cinnamon, clove, fenugreek and control were 7.22, 7.44, 8.13, 7.96, 7.72, 8.00, 7.97, 6.55, 7.75 and 8.23, respectively.

The body and texture of Cottage cheese indicated that the average scores for Cottage cheese samples containing nutmeg, mace, cumin, coriander, mustard, pepper, cinnamon, clove, fenugreek and control were 7.25, 7.42, 8.03, 7.95, 7.78, 8.08, 7.88, 7.22, 7.66 and 8.10, respectively.

The overall acceptability score of Cottage cheese samples containing nutmeg, mace, cumin, coriander, mustard, pepper, cinnamon, clove, fenugreek and control were 6.34, 6.61, 8.15, 8.00, 7.49, 8.09, 7.94, 5.81, 7.05 and 8.10, respectively.

Among the nine spices, flavour and overall acceptability scores of cumin, coriander, pepper and cinnamon was better compared to other experimental samples.

The flavour score of control and Cottage cheese sample containing cumin, coriander, pepper and cinnamon were in range of 7.98 to 8.23; and the difference among them was non-significant ($p>0.05$). Also, they differed significantly from the other experimental sample ($p<0.05$). The difference in the colour and appearance score of Cottage cheese samples containing cumin, coriander, pepper, fenugreek spice powder and control was non-significant ($p>0.05$).

Clove and nutmeg had a negative effect on colour and appearance due to the native colour of spice.

The colour and appearance score of control and Cottage cheese sample containing cumin, coriander, pepper and cinnamon ranged from 7.96 to 8.23; with the difference among them being non-significant ($p>0.05$). Also, they differed significantly from other experimental sample ($p<0.05$).

The body and texture score of Cottage cheese containing cumin, coriander, cinnamon, pepper and control were above 7.75 and the difference among these sample was non-significant (*p*>0.05) and were higher compared to other samples.
The overall acceptability score was highest for Cottage cheese containing cumin (8.15), followed by control (8.10), pepper (8.09), coriander (8.00), and cinnamon (7.94); the difference among these samples was non-significant (*p*>0.05).
The lowest overall acceptability score was for Cottage cheese containing clove powder. Also, they differed significantly from other experimental sample (*p*<0.05).
Based on sensory analysis, spice powders namely pepper, cumin, coriander and cinnamon were selected for further study as these spices were found to be compatible with Cottage cheese.

Proximate chemical composition

The control Cottage cheese was analyzed for their proximate composition and chemical characteristics. The data obtained for chemical composition of fresh Cottage cheese revealed that the cheese contained 72.50% moisture, 5.42% fat, 16.10% protein, 2.05% ash, 3.93% lactose. The fat on dry matter basis was 19.37% while salt content and salt in moisture was 1.02% and 1.41%, respectively. The pH and acidity of Cottage cheese was 5.29 and 0.43% lactic acid, respectively.
As per FSSAI standards (2020) [7], maximum moisture permitted in Cottage cheese is 80% and fat content shall not be less than 4%. The Cottage cheeses prepared in the present study met standards laid by FSSAI.

Total phenolic content of Cottage cheese

Total phenolic content in the cheese samples varies widely as evaluated by FC method (Table 4). The calculation for total phenolic content of all the cheese samples were carried out using the standard curve of Gallic acid and expressed as Gallic acid equivalents mg (GAE) per g of cheese. The Total phenolic content among the spices in selected cheese samples (cumin, coriander, pepper and cinnamon were analysed). The TPC of control cheese, Cottage cheese containing cumin, Cottage cheese containing coriander, Cottage cheese containing cinnamon and Cottage cheese containing pepper was 1.12, 1.32, 1.17, 1.85 and 1.23 mg GAE/ g of cheese, respectively.

Table 4: Total phenolic content of Cottage cheese

Cheese samples	TPC (mg GAE/g)
Control cheese	1.12±0.02
Cumin	1.32±0.01
Coriander	1.17±0.02
Cinnamon	1.85±0.01
Pepper	1.23±0.02

* Mean± SD; n=6

The control cheese also had TPC value inspite of having no spices. This is due to the fact that the total phenolic contents of cheese samples were estimated using Folin-Ciocalteu reagents, which measure only phenols, and can react with many other substances like carbohydrates, amino acids, nucleotides, thiols, unsaturated fatty acids, vitamins, amines, aldehydes, ketones. (Josipovic *et al.*, 2015) [8]. Thus, TPC values of each experimental sample is the net effect of reaction of FC reagent with phenols and other substances present naturally in cheese as mentioned by Everette *et al.* (2010) [6]. This is evident from the higher TPC of Cottage sample containing 0.5% of cumin, coriander, cinnamon and pepper as compared to control.

DPPH Radical-scavenging activity of Cottage cheese

The antioxidant potential of Cottage cheese samples added with different spices were determined by DPPH assay and results are expressed as mM of TEAC/g cheese in the Table 5.

Table 5: DPPH radical scavenging activity of Cottage cheese

Cheese samples	DPPH radical scavenging activity (mM of TEAC/g cheese)
Control cheese	0.53±0.04
Cumin	1.02±0.02
Coriander	0.65±0.01
Cinnamon	1.43±0.03
Pepper	0.83±0.02

* Mean± SD; n=6

The DPPH radical scavenging activity of control cheese, Cottage cheese containing cumin, Cottage cheese containing coriander, Cottage cheese containing cinnamon and Cottage cheese containing pepper was 0.53, 1.02, 0.65, 1.43 and 0.83 mM of TEAC/g of cheese.
Because of their capacity to donate hydrogen atoms to free radicals, phenolic and flavonoid molecules are significant antioxidant components that deactivate free radicals. They also have the ideal structural properties for scavenging free radicals (Amarowicz *et al.*, 2004) [1]. Caleja *et al.* (2015) [4] found that the incorporation of fennel improved the antioxidant activity of Cottage cheese which correlates well with the present study.
Different literature reports indicate a linear correlation of total phenolic and flavonoid content with antioxidant capacity (Shrestha and Dhillion, 2006 and Yoon *et al.*, 2015) [15, 18]. The DPPH radical scavenging activity regulated various biochemical changes involving redox reactions and thereby may exert positive influence on the shelf life of cheese.

Conclusion

Cottage cheese samples were analyzed for the compatibility by the addition of nine different spices. Among the nine spice powders, Cottage cheese containing cumin, coriander, pepper and cinnamon had high sensory scores compared to the other experimental samples. The total phenolic content and DPPH radical scavenging activity was found higher in Cottage cheese samples containing spices than the control cheese sample which not only improved the sensory characteristics of the product but also determined the chemical stability thereby enhancing the shelf life of the product.

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