

Studies of Physicochemical, Microbiological and Sensory characteristics of Paneer (Indian Cottage Cheese) to check the efficacy of clove-oil nanoemulsion in enhancing its shelf-life

D Bhardwaj¹ (✉), B Mann¹, R Sharma¹, R. Kumar^{1a}, Rashmi H.M.², K.Gandhi¹, Sukthija M.P.¹ and Minaxi Sharma¹

Received: 11 January 2024 / Accepted: 15 February 2024 / Published online: 23 August 2024
© Indian Dairy Association (India) 2024

Abstract: Paneer is a popular dairy product in India and is highly vulnerable to spoilage due to its high moisture and nutrient content. In the presented study paneer samples were treated with clove-oil nanoemulsion and its effectiveness in enhancing the shelf-life of paneer was studied by analyzing the physicochemical, microbiological and sensory characteristics of the stored samples. Paneer treated with clove-oil nanoemulsion showed the lesser significant increase ($P < 0.05$) in extent of proteolysis and free-fatty acid content while preventing reduction in moisture and pH of treated samples in comparison to control. Microbiological analysis revealed that paneer samples treated for 15 min with clove oil nanoemulsion followed by packing in nanoemulsion treated High Density Polyethylene Film (HDPE) packaging film) showed reduction in Standard plate count, coliform and Yeast and mold count by 2.02, 2.43 and 1.88 log cycles respectively in comparison to untreated control paneer samples on 21st day of storage. Treated paneer samples were observed to elicit almost constant sensory scores throughout the storage period as compared to control which showed significant reduction ($P < 0.05$) in sensory scores throughout the storage period.

Keywords: Clove-oil; Nanoemulsion; Paneer, Shelf-life

Introduction

India has grown tremendously in the field of dairy sector after the success of the world's largest dairy development program 'Operation-Flood'. Now India is not only self-sufficient but also the largest producer of milk in the world with the production of 230.58 million tonnes in the year 2022-23 (<https://pib.gov.in/PressReleasePage.aspx?PRID=1988609>). The surplus milk is being converted into wide range of value added dairy products such as curd, cheese, paneer, yoghurt and sweets etc. Paneer is one of the most popular and widely consumed traditional Indian dairy product (Kapoor et al. 2021). It is an un-ripened cheese produced by the method of acid and heat coagulation and almost 5% of the total milk produced in India is being converted into paneer. In 2021, International Market Analysis Research and Consulting (IMARC) group reported that market value of paneer was 365.5 billion INR and it was estimated to grow at the rate of 15% in the future. Paneer is manufactured by the method of acid and heat coagulation. It is a rich source of nutrients like proteins, vitamins, minerals and fat. Due to globalization as well as its increasing use as a meat substitute, the demand for paneer is continuously increasing. However, the rich moisture and nutrient content of paneer makes it highly vulnerable to spoilage. The spoilage of paneer is mainly attributed to growth of surface-tainting and off-flavor producing microorganisms such as coliforms, psychrotrophs, yeast and molds (Varalakshmi and Leysen, 2020).

Presently, preservation methods like brining, storage at refrigeration temperature, vacuum-based and addition of synthetic preservatives (benzoates, sorbates, H_2O_2 etc.) and antimicrobial packaging systems are being used to enhance the shelf-life of paneer (Sharma et al, 2019). Due to the increasing awareness of consumer and their concern towards a healthy lifestyle, the demand for plant-based preservation methods is increasing day by day. Therefore, the urge to develop preservation methods that are safer, more sustainable and includes minimum use of preservatives is there (Shashikumar et al. 2011). In this view, US code of Federal Regulations has provided GRAS (Generally Regarded as Safe) status to a number of essential oils and associated bioactive components with them. Different research investigations have shown effective results in

¹ Dairy Chemistry Department, - ICAR-National Dairy Research Institute, Karnal-132001

² Dairy Microbiology Department, ICAR-National Dairy Research Institute, Karnal-132001

D Bhardwaj (✉)
Email- dikshabhardwaj74972@gmail.com

enhancement of shelf-life of paneer by the addition or incorporation of essential-oils for preservation (Badola et al. 2018). Clove oil extracted from the bud, leaf or stem of the spice *Syzygium aromaticum* is one of the most popular essential oil inherited with a wide range of therapeutic properties like antibacterial, antifungal, antiviral, anti-oxidative, anti-inflammatory and analgesic properties (Takahashi et al. 2021 ; Raju and Sasikala, 2016). Therefore conventional methods of preservation could be efficiently replaced by addition of antimicrobial clove essential oil and it could prove to be comparatively better, safer and more sustainable approach for the preservation of paneer. However, several challenges limit its usage when it is directly added to a food system like high volatility, effect on organoleptic properties, high sensitivity towards light, low solubility and destruction of their bioactive component when exposed to different processing conditions (Rani et al. 2014). Nanoencapsulation can prove to be an effective approach to deal with these challenges. It results in improvement of bioavailability due to large surface area of small particles, increases the stability, results in masking the unpleasant taste and also leads to proper release of bioactive component (Chawla et al. 2021). Therefore, to explore the potential of nanoencapsulated clove oil in enhancing the shelf-life of paneer, the present storage study was carried out-(Liao et al. 2019).

Materials & Methods:

Preparation of clove-oil nanoemulsion:

Clove-oil nanoemulsion of oil in water (O/W) type was prepared using non-ionic surfactant 'Tween-80' and 'Clove-oil'. Both the ingredients Tween-80 and clove-oil were added at the rate of 1% followed by making up the volume using distilled water. Mixing of appropriate concentration of ingredients was followed by homogenization using pilot-scale homogenizer with pressure of 2000 psi at first stage and 500 psi at second stage. The prepared clove-oil nanoemulsion was further diluted to 1:1 using distilled water for treatment of paneer.

Treatment of paneer samples:

Paneer blocks of 100 g each with uniform dimensions were obtained from Model dairy plant, ICAR-NDRI Karnal before dipping stage of manufacturing and were treated with prepared clove-oil nanoemulsion. Three different batches of paneer samples were prepared by treating paneer and its packaging material (HDPE) with clove oil nanoemulsion and chilled water for different intervals keeping the total time of dipping constant which was 40 min. First batch was prepared by dipping the paneer samples in chilled water for 40 min, 30 min and 25 min followed by treating with clove-oil nanoemulsion for 0 min, 10 min and 15 min respectively. Second batch was prepared by packing untreated paneer samples in packaging material dipped in clove-oil nanoemulsion (PM). Third batch consisted of paneer samples

dipped in chilled water for 30 min and 25 min and then treating with clove-oil nanoemulsion for 10 min (PM+10) and 15 min (PM+15 min) followed by packing in packaging material treated with clove-oil nanoemulsion. Untreated sample of paneer was kept as control.

Analysis of paneer samples treated with clove-oil nanoemulsion:

Paneer samples treated with clove-oil nanoemulsion were stored at 4°C and were analyzed for different physicochemical, microbiological and sensory characteristics at interval of every three days which was on 0th, 3rd, 6th, 9th, 12th, 15th, 18th and 21st day. All the experiments were performed in triplicates.

Storage Study: Analysis of physicochemical and Microbial parameters of prepared paneer samples treated with clove oil nanoemulsion:

Moisture content:

Moisture content of paneer samples was analyzed using the method of IS: SP 18 (Part XI) (BIS, 1981). About 2.0 g of paneer was weighed in an aluminium dish with a glass rod followed by complete mashing with 4.0 mL of distilled water. Another 1.0 mL distilled water was added to wash-off adhered particles and the aluminium-dishes were kept in hot air oven maintained at 102 ± 1°C for about 4 h. After 4 h, the dishes were transferred to desiccator then cooled and were weighed. Cooling and weighing cycles were repeated after every 30 min until a constant reading was obtained. Moisture content was calculated using the formula:

$$\text{Moisture (\%)} = (w1 - w2) / (w2 - w) \times 100$$

where,

w1 = mass in g of the dish with material before drying

w2 = mass in g of the dish with the material after drying

w = mass in g of the empty dish

pH:

To analyze pH of paneer samples 10.0 g of paneer was taken and was converted into paste using 10.0 mL distilled water and then pH was determined using calibrated pH meter.

Free-fatty acids:

Free-fatty acid content was ascertained using the method developed by Deeth and Fitz-Gerald (1975). About 5.0 mL distilled water was added in 3.0 g grated paneer sample taken in 60 mL stoppered test-tube. Followed by addition of 10 mL extraction mixture (isopropanol, petroleum ether and 4 N sulphuric acid in the proportion 40:10:1) and then 6.0 mL petroleum ether. This

was followed by tempering at 40! for 10 min and the two layers were allowed to separate. 5.0 mL of upper layer was taken and was titrated against 0.02 N methanolic KOH after addition of few drops of phenolphthalein indicator. The formula used to calculate the free-fatty acids is as given below:

$$\text{TFFA } (\mu\text{eq/g of fat}) = \frac{V \times N}{P \times W} \times 1000$$

where,

V= Net titration volume in mL,

N= Normality of methanolic potassium hydroxide solution

P= Proportion of upper layer of aliquot titrated (i.e. volume of aliquot withdrawn / total volume of upper layer)

W = Weight of the sample in g

TFFA=

Extent of proteolysis:

Extent of proteolysis that the paneer samples went through was determined by preparing water soluble extract by method developed by Kuchroo and Fox, (1982). Sixty mL distilled water was added to 20.0 g of paneer followed by sonication. The homogenous solution was centrifuged at 1400 g for 10 min and the upper layer was removed. Followed by this, the pH was adjusted to 4.6 using 1 N HCl and the extract was filtered through 0.22 μ filter. Further protein content was analyzed using the method given by Lowry et al. (1951).

Effect of treatment of clove-oil nanoemulsion on Microbiological-parameters of paneer:

Standard plate count (SPC):

SPC count of paneer samples was taken by pouring 1 mL paneer sample diluted from 10^{-1} to 10^{-5} in a petri plate followed by layering of nutrient agar under aseptic conditions and incubating at 37! for 24 h. Followed by this, colony count was taken and was recorded in log cfu/ mL (Donnelly, 1976).

Coliform count:

Coliform count of paneer samples was taken by pouring 1 mL paneer sample diluted from 10^{-1} to 10^{-3} in a petri plate followed by layering of violet red bile agar under aseptic conditions and incubating at 37! for 24 h. Followed by this, dark red coloured colonies were counted and were recorded in log cfu/ mL (Houghtby et al. 1992).

Yeast and mold count:

Yeast and mold count of paneer samples was taken by pouring 1 mL paneer sample diluted from 10^{-1} to 10^{-3} in a petri plate followed by layering of potato dextrose agar under aseptic conditions and incubating at 25! for 3-5 days. Followed by this, colony count was taken and was recorded in log cfu/ mL (Marshall, 1992).

Storage Study: Analysis of sensory characteristics of prepared paneer samples treated with clove oil nanoemulsion

Paneer samples were cut into cubes of size 2.0 cm x 1.5 cm x 1.5 cm and were evaluated by trained panelists comprising of scientists of NDRI using the 9 point hedonic scale.

Results and Discussion

Analysis of physicochemical and Microbial parameters of prepared paneer samples treated with clove oil nanoemulsion

Moisture content

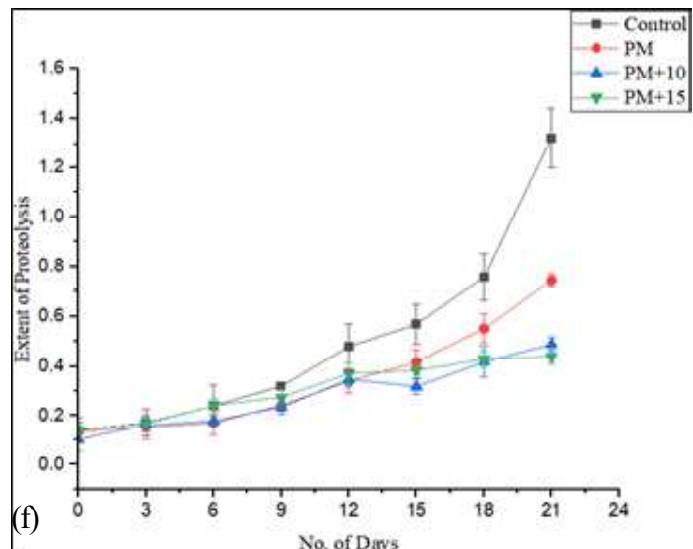
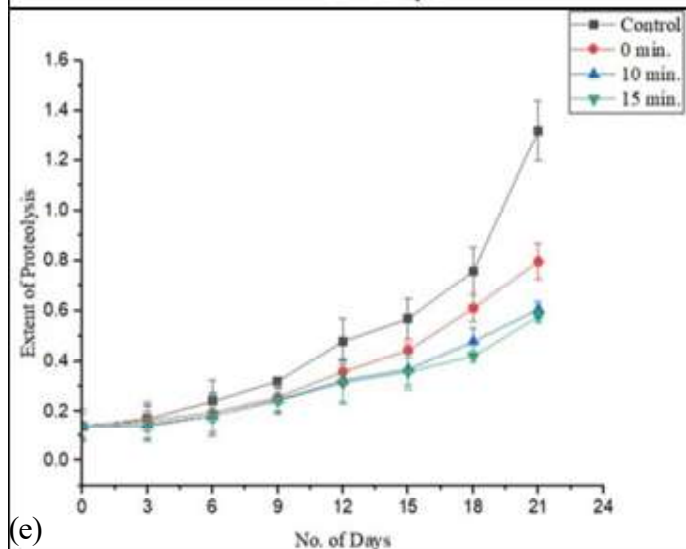
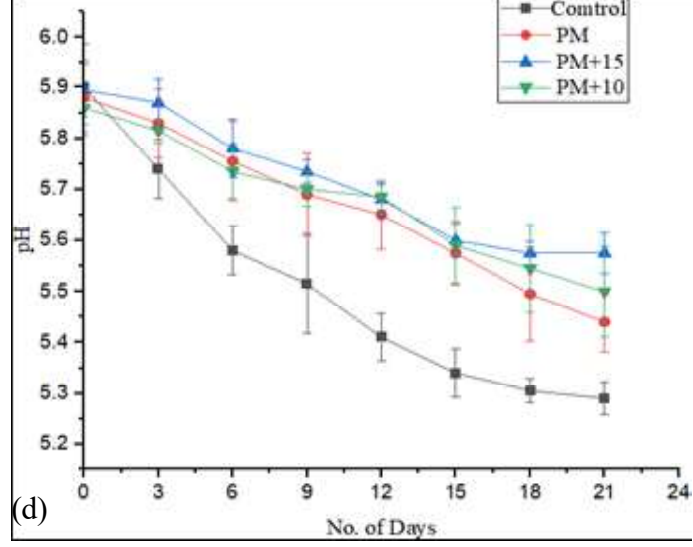
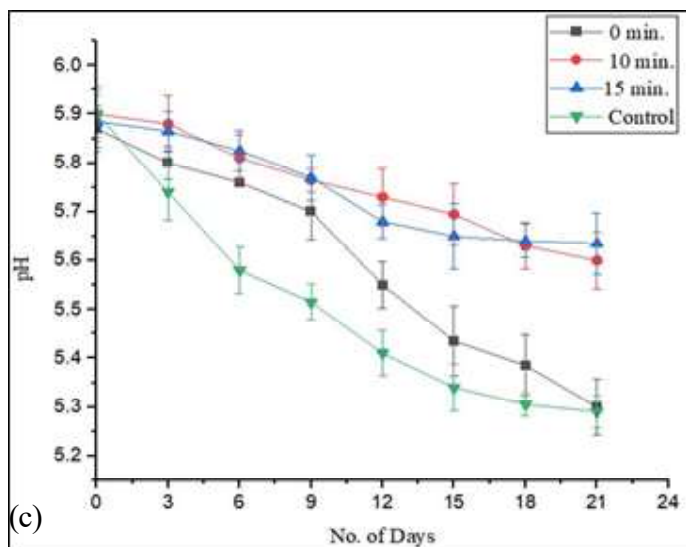
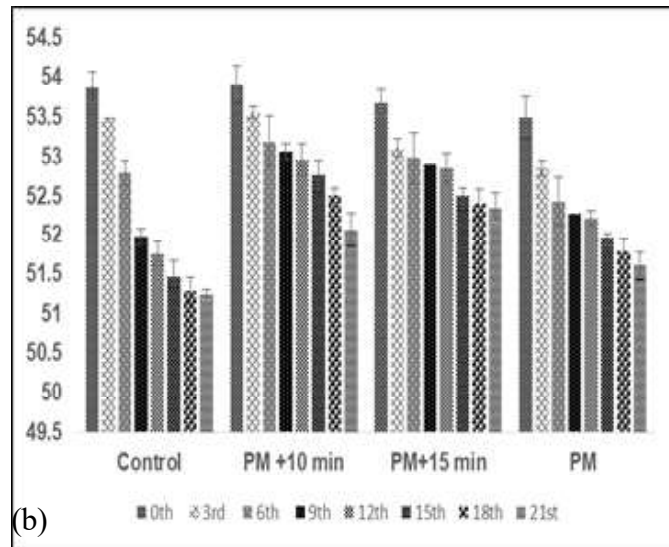
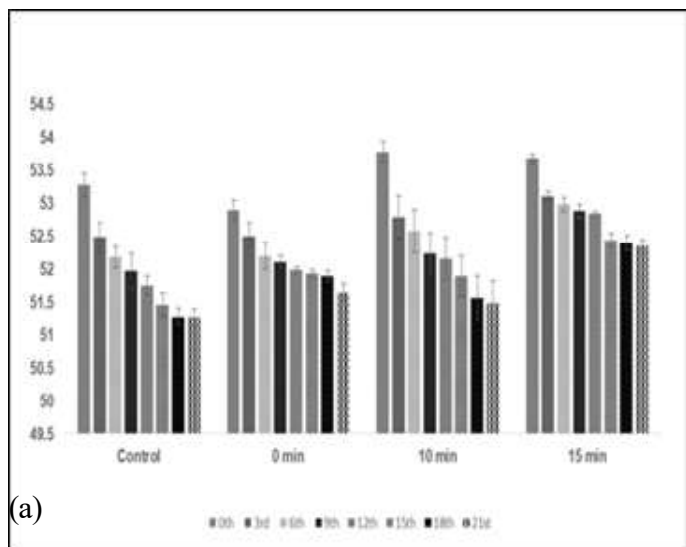
Moisture content of paneer samples treated with clove-oil nanoemulsion for 0 min, 10 min, 15 min and control were observed as $53.78 \pm 0.16\%$, $52.9 \pm 0.15\%$, $53.90 \pm 0.07\%$ and $53.27 \pm 0.18\%$ on the 0th day of storage and got reduced to $51.49 \pm 0.33\%$, $51.65 \pm 0.13\%$, $52.24 \pm 0.18\%$ and $51.27 \pm 0.13\%$ on the 21st day of storage as shown in Fig. 1 (a). While the paneer samples coded as PM, PM+10 and PM+15 showed reduction from $53.49 \pm 0.17\%$, $53.21 \pm 0.00\%$ and $53.68 \pm 0.07\%$ to $51.60 \pm 0.18\%$, $52.07 \pm 0.19\%$ and $52.35 \pm 0.09\%$ respectively as depicted in Fig. 1 (b). Paneer samples were taken as control showed significant reduction ($P < 0.05$) from $53.27 \pm 0.18\%$ to $51.27 \pm 0.13\%$ during 21 days of storage at 4°C. While no significant reduction ($P < 0.05$) was observed in moisture content of paneer samples treated with clove oil nanoemulsion. It was observed that moisture content decreased as the storage period of the paneer was increased which might be because of evaporation of moisture during storage. Due to the treatment with clove oil nanoemulsion, there might be formation of oil layer on the surface of the paneer samples which acted as a barrier and prevented the evaporation of moisture from the paneer. Several other research workers such as Khatkar et al. (2017a) and Masud et al. (2007) also documented similar results of reduction in moisture content during storage of paneer.

pH:

The pH of stored paneer samples was observed to reduce as the storage period was increased. On the 0th day of storage, pH of paneer was 5.90 ± 0.07 , 5.87 ± 0.02 , 5.90 ± 0.03 and 5.89 ± 0.03 for untreated control, samples treated for 0 min, for 10 min and for 15 min respectively. On the 3rd day of storage, the pH of control samples reduced down to 5.74 ± 0.06 , while all the treated paneer samples showed pH values higher than 5.80 ± 0.01 . Further on

the 6th day of storage, there was drastic reduction in pH down to the value 5.58 ± 0.05 while the treated paneer samples continued

to show comparatively much higher values as shown in Fig. 1. Similar trend was observed throughout the storage period when



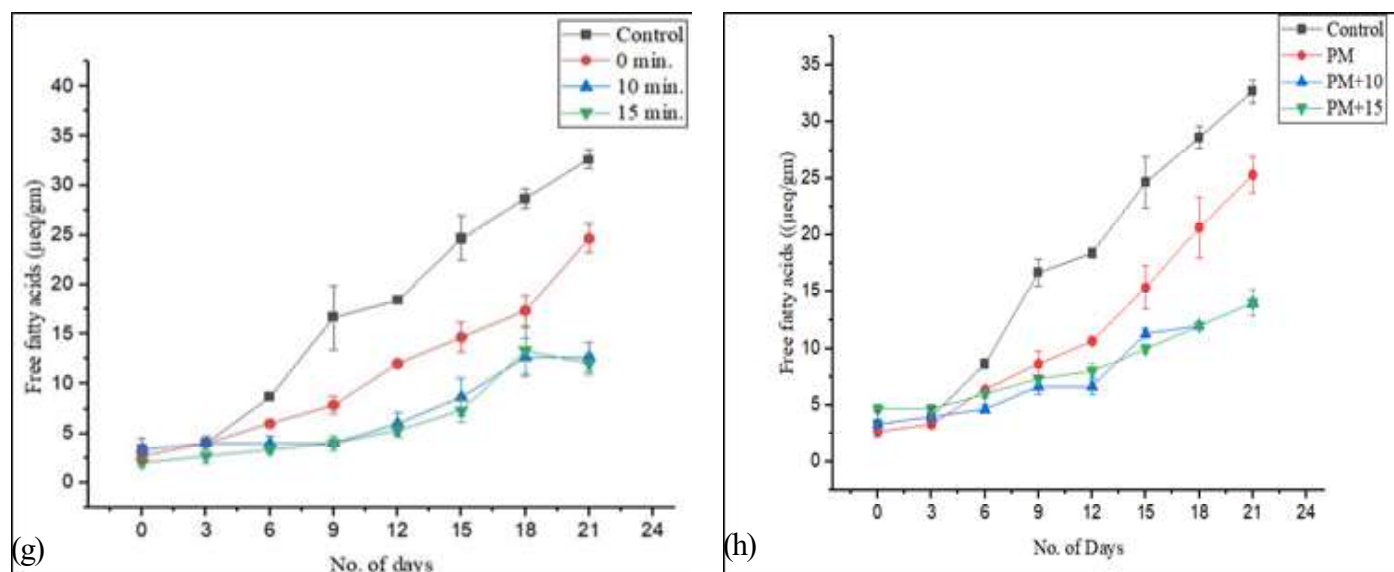


Fig. 1: Physicochemical analysis of Paneer samples treated with clove oil nanoemulsion for different intervals of time such as 0 min, 10 min and 15 min in comparison to untreated control during storage a) Moisture content c) pH e) Extent of proteolysis g) Free fatty acids; Paneer samples treated with clove oil nanoemulsion for different intervals of time such as 10 min and 15 min and packed in packaging material treated with clove essential oil nanoemulsion in comparison to untreated control during storage b) Moisture content d) pH f) Extent of proteolysis h) Free fatty acids

the pH of paneer samples was analyzed on the 9th, 12th, 15th and 18th day of storage. On the 21st day of storage, it was observed to get reduced to 5.29 ± 0.03 , 5.30 ± 0.06 , 5.60 ± 0.06 and 5.65 ± 0.07 for untreated control, samples treated for 0 min, for 10 min and for 15 min as shown in Fig. 1 (c). Similarly reduction in pH from 5.88 ± 0.07 to 5.44 ± 0.06 in case of PM, from 5.90 ± 0.02 to 5.58 ± 0.04 in case of PM+10 and from 5.86 ± 0.03 to 5.50 ± 0.09 for PM+15 samples was recorded as observed in Fig. 1 (d). The reduction in pH in control paneer samples was observed to be significantly higher ($P < 0.05$) in comparison to paneer samples treated with clove-oil nanoemulsion for 10 min, 15 min, PM+10 and PM+15 paneer samples.

The observed reduction in pH could be well-correlated with the SPC and yeast and mold count of paneer samples. On 21st day of storage, the pH of control sample reduced down to minimum value of 5.29 ± 0.03 and showed the highest SPC count of 5.31 ± 0.03 log cfu/ g and yeast and mold count of 4.55 ± 0.00 log cfu/g. Likewise pH of paneer samples treated with clove oil nanoemulsion for 10 min and 15 min was recorded as 5.60 ± 0.06 and 5.65 ± 0.06 respectively and the SPC count of 3.92 ± 0.13 and 3.71 ± 0.08 log cfu/ g and yeast and mold count of 3.61 ± 0.05 and 2.76 ± 0.13 log cfu/g respectively. Similar pattern was observed in the samples treated with clove oil nanoemulsion and packed in treated packaging material which showed pH value of 5.58 ± 0.04 and 5.5 ± 0.09 and SPC count of 3.34 ± 0.05 and 3.29 ± 0.05 log cfu/g and yeast and mold count of 2.67 ± 0.08 and 2.23 ± 0.10 log cfu/g for samples treated for 10 min and 15 min respectively. The decrease in pH was lesser in case of clove oil nanoemulsion

treated samples because of its preservative effect which inhibited the growth of microbes which ultimately led to reduction in pH of paneer (Khatkar et al. 2017 ; Karunamay et al. 2020). While in case of untreated samples, there was growth of acid producing microbes and other spoilage causing bacteria due to which higher reduction in pH was observed (Mishra et al. 2016 ; Dwiwedi et al. 2014).

Extent of proteolysis:

The freshly prepared paneer samples on 0th day of storage were recorded to have water soluble protein content of 0.14 ± 0.06 , 0.14 ± 0.07 , 0.14 ± 0.06 and 0.15 ± 0.05 mg/ mL for samples treated with clove oil nanoemulsions for no time (control), 0 min, 10 min and 15 min respectively as mentioned in Fig 1. On 3rd day of storage, the stored paneer samples started undergoing proteolysis, with the value increasing to 0.17 ± 0.10 mg/ mL in case of control samples while it was observed to be 0.14 ± 0.02 mg/ mL and 0.15 ± 0.03 mg/ mL for samples treated for 10 min and 15 min respectively. Similar pattern of control samples undergoing extensive proteolysis as compared to those given treatment with clove oil nanoemulsion was observed on 6th, 9th, 12th and 15th day of storage. On 18th day of storage, the control samples were recorded to undergo proteolysis as high as 0.76 ± 0.44 mg/ mL while those which were treated for 10 min and 15 min was 0.48 ± 0.01 mg/ mL and 0.42 ± 0.00 mg/ mL treated for 0 min, 10 min and 15 min respectively. On 21st day of storage, the soluble nitrogen content was observed to be 1.32 ± 0.12 , 0.78 ± 0.07 , 0.61 ± 0.03 and 0.58 ± 0.03 mg/ ml for untreated, treated for 0 min, 10 min and 15 min respectively as shown in Fig 1 (e). While soluble nitrogen

content was found to increase from 0.13 ± 0.04 to 0.74 ± 0.03 mg/mL for PM+10 samples and from 0.10 ± 0.05 to 0.49 ± 0.03 mg/mL for PM+15 paneer samples as indicated in Fig. 1 (f). The results obtained by analysis of extent of proteolysis showed that paneer samples taken as control underwent significant increase ($P < 0.05$) in soluble nitrogen as compared to the paneer samples treated with clove oil nanoemulsion for 10 min and 15 min. Degradation of proteins during storage was mainly associated with secretion of enzymes like proteases by the microorganisms (Khatkar et al. 2017b). Similar findings were reported by Sharma (2017) who studied the effect of treatment of clove oil nanoemulsion on extent of proteolysis of paneer during its storage, it was reported that the protein content of water soluble extract was found to reduce from 0.48 ± 0.01 , 0.48 ± 0.01 , 0.49 ± 0.01 , 0.50 ± 0.01 and 0.51 ± 0.01 mg/mL to 0.97 ± 0.02 , 0.76 ± 0.01 , 0.67 ± 0.04 , 0.65 ± 0.02 , 0.80 ± 0.04 and 0.88 ± 0.02 mg/mL for samples treated for 0 min, 10 min, 15 min and 30 min respectively with clove oil nanoemulsion stabilized with milk proteins. Comparable results were pointed by another research study that extent of proteolysis in samples

treated with clove oil nanoemulsion as compared to the control samples increased at a slower rate (Khadka et al. 2021).

Free fatty acid content:

Lipases produced by microorganisms results in hydrolysis of lipids which led to formation of free fatty acids. It was observed that free fatty acid (FFA) content on 0th day of storage was 3.33 ± 0.47 , 2.67 ± 0.39 , 3.33 ± 0.27 and 2.00 ± 0.27 $\mu\text{eq/g}$ of paneer for samples taken as control, treated with clove oil nanoemulsion for 0 min, 10 min and 15 min respectively. On 3rd day of storage, the FFA content of all the paneer samples remained almost same. While on 6th day of storage, a major increase was observed, the FFA content of control samples got increased to 8.67 ± 0.27 $\mu\text{eq/g}$ and those of paneer samples treated for 0 min to 6.00 ± 0.27 $\mu\text{eq/g}$. Similarly, on 9th, 12th, 15th and 18th day of storage, the FFA content of paneer samples taken as control continued to increase at much higher rate as compared to the paneer samples treated with clove oil nanoemulsion. On 21st day of storage, it was observed to increase to 32.67 ± 0.98 , 24.67 ± 0.82 , 12.67 ± 0.81 and

Table 1: Standard plate count, Coliform count and Yeast and mold count (log cfu/g) of different paneer samples treated with clove oil nanoemulsions for different intervals

Day	Microbiological parameters	Control	0 min.	10 min.	15 min.	PM	PM+10	PM+15
0	Standard plate count	4.62 ± 0.14^D	4.63 ± 0.14^C	4.68 ± 0.10^A	4.58 ± 0.03^A	4.55 ± 0.09^{AB}	4.69 ± 0.01^A	4.45 ± 0.09^A
	Coliform count	1.60 ± 0.09^C	1.58 ± 0.11^C	1.36 ± 0.03^B	1.48 ± 0.10^{AB}	1.47 ± 0.03^C	1.45 ± 0.13^B	2.24 ± 0.01^B
	Yeast & mold count	1.74 ± 0.09^E	1.51 ± 0.01^D	1.36 ± 0.00^D	1.46 ± 0.02^C	1.49 ± 0.23^D	1.53 ± 0.07^{DE}	1.70 ± 0.11^B
3	Standard plate count	4.92 ± 0.05^{CD}	4.66 ± 0.06^C	4.62 ± 0.05^C	4.54 ± 0.06^A	4.65 ± 0.01^{AB}	4.62 ± 0.08^A	4.55 ± 0.06^A
	Coliform count	2.08 ± 0.19^{BC}	1.62 ± 0.08^C	1.84 ± 0.15^{AB}	1.00 ± 0.24^B	1.37 ± 0.03^C	0.61 ± 0.01^C	0.95 ± 0.12^B
	Yeast & mold count	1.57 ± 0.14^E	1.49 ± 0.01^D	1.49 ± 0.10^D	0.69 ± 0.15^D	1.27 ± 0.01^D	1.27 ± 0.11^E	1.01 ± 0.03^E
6	Standard plate count	5.31 ± 0.04^{AB}	4.62 ± 0.01^C	4.40 ± 0.05^C	4.31 ± 0.05^A	4.34 ± 0.02^B	4.56 ± 0.02^A	4.48 ± 0.13^A
	Coliform count	2.13 ± 0.00^{BC}	1.62 ± 0.21^B	0.85 ± 0.16^C	0.63 ± 0.13^C	1.21 ± 0.11^B	0.60 ± 0.12^C	0.90 ± 0.09^{BC}
	Yeast & mold count	2.34 ± 0.06^{DE}	1.15 ± 0.02^D	1.05 ± 0.06^E	0.43 ± 0.03^D	1.48 ± 0.05^D	0.87 ± 0.08^{DE}	0.72 ± 0.11^F
9	Standard plate count	5.43 ± 0.06^A	4.97 ± 0.05^B	3.58 ± 0.10^B	3.81 ± 0.02^A	4.39 ± 0.18^B	3.45 ± 0.13^B	3.29 ± 0.12^B
	Coliform count	2.43 ± 0.00^B	2.36 ± 0.10^B	1.25 ± 0.07^C	1.08 ± 0.10^{AB}	2.14 ± 0.13^B	1.19 ± 0.09^B	1.45 ± 0.06^B
	Yeast & mold count	2.71 ± 0.04^{CD}	1.97 ± 0.05^C	1.39 ± 0.01^D	1.19 ± 0.03^C	2.02 ± 0.12^C	1.41 ± 0.11^D	1.11 ± 0.04^E
12	Standard plate count	5.21 ± 0.16^{ABC}	5.15 ± 0.02^A	3.23 ± 0.08^A	3.99 ± 0.12^A	4.75 ± 0.01^{AB}	3.28 ± 0.01^B	3.05 ± 0.01^{BC}
	Coliform count	2.60 ± 0.04^B	2.45 ± 0.14^B	1.37 ± 0.02^B	1.22 ± 0.10^{ABC}	2.20 ± 0.12^{BC}	1.47 ± 0.07^B	1.80 ± 0.10^{AB}
	Yeast & mold count	3.26 ± 0.11^{BC}	2.78 ± 0.00^B	2.26 ± 0.02^C	1.49 ± 0.02^C	2.59 ± 0.14^B	2.18 ± 0.08^C	1.37 ± 0.10^D
15	Standard plate count	5.03 ± 0.04^{BC}	5.68 ± 0.06^C	3.84 ± 0.08^C	3.93 ± 0.14^A	4.93 ± 0.06^A	3.27 ± 0.12^B	2.97 ± 0.10^B
	Coliform count	2.59 ± 0.16^B	2.52 ± 0.12^B	1.61 ± 0.16^A	1.23 ± 0.16^A	2.46 ± 0.19^{AB}	2.36 ± 0.04^{AB}	2.04 ± 0.07^A
	Yeast & mold count	3.29 ± 0.00^B	2.77 ± 0.01^B	2.05 ± 0.03^C	2.00 ± 0.04^B	2.92 ± 0.08^B	2.20 ± 0.07^B	1.60 ± 0.04^{BC}
18	Standard plate count	5.16 ± 0.12^{ABC}	4.49 ± 0.12^{BC}	3.86 ± 0.02^C	3.74 ± 0.16^A	4.36 ± 0.13^B	3.69 ± 0.03^B	3.43 ± 0.02^B
	Coliform count	3.17 ± 0.18^A	2.83 ± 0.13^{AB}	1.88 ± 0.11^A	1.25 ± 0.09^{AB}	2.92 ± 0.00^A	1.82 ± 0.10^A	1.35 ± 0.01^B
	Yeast & mold count	3.62 ± 0.00^B	3.60 ± 0.01^A	2.78 ± 0.03^B	2.24 ± 0.01^B	3.58 ± 0.00^A	2.49 ± 0.11^B	1.83 ± 0.07^B
21	Standard plate count	5.31 ± 0.03^{AB}	4.42 ± 0.12^C	3.92 ± 0.13^C	3.71 ± 0.08^A	4.54 ± 0.01^B	3.34 ± 0.05^B	3.29 ± 0.05^B
	Coliform count	3.38 ± 0.00^A	3.04 ± 0.05^A	2.11 ± 0.14^A	1.17 ± 0.10^{AB}	2.77 ± 0.10^A	1.49 ± 0.08^A	0.95 ± 0.03^B
	Yeast & mold count	4.55 ± 0.03^A	3.78 ± 0.01^A	3.61 ± 0.05^A	2.76 ± 0.13^A	3.76 ± 0.03^A	2.73 ± 0.08^A	2.12 ± 0.10^A

12.00 ± 0.67 µeq/g for paneer samples taken as control, treated with clove oil nanoemulsion for 0 min, for 10 min and for 15 min respectively as shown in Fig. 1 (g). Similar trend was observed for paneer samples packed in treated packaging material after treating with clove oil nanoemulsion such as 25.33 ± 0.94 µeq/g in PM, 14.00 ± 0.47 µeq/g for PM+10 and 14.00 ± 0.47 µeq/g for PM+15 paneer samples as depicted in Fig. 1 (h).

FFA content showed direct relation with the SPC and yeast and mold count of paneer samples. It was observed that paneer samples taken as control showed maximum increase in SPC count to 5.29 ± 0.03 log cfu/g and yeast and mold count got increased to 4.55 ± 0.00 log cfu/g. While paneer samples which were treated with clove oil nanoemulsion showed a very low SPC count of 3.92 ± 0.13 log cfu/g and 3.71 ± 0.08 log cfu/g and yeast and mold count of 3.61 ± 0.05 log cfu/g and 2.76 ± 0.13 log cfu/g for samples treated for 10 min and 15 min with clove oil nanoemulsion. This clearly indicated that increase in FFA content was directly related with the microbial growth in the paneer samples which secreted the enzymes like lipases and ultimately resulted in lipolysis of paneer samples. Treatment of paneer samples with clove oil nanoemulsion for 15 min reduced the FFA content by 63.2% as compared to control.

Similar results were reported, when Sharma (2017) studied the effect of treatment of clove oil nanoemulsion in reducing the FFA content of the paneer samples. It was reported that FFA content was significantly lower in those samples which were treated with the nanoemulsion for 10 min. and 15 min. as compared to those which were kept untreated. In an another study, it was observed that when the efficiency of turmeric was tested for its ability to enhance the shelf life of paneer, FFA content of the untreated samples increased at a much faster rate as compared to samples treated with turmeric. Also it was documented that addition of 0.6% turmeric in paneer samples resulted in reduction of its FFA content (Buch et al. 2014).

Effect of treatment of clove-oil nanoemulsion on Microbiological-parameters of paneer:

Standard plate count:

Steep rise was observed in SPC count of paneer samples with the increase in storage time. SPC count of the clove oil nanoemulsion treated paneer samples was observed to increase at a gradual rate as compared to the untreated samples. On 0th day of storage, it was observed as 4.62 ± 0.14, 4.63 ± 0.14, 4.68 ± 0.10 and 4.58 ± 0.03 log cfu/g for samples taken as control, treated with clove oil nanoemulsion for 0 min, for 10 min and for 15 min. respectively as shown in Table 1. It remained almost same on 6th day of storage. On 9th day of storage, the SPC count of the control paneer samples increased abruptly to 5.43 ± 0.06 log cfu/g while a sharp decrease in SPC count to 3.58 ± 0.10 and 3.81 ± 0.02 log cfu/g was recorded for samples treated with clove oil

nanoemulsion for 10 min and 15 min. respectively. It was notified that the antimicrobial effect of added clove oil became evident at the end of the lag phase of microbial growth, when the exponential phase of growth of microbes just got started.

On further storage of paneer samples, the SPC count of all the samples observed to increase when assessed on 12th, 15th and 18th day of storage as shown in Table 1. On 21st day of storage, the SPC count was recorded as 5.31 ± 0.03, 4.42 ± 0.12, 3.92 ± 0.13 and 3.71 ± 0.08 log cfu/g for paneer samples taken as control, treated with clove oil nanoemulsion for 0 min, 10 min and 15 min respectively. Similar pattern was observed in paneer samples packed in treated packaging material after the treatment of samples with clove oil nanoemulsion like SPC count remained static in case of untreated samples packed in treated packaging material, reduced from 4.69 ± 0.01 to 3.34 ± 0.05 log cfu/g when packed after treatment for 10 min and reduced from 4.45 ± 0.09 to 3.29 ± 0.05 log cfu/g when treated with clove oil nanoemulsion for 15 min as shown in Table 1. The observed significant difference (P<0.05) between the increase in SPC count of treated and untreated samples could be justified by the antimicrobial effect associated with clove oil. Devaki et al. 2021, reported that star anise incorporated paneer samples showed an increase from 12x10² cfu/g to 51x10² cfu/g in 30 days while for control samples it was reported to increase from 28x10³ to 75x10³ cfu/g after 30 days. It was observed that paneer incorporated with spices showed lower microbial count as compared to the control samples. Sharma (2017) reported that shelf-life of nanoemulsion treated paneer increased to more than 15 days as compared to 6 days in case of control. It was reported that paneer samples treated for 10 min and 15 min with clove essential oil showed four log cycle lower counts for SPC as compared to the control when stored for 20 days.

Coliform count

On the 0th day of storage study, coliforms count were recorded to be 1.60 ± 0.09, 1.58 ± 0.11, 1.36 ± 0.03 and 1.48 ± 0.10 log cfu/g for control samples, samples treated for 0 min, for 10 min and for 15 min respectively. It was observed that on 6th day of storage of paneer samples, there was a steep rise in coliform count from 1.60 ± 0.09 to 2.13 ± 0.00 log cfu/g in case of control while the paneer samples treated with clove oil nanoemulsion showed a reduction in coliform count from 1.36 ± 0.03 to 0.85 ± 0.16 log cfu/g for samples treated with clove oil nanoemulsion for 10 min and from 1.48 ± 0.10 to 0.63 ± 0.13 log cfu/g for samples treated for 15 min with clove oil nanoemulsion as observed from the Table 1. Increase in coliform count was observed, when the paneer samples were analyzed further during the storage period on 9th, 12th, 15th and 18th day of storage. However, increase in coliform count in treated paneer samples was lesser significant (P<0.05) compared to the untreated samples as depicted in Table 1. Coliform count on 21st day of storage was reported to be 3.38 ± 0.00, 3.04 ± 0.04, 2.11 ± 0.14 and 1.17 ± 0.10 log cfu/g for control

samples, for samples treated for 0 min, for 10 min and for 15 min respectively as shown in Table 1.

Similar trend was observed, when paneer samples were packed in treated packaging material after being treated with clove oil nanoemulsion such as coliform count on 21st day of storage was observed to be 2.77 ± 0.10 log cfu/g for untreated paneer samples packed in treated packaging material, 1.49 ± 0.08 log cfu/g for samples treated with clove oil nanoemulsion for 10 min before packaging. The results obtained during the study showed that treatment of paneer with clove oil nanoemulsion reduced the coliform count by 1.27 and 2.21 log cycles respectively for samples treated for 10 min and 15 min respectively. While for samples packed in treated packaging material the reduction was estimated to be 1.89 and 2.43 log cycles for samples treated for 10 min and 15 min respectively.

Similarly, Khatkar et al. 2017 reported that coliform count increased from 1.17 to 1.84 log cfu/g during the storage period of 15 days. Another research study pointed that cardamom treated paneer samples showed a sharp decrease in coliform count after 14 days of storage while it was reported to increase abruptly in case of control samples. Shelf-life of paneer samples was reported to increase upto 28 days after treatment of paneer (Eresam et al. 2015).

Yeast and mold count:

Yeast and mold count was 1.74 ± 0.09 , 1.51 ± 0.01 , 1.36 ± 0.00 , 1.46 ± 0.02 and 1.49 ± 0.23 log cfu/g for untreated paneer samples, samples treated for 0 min, for 10 min, for 15 min and packed in treated packaging material respectively on 0th day of storage as shown in Table 1. Initially there was reduction in yeast and mold count on 3rd day of storage and then yeast and mold count started to increase. On 6th day of storage, it was observed that the count increased to 2.34 ± 0.06 log cfu/g in case of control while it got reduced to 1.05 ± 0.6 log cfu/g and 0.43 ± 0.03 log cfu/g in samples treated with clove oil nanoemulsion for 10 min and 15 min respectively. Increase in yeast and mold count was observed when paneer samples were further analyzed on interval of every 3 days like on 12th, 15th and 18th day of storage as observed from Table 1.

On 12th day of storage, black coloured spots which suggested the growth of *Aspergillus spp.* or *Cladosporium spp.* were visible on the control samples. No such growth was witnessed on paneer samples treated with clove oil nanoemulsion as shown in Plate 1. On 21st day of storage, the count was reported to be 4.55 ± 0.03 , 3.78 ± 0.01 , 3.61 ± 0.05 , 2.76 ± 0.13 and 3.76 ± 0.03 log cfu/g for control samples, samples treated for 0 min, for 10 min, for 15 min and for the packaging material dipped in clove oil nanoemulsion as shown in Table 1. While paneer samples dipped in clove oil nanoemulsion for 10 min and 15 min followed by packing in treated packaging material were ascertained to have yeast and

mold count of 2.73 ± 0.08 and 2.12 ± 0.10 log cfu/g respectively as shown in Table 1. On 21th day of storage, spots of pink, green, black and brown colour were observed on paneer samples taken as control, dipped in clove oil nanoemulsion for 0 min and packed in packaging material dipped in the nanoemulsion as shown in Plate 1. The spots of pink, green, black and brown might be because of growth of yeast *Rhodotorula*, yeast *Candida albicans*, mold *Aspergillus* and mold *Penicilli* respectively (Khatkar et al. 2017 ; Goyal and Goyal 2016).

Sharma (2017) documented similar results that treatment of paneer samples with clove oil nanoemulsions prepared using whey protein concentrate and maltodextrin conjugate resulted in reduction in yeast and mold count by one and three log cycles after storing the samples in refrigerator for three and twelve days respectively as compared to control. Also it was reported that shelf-life of treated paneer samples increased to 15 days as compared to 6 days in case of control. Eresam et al. 2015 reported that yeast and mold count increased sharply in case of control samples and no such steep rise in cardamom treated paneer samples was observed.

Storage Study: Analysis of sensory characteristics of prepared paneer samples treated with clove oil nanoemulsion

Flavor:

Paneer samples treated with clove oil nanoemulsion elicited comparatively lower scores of 7.33 ± 0.13 and 7.10 ± 0.11 for samples treated for 10 min and 15 min in comparison to the untreated sample which received the scores of 8.33 ± 0.18 on 0th day of storage. Clove-oil flavor was prominent in samples treated with clove-oil nanoemulsion for longer time and therefore these samples received lower scores. However there was reduction in clove-oil flavor with time and the treated samples received higher scores on 3rd day of storage of 7.47 ± 0.11 and 7.20 ± 0.11 for samples treated for 10 min. and 15 min. which may be because of volatile nature of clove-oil.

While the sensory scores of untreated sample got diminished to 8.27 ± 0.20 . On 6th day of storage, there was a tinge of acidic flavor and the overall acceptability score was recorded as 7.73 ± 0.31 which might be because of the growth of lactic acid bacteria. No such flavour was perceived in paneer samples treated with clove oil nanoemulsions for different intervals of time and sensory scores remained the same. The reason may be preservative action due to antimicrobial activity of clove essential oil which inhibited the growth of lactic acid bacteria and prevented paneer from spoilage. The overall acceptability score of control samples diminished drastically on 9th day of storage to 6.27 ± 0.87 while the paneer samples treated for 10 min received 7.37 ± 0.13 and those treated for 15 min received 7.17 ± 0.13 as sensory scores. Paneer samples treated for 0 min. were given 7.03 ± 0.94 scores.

Similarly, it was observed that the acceptability scores of control samples diminished continuously while those of paneer samples treated with clove oil nanoemulsion remained almost constant when assessed on 12th, 15th and 18th day of storage as shown in Fig. 2 (a). On 21st day of storage, it was observed that paneer samples taken as control and dipped in nanoemulsion for 0 min. became unacceptable on the basis of their organoleptic characteristics with the sensory scores of 0.67 ± 0.31 and 4.30 ± 0.69 respectively. On the other hand, paneer samples treated for 10 min and 15 min remained acceptable with sensory scores of 6.43 ± 0.20 and 6.63 ± 0.15 respectively and no change in flavour or taste was perceived by the panelists. Similarly, paneer samples

packed in treated packaging material became unacceptable while those packed after giving treatment for 10 min and 15 min remained acceptable with sensory scores of 6.30 ± 0.14 and 6.30 ± 0.13 respectively as shown in Fig. 2(b).

Body and Texture

Textural characteristics of treated paneer samples were ascertained to be similar to the control sample because the total time of dipping the paneer samples collectively in water and clove oil nanoemulsion was kept constant. No deterioration in texture of

Plate 1: Appearance of paneer samples treated with clove essential oil nanoemulsions for different intervals of time on 21st day of storage

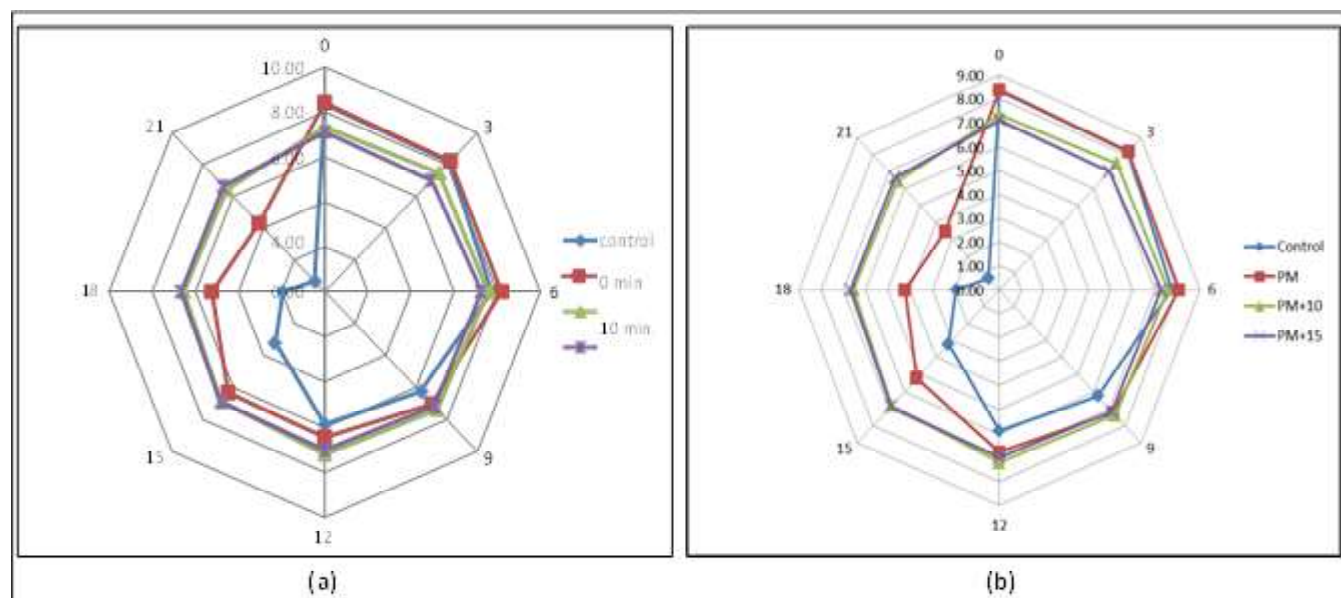
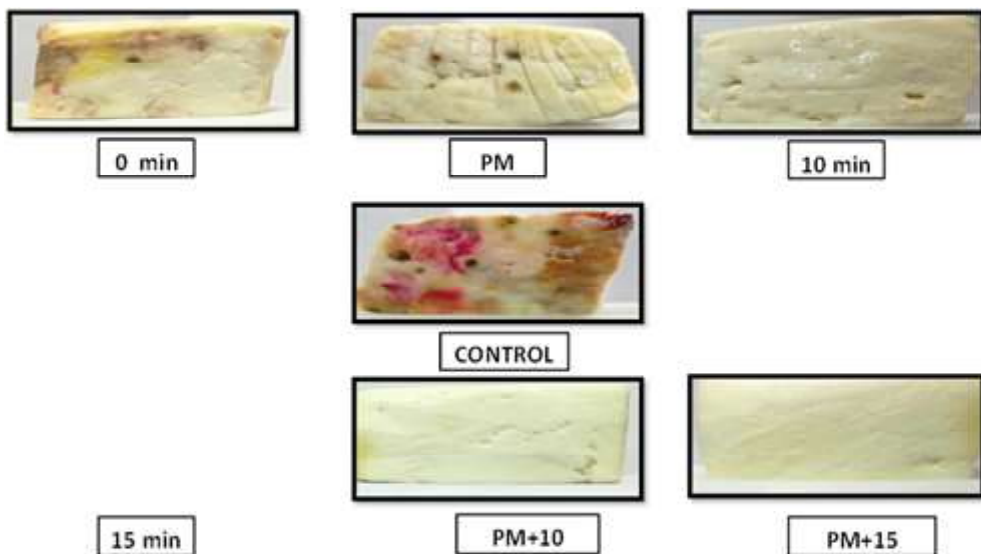


Fig. 2 Change in sensory scores of paneer samples treated with clove oil nanoemulsion for a) different intervals of time such as 0 min, 10 min. and 15 min. in comparison to untreated control during storage b) different intervals of time such as 10 min. and 15 min. and packed in packaging material treated with clove essential oil nanoemulsion in comparison to untreated control during storage

the treated paneer sample was noticed on 3rd and 6th day of storage. On the other hand, greenish-yellow colored slime formation became evident on 9th day of storage which might be because of the growth of slime secreting bacteria such as *Escherchia spp.*, *Streptococcus spp.* and *Staphylococcus spp.* (Yashwantha et al. 2020). Similar slime growth was observed in paneer samples treated for 0 min and samples packed in treated packaging material with clove oil nanoemulsion on 15th day of storage. Body and texture of paneer samples treated for 10 min. and 15 min. remained almost similar and was acceptable by all the panellists.

Appearance

Appearance of different paneer samples was also recorded during the storage study and it was observed that in case of control samples black coloured spots became visible on 12th day of storage. Growth of different molds which could be ascertained by different pink, yellow, brown and green coloured spots became evident on subsequent days of storage. Appearance of these spots could be correlated with growth of different yeast and molds such as yeast *Rhodotorula*, yeast *Candida albicans*, mold *Aspergillus* and mold *Penicillium* (Yashwantha et al. 2020).

No such development of coloured spots was witnessed in case of paneer samples treated for 10 min. and 15 min. and also in samples followed by packing in treated packaging material as evident from Plate 1. Due to which the paneer samples treated for longer time elicited higher scores as compared to other mentioned samples on the 21st day of storage as shown in Fig. 2 (a) and Fig. 2 (b). Based on the sensory characteristics of paneer in terms of flavour, texture and appearance of paneer samples, it was observed that the paneer samples treated with clove oil nanoemulsion for 10 min. and 15 min. and those packed in nanoemulsion treated packaging material after treating the paneer samples with clove oil nanoemulsion for 10 min and 15 min were found to be shelf-stable during twenty-one days of storage.

Paneer spread containing 1% salt with sodium benzoate preservative reported that acceptability score diminished from 8.20 to 4.00 over a period of 20 days of storage. It was reported that sensory characteristics of paneer spread remained almost unaltered when preservatives like sodium benzoate and potassium bisulphate were added (Dongare et al. 2019; Singh et al. 2020).

Conclusion

Findings of the present study revealed that treatment of paneer with clove oil nanoemulsion resulted in enhancement of shelf-life of paneer by efficiently inhibiting the growth of coliforms, yeast and molds and other microbes without having negative effect on sensory characteristics of treated samples. Physicochemical characteristics such as moisture content, pH, free fatty acid content and extent of proteolysis were ascertained to be $52.35 \pm 0.09\%$, 5.50 ± 0.09 , $14.00 \pm 0.47 \mu\text{eq./g}$ and 0.44 ± 0.04

mg/ mL for paneer samples treated with clove-oil nanoemulsion for 15 min. followed by packing in treated packaging material while $51.27 \pm 0.13\%$, 5.29 ± 0.03 , $24.67 \pm 0.82 \mu\text{eq./g}$ and $1.32 \pm 0.76 \text{ mg/ mL}$ respectively in case of untreated control samples on 21st day of storage. Similarly studies of microbiological parameters revealed reduction in SPC, coliform and Yeast and mold count by 2.02, 2.43 and 1.88 log cycles in comparison to control. It was observed that as the time of treatment of paneer samples was increased, there was improvement in physicochemical, microbiological and sensory characteristics of paneer and hence it could be prevented from spoilage for longer time. Clove-oil nanoemulsion treatment of paneer resulted in increase in its shelf-life by almost two-weeks. Therefore, it may be stated that clove oil, a natural antimicrobial essential oil in its nanoemulsion form may prove to be an efficient method in replacing the existing chemical methods of preservation of paneer.

References

- Badola R, Danish M, Kumar S, Fahad M, Kanade PP, Upadhayay S, Kohli D, Rautela I (2018) Effect of incorporation of black pepper and cardamom on quality characteristics of paneer. *Int J Appl Sci Eng* 6(2):121-127
- BIS (1981) Handbook of Food analysis, SP- 18, Part XI- Dairy Products Bureau of Indian Standards. Manak Bhavan, New Delhi
- Buch S, Pinto S, Aparnathi K D (2014) Evaluation of efficacy of turmeric as a preservative in paneer. *J Food Sci Technol* 51(11): 3226-3234
- Chawla P, Najda A, Bains A, Nurzyńska-Wierdak R, Kaushik R, Tosif MM (2021) Potential of gum arabic functionalized iron hydroxide nanoparticles embedded cellulose paper for packaging of Paneer. *Nanomaterials* 11(5):1308
- Deeth HC, Fitz-Gerald CH, Wood AF (1975) A convenient method for determining the extent of lypolysis in milk. *Aus J Dairy Technol* 30:109-111.
- Devaki CS, Rashmi HS, Pallavi R, Shekhara RN (2021) Development and Storage Studies on Ready to Use Spice-Based Paneer. *J Adv Dairy Res* 9(3) 250: 1-6
- Dongare SA, Dige YP, Syed HM (2019) Storage study and textural profile analysis of paneer at different temperature. *J Pharma Phyto* 8(2): 864-868
- Dwivedi B, Yadav BL, Gupta MP (2014) Storage related changes in sensory profile of paneer spread. *J Rur Agric Res* 14(1) 9-11
- Donnelly CB, Gilchrist JE, Peeler JT, Campbell JE (1976) Spiral plate count method for the examination of raw and pasteurized milk. *Appl Environ Microbiol* 32(1): 21-27
- Eresam E, Pinto S, Aparnathi KD (2015) Concise and informative title: evaluation of selected spices in extending shelf life of paneer. *J Food Sci Technol* 52(4): 2043-2052
- Goyal S, Goyal GK (2016) Maximizing shelf life of paneer—A review. *Crit Rev Food Sci Nutri* 56(8):1253-1261
- Houghtby GA, Maturin LJ, Koenig EK (1992) Microbiological count methods. *Standard methods for the examination of dairy products* 16: 213-246.
- <https://pib.gov.in/PressReleasePage.aspx?PRID=1988609>
- IMARC (2021) Paneer Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2022-2027.
- Kapoor R, Jash A, Rizvi SS (2021) Shelf-life extension of Paneer by a sequential supercritical-CO₂-based process. *LWT* 135: 110060

- Karunamay S, Badhe S R, Shulka V, Jaiswal S (2020) Effect of essential oil of clove and oregano treated edible packaging film in extending the shelf life of paneer. *J Pharm Innov* 9(7): 317-322
- Khadka B (2021) Effect of Herbal Extract on the Shelf Life of Paneer (Doctoral dissertation, Department of Food Technology Central Campus of Technology Institute of Science and Technology Tribhuvan University, Nepal)
- Khatkar AB, Ray A, Kaur A (2017a) Effect of addition of clove essential oil on the storage stability of paneer. *Phar Inno* 6(9): 39
- Khatkar AB, Ray A, Kaur A (2017b) Studies on shelf life extension of paneer with the addition of plant essential oil and different packaging materials. *Int J Curr Microbiol Appl Sci* 6:376-389
- Kuchroo CN, Fox PF (1982) Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. *Milchwissenschaft* 37:331-335
- Liao W, Badri W, Dumas E, Ghnimi S, Elaïssari A, Saurel R, Gharsallaoui A (2021) Nanoencapsulation of essential oils as natural food antimicrobial agents: an overview. *Appl Sci* 11(13): 5778
- Lowry OH, Rosebrough NF, Farr AL and Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem* 193 265-275
- Marshall RT (1992) Standard methods for the examination of dairy products. American Public Health Association, Washington D.C.
- Masud T, Shehla S, Khurram M (2007) Paneer (White cheese) from buffalo milk. *Biotech Biotechnol Equi* 21(4): 451-452
- Mishra D, Rao KJ, Bhardwaj R, Sutariya H, Kavitar RS, Subhash WS (2016) Effect of pH on sensory, textural, microbial quality and shelf-life of paneer. *Inter J Food Ferment Tech* 6(2): 405-414
- Raju A, Sasikala S (2016) Natural Antimicrobial Edible Film for Preservation of Paneer. *Biosci Biote Res Asia* 13(2): 1083-1088
- Rani M, Dabur RS, Khanna S, Potliya S, Verma S (2014) Application of pickling for preservation of paneer. *Asi J Dairy Food Res* 33(3): 183-186
- Sharma A, Shivaprasad DP, Chauhan K, Taneja NK (2019) Control of *E. coli* growth and survival in Indian soft cheese (paneer) using multiple hurdles: Phytochemicals, temperature and vacuum. *LWT* 114: 108350
- Sharma M, Mann B, Sharma R, Bajaj R, Athira S, Sarkar P, Pothuraju R (2017) Sodium caseinate stabilized clove oil nanoemulsion: physicochemical properties. *J Food Eng Biot Rep* 34
- Shashikumar CSS and Puranik DB (2011) Study on use of lactoferrin for the biopreservation of paneer. *Trop Agric Res* 23 (1): 70 – 76
- Singh NK, Dwiwedi N, Gupta M (2020) Storage related changes in microbiological quality of filled milk paneer. *J Rur Agric Res* 20(2): 18-22
- Takahashi H, Nakamura A, Fujino N, Sawaguchi Y, Sato M, Kuda T, Kimura B (2021) Evaluation of the antibacterial activity of allyl isothiocyanate, clove oil, eugenol and carvacrol against spoilage lactic acid bacteria. *LWT* 145: 111263
- Varalakshmi S, Leysen S (2020) Evaluation of the effectiveness of different preservation techniques on the inactivation of *Listeria monocytogenes* by using challenge testing protocol in the fresh, soft cheese – paneer. *LWT-Food Sci Technol* 125:109359
- Yashavantha R, Pinto S, Patel D, Paul P, Chaudhary M (2020) Development of lemon flavoured paneer. *J Pharm and Phyto* 9(5): 1320-1324