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Enhancement of Shelf Life of Low Cholesterol Paneer using Thyme Essential Oil

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ABSTRACT: Thyme essential oil was tested for its relative efficiency in improving the shelf life of paneer. The physico-chemical and biofunctional properties of thyme essential oil were evaluated. The total polyphenolic value and thymol content of essential oil was 98.19 mg/g GAE and 31.30% respectively. The essential oil was also found to have exceptional antimicrobial and antioxidant activity. The percentage radical scavenging activity of essential oil was found to be 57.80% RSA at 1 g/L concentration. The antimicrobial properties of the essential oil were assessed against three indicator organisms, namely Serratia marcescens, Staphylococcus aureus, and Escherichia coli, by agar-well diffusion assay. The essential oil exhibited inhibitory action against all of the organisms. Thyme essential oil at different concentrations ranging from 0.05 to 1% v/v was added to buffalo milk treated with beta-cyclodextrin for the preparation of low cholesterol paneer. Out of eleven treatments, paneer prepared from milk treated with 0.1 and 0.05% thyme essential oil had the highest sensory score. These paneer samples had an antioxidant activity of 57.74 and 31.93% RSA, respectively. Therefore, the optimum level of addition of thyme essential oil was fixed at 0.1%. Optimized and control paneer were packaged in LDPE pouches and subjected to shelf-life studies under refrigerated conditions. The functional paneer showed a shelf life of 17 days, while for control, it was 11 days.

Keywords: Low cholesterol paneer, shelf life enhancement, thyme essential oil.

INTRODUCTION

The native soft cheese called paneer is made by heating milk to a high temperature and by imparting acidic coagulation. Paneer act as a good source of high-quality animal protein which can be consumed by the vegetarians who constitutes a sizable population of India. The average fat content of paneer is 23-26% (Mamata *et al*., 2019). Cholesterol accounts for 0.25-0.45% of the total lipids in milk (Shingla and Mehta 2018). Consumption of high-fat dairy products is associated with an increased risk of cardiovascular disease due to the content of saturated fatty acids and cholesterol (Schlimme and Kiel 1990). Low cholesterol products are designed to meet the dietary requirements of persons at risk of cardiovascular diseases, in terms of lowering total daily cholesterol intake. Recently, β-cyclodextrin (a starch hydrolysed product) has been effectively used for cholesterol removal from buffalo milk paneer (Reji *et al*., 2024). β-cyclodextrin is found to be non-toxic, non hygroscopic, chemically stable and edible. The selective elimination of cholesterol can be achieved through the use of βcyclodextrin (β-CD), without appreciably affecting the amount of other nutritional and taste components (Kolaric *et al*., 2022).

Although the process of making paneer involves heating milk to almost boiling point in a mildly acidic environment, this process also exposes the milk to activities like manual handling, pressing, cooling, and packaging. This may lead to the loss of the bactericidal advantage gained from thermal processing and reduces the shelf life of paneer. The lower shelf life of paneer can be attributed to several factors like high moisture content (53-55%), rich supply of nutrients, and the relatively high temperatures experienced in many parts of the nation (Gokhale *et al*., 2016). The comparatively short shelf life of paneer poses a significant obstacle to the commercial use of this product. To extend the shelf life of paneer, food additives such sorbic acid, potassium sorbate, brine, H_2O_2 solutions, and delvocid (Sachdeva and Singh 1990) have been tried and proven effective. While turmeric was added to paneer at a weight percentage of 0.6%, the food's shelf life was found to be increased to 12 at 7±2 °C (Sachdeva and Singh 1990). The relative effectiveness of clove, cardamom, cinnamon, and black pepper in extending the shelf life of paneer was investigated by Eresam *et al*. (2015). Cardamom was the most effective spice among those examined for extending paneer's shelf life to 28 days at 7±2 °C.

Thyme essential oil has been widely used in traditional medicines and functional foods for its anti-inflammatory, antihypertensive, antibacterial, antifungal, antioxidant, and therapeutic effects. Therefore, thyme essential oil can be used to extend the shelf life of paneer. The genus Thymus belong to Lamiaceae family. These plants, which are native to the Mediterranean region, are widely utilised for food, cosmetics, and medicine. The most well-known herbal ingredient in the pharmaceutical sector is thyme herb derived from *Thymus vulgaris* L. and *Thymus zygis* L. Thyme herb, its essential oil (EO), and their principal volatile components—thymol and carvacrol—have been widely used for therapeutic purposes. The Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) summarised and published the scope of medical usage, preclinical, and clinical data, as well as the chemical composition of thyme herb and EO. In recent years, there has been a surge of interest in essential oils and their use as dairy preservatives as viable alternatives to synthetic preservatives. Several researches have been conducted to investigate the antibacterial activity of essential oils and their active components, as well as their mode of action (Mishra *et al*., 2020). Gouvea *et al*. (2017) reported antibacterial action of essential oils and plant extracts when added to cheeses. According to Jemaa *et al*. (2018), including essential oils from Thymus inhibits bacterial development in pasteurised milk, extending its shelf life. According to Xue *et al*. (2017), thymol is a regularly utilised essential oil component. Thymol has been shown to be a broad-spectrum antibacterial against gram-positive and gram-negative bacteria (Burt, 2004).

The microbial spoilage in paneer occurs due to the surface growth of microorganisms. The surface gets covered in a greenish yellow slime, and the coloring and odd flavor go hand in hand. Because of this, when food is refrigerated for a longer period of time, the interior usually stays fresh but the surface inevitably deteriorates sooner. Therefore, the study was planned to treat the buffalo milk intended for the production of low cholesterol paneer with certain levels of thyme essential oil to evaluate its effectiveness in extending shelf life of low cholesterol paneer. It was hypothesized that such treatment would result in an improved shelf life

MATERIALS AND METHODS

Milk: the buffalo milk used for paneer preparation was procured from University Dairy plant, Mannuthy, Thrissur. β Cyclodextrin 97% pure (Food grade) was purchased from RP Chemicals, Issaji Street Masjid Bandar West, Mumbai. Citric Acid: Food grade citric acid was purchased from local market, Thrissur. Thyme Essential oil: Thyme essential oil with 31.30% thymol was purchased from Kayani Exports, New Delhi. Packaging Material: LDPE pouches of size 10 cm × 5 cm (50 μ thickness) were purchased from Jaihind Market, Thrissur, was used for packaging of samples during storage study

Procedure: The experiment was conducted in the following sequence.

ANALYSIS OF THYME ESSENTIAL OIL

• Physical properties

Specific gravity. Specific gravity was done as per the method of Guenther *et al.* (1967) using pycnometer.

Refractive index. Refractive index was measured as described by Saputro *et al.* (2016). The refractive indices were carried out using a refractometer (PR-301α, Atago, Japan).

• Chemical properties

Total polyphenolic content. Total polyphenolic content was measured as per the procedure by Abdul-Hafeez *et al.* (2014). Thyme essential oil (100 μL) was dissolved in 80% ethanol (1 mL); 0.2 mL of this solution was made up with 0.3% HCl to 0.5 mL. An aliquot (100 μL) of the resulting solution was added to 7% Na_2CO_3 (2 mL) and, after 2 min, the Folin-Ciocalteau reagent diluted with methanol 1:1 (100 μL) was added and mixed well. After 30 min incubation, the absorbance of exactly 2 mL of the sample was recorded at 735 nm. The total phenolic content was calculated in terms of gallic acid equivalents (GAEs) from a calibration curve of gallic acid standard solutions, and the results were expressed as milligrams of gallic acid per 100 μL of essential oil. The total phenol value was obtained from the regression equation and expressed as milligrams per gram GAE using the formula, T = CV/M, where T = total content of phenolic compounds (mg/g GAE), C = concentration of gallic acid (mg/mL) established from the calibration curve, V = volume of extract and m = the weight of plant extract.

• Biofunctional properties

Antimicrobial activity. The antimicrobial potential of the essential oil was tested against *Staphylococcus aureus*, *Escherichia coli* and *Serratia marcescens* (Culture stock, Department of Dairy Microbiology, VKIDFT, Mannuthy) by agar well diffusion bioassay as described by Sipahelut *et al.* (2019). Overnight incubated cultures of indicator organisms were adjusted to an optical density (OD) of 0.3 (which corresponds to 10^7 to 10^8 cfu/ml cell) at 540 nm and were spread on pre-set Mueller Hinton agar (MHA, HiMedia Laboratories Pvt. Ltd., Mumbai) plates. Wells were bored on the plates using a sterile borer and loaded with 80 μL of essential oil. Broth which was not inoculated was used as the negative control. The plates were incubated at 37°C for 24 h and the zones of clearance developed around the wells were measured to evaluate the antimicrobial property of the isolates.

Antioxidant activity. The antioxidant activity of the thyme essential oil was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical, DPPH (2,2´-diphenyl-1-picrylhydrazyl) according to the method of Kulisic *et al.* (2005).

SELECTION OF LEVEL OF ADDITION OF THYME ESSENTIAL OIL

• Sensory Evaluation. The paneer samples prepared from buffalo milk added with 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1%v/v of thyme essential oil were evaluated organoleptically for different quality attributes like flavour, body and texture, colour and appearance by a selected panel of judges of five members. The paneer was evaluated in raw form based on the guidelines of IS: 15346-2003

• **Antioxidant assay.** Paneer samples with highest sensory scores were tested for antioxidant properties as per Qureshi *et al.* (2019) with some modifications. Crushed paneer sample (20 g) was mixed with 40 mL of distilled water. To make homogenous mixture, the sample was sonicated (20 kHz frequency, 70% amplitude level (525W power), and pulse duration 5s on and 5s off, 2 min at lab temperature) using an ultrasonic processor (Vibrocell TM, Sonics, USA) with a probe inserted up to 2 inch inside the sample. The mixture was centrifuged (Hermle Labortechnik GmbH Siemensstr-25 D-78564, Germany) at 14,000 g for 15 min at 4°C after adjusting pH 4.6 by using 1 N HCl. The fat layers were removed and the supernatants were filtered through Whatman No. 1 filter paper. Two mL of DPPH (60 µmol/L in absolute ethanol) solution was added into 2 mL of WSEs of paneer. The mixture was vortexed (Spinix, Tarsons products Pvt Ltd, India for 1 min. After incubation for 30 min at room temperature in dark, the absorbance was taken at 517 nm using a spectrophotometer (Systronics, India). The blank (absolute ethanol) was also prepared in the same manner. The radical scavenging activity was calculated as a percentage of DPPH discolouration.

PREPARATION OF PANEER

The paneer was prepared from buffalo milk standardized to 5% fat, as per the procedure by Bhattacharya *et al.* (1971). β-cyclodextrin treatment was performed according to Reji *et al*. (2024). Thyme essential oil was added to the standardized milk just before heating process. Low cholesterol paneer was used as control paneer.

SHELF-LIFE STUDIES

The paneer prepared with buffalo milk added with TEO and control paneer samples were packed in LDPE pouches under laboratory conditions and subjected to storage studies at refrigerated temperature. Stored samples were analysed for the following parameters at three days interval, till significant spoilage occurred.

• **Sensory attributes.** The paneer prepared with buffalo milk added with TEO and control paneer were evaluated organoleptically for different quality attributes like flavor, body and texture, color and appearance by a selected panel of judges comprising of five members as per the guidelines given by IS: 15346-2003.

• **Physico chemical attributes**

Preparation of Sample. Paneer samples were grated to uniform mass in a glass pestle and mortar, mixed thoroughly and transferred to an air-tight container prior to analysis. Samples were kept in an airtight container until the time of analysis. The storage temperature was below 10°C.

Determination of Moisture, Fat, Protein, Free Fatty Acids, Titratable acidity and Thiobarbituric Acid (TBA) Value. Moisture content was estimated by garavimetric method. Protein and Fat content were analysed by Microkjeldahl method (AOAC, 1990) and Mojonnier method respectively. The FFA content of paneer samples was determined by extraction titration method suggested by Deeth *et al*. (1975). The titratable acidity was determined by the method recommended by AOAC (1990) for cheese. TBA value of paneer sample was determined according to the method recommended by Sidwell *et al*. (1955). Tyrosine value of paneer was estimated as per the modified method of Juffs (1973).

RESULTS AND DISCUSSION

Analysis of Thyme Essential Oil

• **Physical properties.** The visual observation implies that thyme essential oil (TEO) had a liquid appearance, light yellow colour and a spicy pungent typical odour of thyme. The physical characteristics of TEO are given in Table 1. Values for Optical rotation were obtained from the details provided by the manufacturer.

Table 1: The physical characteristics of Thyme essential oil.

Characteristics	Value
Refractive index	1.494 ± 0.00
Specific gravity	0.891 ± 0.00
Optical rotation	+1.58 ± 0.00

Figures are mean ± standard error of four replications

Physical characteristics are influenced by the chemical composition of thyme essential oil. As shown in Table 1, the specific gravity of essential oil was 0.891, optical rotation obtained was + 1.58 and refractive index was 1.494. a similar work by Mossa *et al.* (1987) characterized the physical properties of thyme essential oil and found that three varieties of thyme from Saudi origin, Spanish origin and Moroccan origin had bright yellow, yellow-reddish and red colour, respectively. They determined that essential oils of Saudi, Spanish and Moroccan thyme have specific gravity in the range of 0.910 to 0.960; refractive indices of the three varieties were in the range of 1.497 to 1.5045.

• **Chemical properties**

Total polyphenolic content. The standard curve for gallic acid as plotted and used for expressing total polyphenolic content in thyme essential oil is given in the Fig. 1.

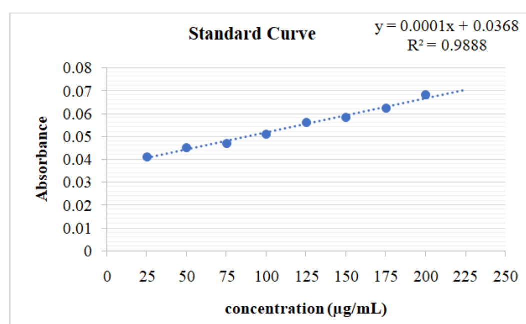

Fig. 1. Standard curve for gallic acid.

The total phenol value was obtained from the regression equation and expressed as milligrams per gram GAE using the formula, T = CV/M, where T = total content of phenolic compounds (mg/g GAE)C = concentration of gallic acid (mg/mL) established from the calibration curve, (since OD was obtained as 1.12, concentration obtained from the graph as 10832 µg/mL, which equals 10.83 mg/mL) V = volume of extract (2 mL) and m = the weight of plant extract (0.235 g). This gives the result as 98.19mg/g GAE. The chemical characteristics are expressed in Table 2.

Table 2: The chemical characteristics of Thyme essential oil.

Characteristics	Value
Thymol content	31.30%
Total polyphenolic content	98.19 mg/g GAE

Figures are mean ± standard error of four replications.

Value for thymol content was obtained from the details provided by the manufacturer.

As shown in Table 2, the thymol content of essential oil was 31.30% and total poly phenolic content was 98.19 mg/g GAE. This is in accordance with the following works. Aljabeili *et al*. (2018) studied the components of thyme essential oil using GC-MS and reported that the major compound of TEO was Thymol (41.04%) whereas, 1,8-Cineole (14.26%), γ-Terpinene (12.06%), p-Cymene (10.50%), α-Terpinene (9.22%), Linalool (2.80%) and Carvacrol (2.77%) were observed in valuable amounts. The value obtained for total phenolic content is this study is in accordance with Hafeez *et al*. (2014).

• **Bio - functional properties**

Antimicrobial activity. Assessment of antimicrobial activity of TEO was performed using well diffusion assay. Antimicrobial potential against the indicator organisms *Serrata marcescens, Staphylococcus aureus* and *Escherichia coli* were tested. The following data shows the zone clearances obtained for each indicator microbes. This clearly indicates the antimicrobial property of thyme essential oil.

*Inclusive of well diameter 6 mm.

Fig. 2. Zone of clearance of *S. marcescenes.*

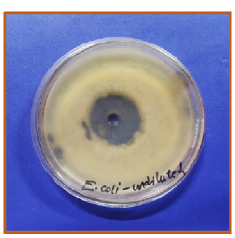

Fig. 3. Zone of clearance of *E. coli.*

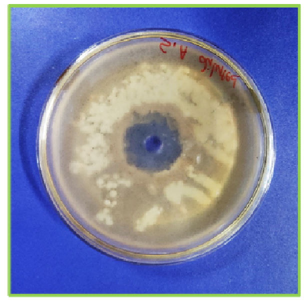

Fig. 4. Zone of clearance of *S. aureus.*

Antimicrobial potential of TEO against the indicator organisms *Serrata marcescens, Staphylococcus aureus* and *Escherichia coli* was evident with the obtained zone of clearance of 30.2, 23.5 and 19.7mm respectively. This is in accordance to the findings of Aljabeili *et al*. (2018) who studied the antimicrobial potential of thyme essential oil from *Thymus vulgaris* and reported that TEO showed inhibition zone ranged from 25 to 38 mm with relative MIC ranged from 40 to 270 mg·L^{-1} against pathogenic strains of *Bacillus cereus, Escherichia. coli, Listeria monocytogenes, Salmonella typhimurium, Salmonella typhi, Staphylococcus aureus* and Yersinia spp. Falcone *et al.* (2005) found that thymol has a nonlinear dose-dependent inhibitory effect on the microbial growth of the food spoilage bacteria and yeasts studied.

Table 3: Antimicrobial property of TEO against indicator organisms.

Indicator microorganisms	Zone of clearance*(mm)
S. marcescens	30.2
E. coli	23.5
S. aureus	19.7

• **Antioxidant activity of Thyme Essential oil.** Antioxidant activity was determined by DPPH RSA method. This is a commonly used method since it is both simple and sensitive. In this method, the results are expressed as the radical scavenging activity (RSA). RSA is calculated as a percentage of DPPH discolouration. The % DPPH RSA of thyme essential oil at 0.1, 1 and 2 g/L methanolic solutions is shown in the Table 4.

Table 4: Antioxidant activity of thyme essential oil.

Concentration of Thyme essential oil(g/L)	% RSA
0.1	30.17 ± 0.04
1	57.80 ± 0.08
2	83.60 ± 0.06

Figures are mean ± standard error of four replications

Per cent of inhibition was plotted against the sample concentration to get IC_{50} value, the amount of sample concentration necessary to decrease absorbance of specified concentration of DPPH by 50 percent.

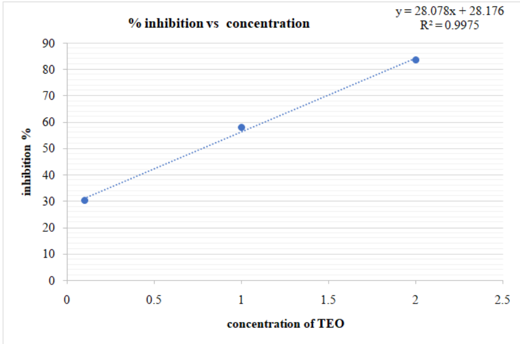

Fig. 5. Plot between percent inhibition and concentration.

From the plot, IC_{50} value of 0.77 was obtained.

Free radicals and reactive oxygen species cause lipid oxidation, which is the major chemical change involved in the deterioration of food during storage. Results showed that thyme essential oil had 30.17, 57.80 and 83.60% RSA at 0.1, 1 and 2 g/L concentrations respectively. From the plot, IC_{50} value was also obtained as 0.77. This is in accordance with the findings of Kulisic *et al.* (2005) who reported that essential oil from *Thymus vulgaris* had 38.80 and 91.30 % RSA at 0.1 and 2 g/L concentrations. Aljabeili *et al.* (2018) studied the anti-oxidant potential of thyme essential oil from *Thymus vulgaris* and reported that TEO had an excellent antioxidant property of 149.8 and 192.4 for DPPH (μmol of TE g−1) and ABTS (μmol of TE g^{-1}), respectively.

SELECTION OF LEVEL OF ADDITION OF THYME ESSENTIAL OIL

Sensory Evaluation. Effect of thyme essential oil on the sensory quality of paneer is given in Table 5.

Table 5: Effect of TEO treatment on the sensory quality of paneer.

Characteristics	Max Score	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
Flavour	50	NA*	NA*	NA*	32	34	36	37	39	42	45	45
Body and Texture	35	31	31	31	31	31	32	32	33	33	33	33
Colour and Appearance	10	7	7	7	7	8	8	8	8	8	8	8

Figures are mean of four replications.
*The paneer was unacceptable since the flavour was too intense.
S1-buffalo milk was treated with 1%v/v of TEO before paneer making; S2-buffalo milk was treated with 0.9%v/v of TEO before paneer making; S3-buffalo milk was treated with 0.8%v/v of TEO before paneer making; S4-buffalo milk was treated with 0.7%v/v of TEO before paneer making; S5-buffalo milk was treated with 0.6%v/v of TEO before paneer making; S6-buffalo milk was treated with 0.5%v/v of TEO before paneer making; S7-buffalo milk was treated with 0.4%v/v of TEO before paneer making; S8-buffalo milk was treated with 0.3%v/v of TEO before paneer making; S9-buffalo milk was treated with 0.2%v/v of TEO before paneer making; S10-buffalo milk was treated with 0.1%v/v of TEO before paneer making; S11-buffalo milk was treated with 0.05%v/v of TEO before paneer making' S10 and S11 had the highest sensory score and were selected for testing for antioxidant properties.

Paneer was prepared from buffalo milk added with eleven different levels of thyme essential oil and sensory scores were shown in Table 5. It was observed that the significant difference among these treatments was only in terms of flavour. This was due to the intense flavour contributed by TEO. This was in accordance with Hachana *et al.* (2019), who prepared farm cheese by incorporating 1% thyme essential oil as an antibacterial agent. In this study, S10 and S11 added with 0.1 and 0.05% TEO respectively on milk basis had the highest sensory scores and hence, were selected.

Antioxidant assay. S10 and S11 was tested for their antioxidant properties along with control paneer in order to finalize the level of addition of TEO (Table 6 and Fig. 6). The anti-oxidant potency of the samples was determined using the DPPH assay.

Table 6: Antioxidant activity of control, S10 and S11.

Sample	A_0	A_S	% RSA
Control	0.523±0.00	0.385±0.01	26.38
S10	0.523±0.00	0.221±0.00	57.74
S11	0.523±0.00	0.356±0.03	31.93

Figures are mean ± standard error of four replications.

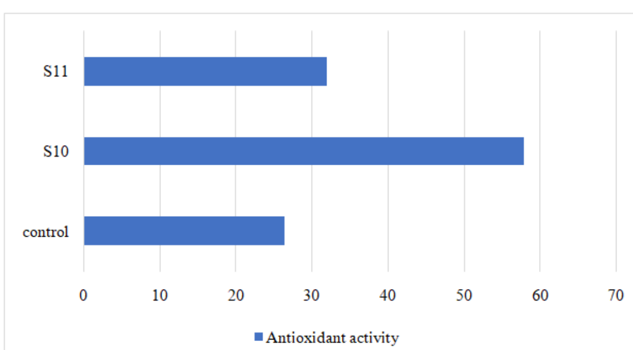

Fig. 6. Antioxidant activity of control, S10 and S11.

Since S10 had the highest antioxidant ability it was selected as the optimum level of addition of TEO.

Shelf-life studies. The optimized functional Paneer was packaged in LDPE pouches and was stored under refrigeration (7±1°C). In comparison to the control sample, changes in the sensory appeal and physico-chemical properties were observed at every 4 days interval. The rate of deterioration of a product is determined by interactions between food ingredients and storage conditions. When performing physicochemical and microbiological analysis, the comparison between the periods within each sample was done using repeated measures ANOVA and Independent t-test. For sensory tests chi square was used.

• **Sensory attribute**

Table 7: Effect of essential oil treatment on the sensory quality of Functional paneer.

Attributes		Days of storage						
		0	4	8	12	16	20	Chi square value
Flavour	Control	47.75±0.25	47.25±0.25	41.50±0.28	Spoiled	Spoiled	Spoiled	8.66*
	Functional paneer	46.55±0.25	46.25±0.25	47.00±0.41	45.00± 0.41	45.25±0.48	Spoiled	11.55*
	U value	0.5*	1.5^{ns}	16*				
Body and texture	Control	33.75±0.25	32.00±0.41	32.25±0.25	Spoiled	Spoiled	Spoiled	7.29*
	Functional paneer	33.75±0.25	32.75±0.48	32.50±0.28	31.25±0.48	30.50±0.64	Spoiled	13.68**
	U value	8^{ns}	11.5^{ns}	10^{ns}				
Colour and appearance	Control	10.00±0.00	10.00±0.00	9.00±0.00	Spoiled	Spoiled	Spoiled	11.00**
	Functional paneer	10.00±0.00	10.00±0.00	9.00±0.00	8.50±0.28	8.25±0.75	Spoiled	16.86**
	U value	8^{ns}	8^{ns}	8^{ns}				
Package	Control	5.00±0.00	5.00±0.00	5.00±0.00	Spoiled	Spoiled	Spoiled	0.00^{ns}
	Functional paneer	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00	Spoiled	0.00^{ns}
	U value	8^{ns}	8^{ns}	8^{ns}				

Values are mean ± standard error of three replications, *Significant at five per cent level ($p<0.05$) **-Significant at one per cent level ($p<0.01$), ns-non-significant ($p>0.05$), a-d -Means with different superscript vary significantly within a row.

• **Physico chemical attributes.** Effect of refrigerated storage on different physico-chemical and compositional parameters like moisture content, pH, acidity, tyrosine content, TBA value, FFA value were analyzed for both control and functional paneer. The results are shown in tables below.

Table 8: Effect of refrigerated storage (7 ± 1°C) on the moisture content of Functional paneer.

	Days of storage						F-value
	0	4	8	12	16	20	
Sample	Moisture Content(per cent)						
Control	54.98± 0.61^{a}	$54.47±0.42^{a}$	$54.22±0.11^{a}$	Spoiled	Spoiled	Spoiled	0.33^{ns}
Functional paneer	55.31± 0.48^{a}	55.23± 0.42^{ab}	54.61± 0.57^{ab}	53.82± 0.44^{ab}	53.27± 0.36^{b}	Spoiled	3.65^{ns}
t-value	-1.54^{ns}	-0.92^{ns}	-1.34^{ns}				

Values are mean ± standard error of three replications, *-Significant at five per cent level ($p<0.05$) **-Significant at one per cent level ($p<0.01$), ns-non- significant ($p>0.05$). a-d -Means with different superscript vary significantly within a row.

Table 9: Effect of refrigerated storage (7 ± 1°C) on the acidity content of Functional paneer.

	Days of storage						F-value
	0	4	8	12	16	20	
Sample	Acidity (% lactic acid)						
Control	$0.18±0.00^{a}$	$0.21±0.01^{a}$	$0.30±0.01^{b}$	Spoiled	Spoiled	Spoiled	23.30**
Functional paneer	$0.18±0.00^{a}$	$0.19±0.00^{a}$	$0.21±0.01^{b}$	$0.24±0.00^{c}$	$0.29±0.00^{d}$	Spoiled	144.20**
t –value	0.00^{ns}	5.66**	3.98**				

Values are mean ± standard error of four replications, *-Significant at five per cent level ($p<0.05$) **-Significant at one per cent level) ($p<0.01$), ns-non- significant ($p>0.05$). a-d -Means with different superscript vary significantly within a row.

Table 10: Effect of refrigerated storage (7 ± 1°C) on the tyrosine content of Functional paneer.

	Days of storage						F-value
	0	4	8	12	16	20	
Sample	Tyrosine content(mg/100mL)						
Control	$18.95±0.36^{a}$	$21.82±0.29^{b}$	$35.95±0.57^{c}$	Spoiled	Spoiled	Spoiled	459.85**
Functional paneer	18.68± 0.26^{a}	21.72± 0.21^{b}	28.95± 0.31^{c}	32.92± 0.66^{d}	$39.39±0.43^{e}$	Spoiled	417.65**
t –value	0.59^{ns}	0.27^{ns}	10.81**				

Values are mean ± standard error of four replications, *-Significant at five per cent level ($p<0.05$) **-Significant at one per cent level ($p<0.01$), ns-non-significant ($p>0.05$), a-e -Means with different superscript vary significantly within a row.

Table 11: Effect of refrigerated storage (7 ± 1°C) on the TBA value of Functional paneer.

	Days of storage						F-value
	0	4	8	12	16	20	
Sample	TBA Value (OD)						
Control	0.012±0.00[a]	0.015±0.00[b]	0.029±0.01[c]	Spoiled	Spoiled	Spoiled	101.03**
Functional paneer	0.012± 0.00[a]	0.015± 0.00[a]	0.016± 0.00[b]	0.020± .00[c]	0.031±0.00[d]	Spoiled	34.67**
t –value	-0.65[ns]	0.00[ns]	8.33**				

Values are mean ± standard error of four replications, *-Significant at five per cent level (p<0.05) **-Significant at one per cent level (p<0.01), ns-non-significant (p>0.05), a-d -Means with different superscript vary significantly within a row

Table 12: Effect of refrigerated storage (7 ± 1°C) on the FFA value of Functional paneer.

	Days of storage						F-value
	0	4	8	12	16	20	
Sample	FFA Value (µeq/g)						
Control	0.98±0.01[a]	1.12±0.03[b]	1.64±0.09[c]	Spoiled	Spoiled	Spoiled	117.57**
Functional paneer	0.99±0.01[a]	1.04±0.01[a]	1.28±0.11[b]	1.31±0.01[b]	1.67±0.06[c]	Spoiled	25.68**
t –value	-0.12[ns]	5.49*	3.91**				

Values are mean ± standard error of four replications, *-Significant at five per cent level (p<0.05) **-Significant at one per cent level (p<0.01), ns-non-significant (p>0.05), a-c -Means with different superscript vary significantly within a row.

A significant increase in acidity, tyrosine, TBA, and FFA values and a significant decrease in pH of the control and treated paneer samples were observed throughout the storage period. There were no significant change in the moisture content. For This change was more rapid in the control paneer, which indicated faster deterioration. The paneer with added thyme essential oil had a shelf life of 12 days, while for control, it was 8 days. The sensory data also shows that thyme essential oil does not seriously affects the sensorial qualities. The results were in accordance with Youdim *et al.* (2002) who studied the useful antioxidant properties of thyme essential oil and suggested that it may be utilized in the food industry and as a dietary supplement.

CONCLUSIONS

Low cholesterol paneer with prolonged shelf life was prepared from buffalo milk treated with β-cyclodextrin, with the addition of thyme essential oil. It was found that the addition of 0.1 % of the same had the highest sensorial quality. Low cholesterol paneer with prolonged shelf life showed no significant difference in sensorial properties from that of control paneer. The shelf life was improved to 12 days, while control paneer had only 8 days of shelf life. For functional products like low cholesterol paneer, the shelf life enhancement is of importance from the technical as well as marketing point of view. It opens a wide window for a range of shelf life enhanced dairy products.

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