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Extraction and characterization of essential oil of nutmeg (*Myristica fragrans*) fruit pericarp and utilization as a surface coating material to improve the shelf life of paneer

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Abstract

Paneer is a traditional dairy product of India and is similar to unripened soft cheese. It is used as a raw material for the preparation of a variety of culinary dishes and snacks. Paneer is marble white in appearance, with a firm, cohesive and spongy body and a sweetish-acidic-nutty flavor. But it is highly perishable with a limited shelf- life like other indigenous dairy products. Its shelf life was reported to be only six days under refrigeration, though its freshness is lost within three days. The spoilage of paneer occurs mainly due to the growth of microorganisms, which bring about various physico-chemical changes. In the present study, attempts were made to increase the shelf-life of paneer at refrigerated storage by the application ofnutmeg pericarp essential oil surface coating. The essential oil was prepared from fresh nutmeg fruit flesh by steam distillation method. The physico-chemical and bio functional properties of essential oil were determined. The specific gravity and refractive index of the essential oil were found to be 0.903 and 1.477 respectively. The acid value and total polyphenolic value of essential oil were 3.36 and 4.04 mg GAE/g respectively. The essential oil was also found to have good antimicrobial and antioxidant activity. The shelf life of samples coated with essential oil packaged in LDPE pouches had more shelf life than the control paneer, as the coated paneer kept well for 9 days while the control paneer spoiled after the 6th day of refrigerated storage. The paneer with essential oil coating obtained satisfactory sensory scores.

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Keywords: Paneer, surface coating, nutmeg, essential oil, antimicrobial, DPPH, shelf life

Paneer is one of the main indigenous dairy products prepared by heating followed by acid coagulation of milk. About 5per cent of liquid milk produced in India is converted into paneer. It contains all milk solids except some soluble constituents like lactose, whey proteins and minerals. According to the Food Safety and Standards Authority of India paneer is 'the product obtained from any variant of milk with or without added milk solids, by precipitation with permitted acidulants and heating'. The regulations also set a maximum moisture percentage (m/m) of 60.0 and a minimum milk fat percentage (dry matter basis) of 50.0. Due to its high levels of fat and protein, as well as the abundant presence of minerals like calcium and phosphorus, this indigenous product is regarded as being very important in the diet, especially in the context of Indian vegetarian food. However, due to a short shelf life, paneer is not widely available in the Indian market. Paneer can only be kept at ambient temperature for one day and in the refrigerator for around six days without losing its chemical and microbiological quality.

The technology of paneer manufacturing allows considerable exposure of paneer with large volumes of water and air during manufacture and packaging. The development of microorganisms, causes numerous physico-chemical changes and the emergence of various off-flavours in the product and eventually its spoilage. This is the main obstacle to paneer manufacturing being adopted industrially. Therefore, it is imperative to discover effective methods of extending the shelf life of paneer. The main methods for preventing microbiological, chemical, and physical deterioration of food are heat processing, freezing, drying, concentration and irradiation. However, additional processing methods also have to be used due to a number of restrictions related to certain food kinds and the ineffectiveness of some physical methods. One such method is the use of food additives, which are typically referred to as food preservatives and are either natural or synthetic chemical agents. The main aim of using food preservatives, is to inhibit microbial development through their antimicrobial action and also to protect food constituents from chemical reactions.

Nutmeg (*Myristica fragrans*) is a dark leaved evergreen tree spread across tropical countries. Its fruit consists of seed (nutmeg), mace (the outer covering of seed) and pericarp or rind (outer fruit flesh). Out of these, the nutmeg pericarp is the largest part of about 80-85 per cent of the total weight of nutmeg fruit. The seed and mace are the primary product of *Myristica fragrans* as spice in food products and in traditional medicine. But the utilization of nutmeg pericarp is still limited and a lot of it is wasted as agricultural waste due to its astringent taste and aromatic flavor. At the same time, the essential oil from nutmeg pericarp has gained therapeutic interest, especially its potential antioxidant and antimicrobial effects. Its main phytochemicals include monoterpenes, monoterpene alcohols, myristicin, sabinene, limonene, eugenol and safrole (Ashokkumar *et al*., 2022). This pericarp can be utilised as a socio-economic food, which promises to contribute good nutritional properties, for which more research studies are required. They can be used for their conversion into value-added goods by using the appropriate techniques.

For both the food sector and consumers, food security is a fundamental concern. The demand for antimicrobials appropriate for dairy products has increased due to the rise in food-borne illnesses. Studies on the essential oil derived from the pericarp of nutmeg fruit reported that the extract has the bioactivities like anti-inflammatory, antimicrobial, antifungal and antioxidant activities. It is reported that, *Myristica fragrans* possess good antimicrobial activity against both the gram positive and gram negative microorganisms (Ameen, 2011). But the antimicrobial potency of the nutmeg pericarp essential oil has yet to be exploited in food products. Therefore, the current study aims to determine the effect of essential oil derived from nutmeg fruit pericarp as an antimicrobial coating to improve the shelf life of paneer.

Materials and methods

The buffalo milk used for paneer preparation was procured from University Dairy

Plant, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur. LDPE pouches of size 10 cm x 5 cm were purchased from the local market. Completely ripened nutmeg pericarp, after separating the seed and mace during harvesting, was collected from the farmers of Thrissur district. All analytical grade chemicals and microbiological media were procured from the approved firms.

Extraction of essential oil from nutmeg pericarp

Distillation was done in order to separate oil content using hydro distillation technique. The flesh of the nutmeg fruit was washed and crushed in a mixer. Samples (200 grams) were put inside a round bottom flask equipped with Clevenger apparatus. Afterwards, water was added until the ratio of water to sample was 6:1. The flask was then placed on top of a heating mantle. Distillation was done for six hours or until the oil content inside distilled water had run out. Separation was conducted for the purpose of separating oil from water. Oil that has lighter specific gravity than water would be on top. The obtained distillate was separated from the water phase using anhydrous Na_2SO_4, collected in amber coloured bottles and stored at 4˚C until usage.

Analysis of essential oil

The collected nutmeg pericarp essential oil was tested for the following properties.

Specific gravity

Specific gravity was done with a method carried out by Chophi *et al*. (2019) using a pycnometer. The pycnometer was washed and cleaned with ethanol followed by flushing with ether. Once dried it was weighed on a digital scale, following which the pycnometer was then filled with distilled water until the given mark and closed. The pycnometer filled with distilled water was left for a while before it was weighed once more. The water equivalent pycnometer was the weight of the pycnometer filled subtracted from the empty weight. This method was then conducted with the nutmeg oil and the density was calculated with the following formula:

Specific gravity (T^0C) =

$$\frac{\textit{Weight of nutmeg oil}}{\textit{Weight of distilled water}}$$

Refractive index

The refractive index was measured as described by Saputro *et al.* (2016). The refractive indices were assessed using a refractometer. The nutmeg oil sample was placed on the prism of the refractometer at about 2-3 drops to cover the surface of the prisms. The refractometer displayed the refractive index value.

Total polyphenolic content

Total polyphenolic content was measured as per the procedure by Singleton *et al.* (1999)

Acid value

The acid value of essential oil was measured as per the method of Low and Ng (1987). Around 0.3 g of essential oil was taken in a 100 mL Erlenmeyer flask. Mixed with 9.7 mL of n-Hexane (Total volume should be 10 mL) and 1-2 drops of indicator (phenolphthalein). Titrated the contents against 0.02N KOH solution. The endpoint was reached when pink colour persisted for 30 seconds. Carried out a blank titration using 10 mL of n-Hexane.

Acid value (mg/g) =

$$\frac{56.11 \; X \; 0.02 \; X \; (Vs–Vb) \; X \; F}{W}$$

Where Vs = titration volume of sample (mL).

Vb = titration volume of blank (mL).

W = weight of essential oil used (g).

F = factor of 0.02 KOH solution, where F = 5/ Vf

Vf is the volume of 0.02N KOH required to neutralize 5 mL of the 0.02N H_2SO_4solution.

Antimicrobial activity

The antimicrobial potential of the

essential oil was tested against *Staphylococcus aureus* (Culture stock, Department of Dairy Microbiology) and *Escherichia coli* 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 plates. Wells were bored on the plates using a sterile borer and loaded with 80 µl of essential oil. Uninoculated MRS broth was used as the negative control. The plates were incubated at 37^{0}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

Radical scavenging activity was determined using DPPH free radical assay as described by Assa *et al*. (2014). One mL of essential oil was mixed with 1 mL of a 90 µM DPPH solution in methanol and the final volume was made to 4 mL with methanol. The mixtures were well shaken and kept at 25^{0}C in the dark for 1 h. The absorbance was measured at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration, using the equation:

$$\% \text{ RSA} = \frac{Ao - As}{Ao} X\ 100$$

Where, Ao is the absorbance of the control (containing all reagents except the essential oil) As is the absorbance using essential oil

Coating of nutmeg pericarp essential oil on paneer

The paneer was cut into uniform pieces (1 cm^3) and the essential oil was applied onto the surface of the paneer under aseptic conditions by gentle brushing. Care was taken to ensure that the coating had uniformly spread onto the sample surface. The samples were then dried at room temperature for an hour. The coated paneer samples were aseptically packed into the pre-sterilized pouches, sealed and stored at refrigerated temperature (7±1^{o}C).

Sensory evaluation of paneer coated with essential oil during refrigerated storage

The treated and control paneer samples were packed in LDPE pouches and stored at refrigerated temperature. Stored samples were analysed for sensory parameters for a period of 12 days at three days intervals. Evaluated organoleptically for different quality attributes like flavour, body and texture, colour and appearance and overall acceptability by a selected panel of judges comprising five members. The paneer was evaluated in raw form. A nine-point hedonic scale scorecard was used for evaluation.

Statistical analysis

The result obtained from the analysis were verified using One-way Analysis of Variance (ANOVA) and T-test statistically using SPSS (Statistical Packages for Software Solutions) software, version 21.0 designed by IBM Company, USA and data were expressed as Mean ± Standard Error.

Results and discussion

The present study was carried out to extract the essential oil from nutmeg fruit pericarp and determine its effect on the sensory quality of paneer during refrigerated storage.

Properties of nutmeg pericarp essential oil

The visual observation implied that the produced essential oil ranged from colourless to pale yellow. Physical characteristics like specific gravity and refractive index are influenced by the chemical composition of nutmeg oil. As shown in Table 1, the specific gravity of nutmeg fruit flesh essential oil is 0.903. The refractive index value is 1.4772. These results are comparable with the research conducted by Ma'mun (2013) using nutmeg oil samples from Papua which was 0.909 specific gravity and 1.487 refractive index value. Similarly, the specific gravity obtained from nutmeg mace, young seed, medium seed and old seed respectively were 0.919, 0.902, 0.923 and 0.930. The refractive index values are 1.487, 1.481, 1,486, and 1.487. This showed that the older the harvesting age, the higher the value of specific gravity and refractive index

of nutmeg seed (Mimbar *et al*., 2016). Higher specific gravity and refractive index would be present with heavier molecules.

The acid value and total polyphenolic content of essential oil from nutmeg fruit flesh was 3.36 and 3.49 mg GAE/g respectively. It would be better for the acid value if the oil had less acid in it. Since acid is quickly modified by air oxidation and changes the aroma of essential oils, it is not wanted in essential oils. Several researchers examined the relationship between phenolic compounds content and antioxidant action. In the extracts of *Kappaphycusalvarezi*i, Kumar (2008) found a very strong correlation (r = 0.937) between the percent of DPPH free radical inhibition and total phenolic content. Assa *et al.* (2014) indicated that the antioxidant activity of nutmeg flesh, seeds and mace indicated a very strong correlation (r= 0.862) between the amount of total phenols with the capacity to scavenge DPPH radicals. The ability of the extracts to scavenge DPPH radicals was also enhanced by the increased phenolic component concentration of the extracts. The effectiveness of the extracts to reduce ferrous ions was further enhanced by the higher phenolic component concentration of the extracts (Assa *et al.*, 2014). The chelating capacity of ferrous ions and the total phenolic extract of nutmeg had a negative correlation (r = -0.984). Since the mace and seed extract of nutmeg had more total phenols than the flesh extract, the latter had a greater capacity to chelate substances.

Antimicrobial activity

The essential oil from nutmeg fruit flesh exhibited antimicrobial activity against both indicator organisms (Table 2). Zone of clearance (ZOC) was observed around the wells loaded with essential oil. This is in agreement with the observation of Sipahelut *et al*. (2019). According to Elgayyar *et al.* (2001), three categories are used to categorise the antibacterial activity of essential oils: strong activity (inhibition zone >8 mm), medium activity (inhibition zone 6 to 8 mm) and poor activity (no inhibition zone <6 mm). The Table 2 showed that the use of essential oil derived from nutmeg fruit flesh showed strong antibacterial power against the *Escherichia coli* and *Staphylococcus aureus*.

Table 1. The physico-chemical characteristics of essential oil of nutmeg fruit flesh

Characteristics	Result
Refractive index	1.4772 ± 0.002
Specific gravity	0.903 ± 0.007
Acid value	3.36 ± 0.031
Total Polyphenolic content	4.02 mg gallic acid equivalent/g

Hydroxyl (-OH) and carbonyl groups are frequently found in essential oils that actively have antibacterial activity. Through an adsorption mechanism involving hydrogen bonds, phenol derivatives interact with bacterial cells. When the concentration is low, it promptly decomposes after forming weakly bound phenol-protein complexes. This is followed by phenol penetration into the cells, which results in protein precipitation and denaturation. When phenol concentrations are high, it induces protein coagulation and lyses membrane cells (Parwata and Dewi, 2008).

Cell membranes are harmed by essential oils. The most significant chemical component is the presence of hydrophobic properties, which have accumulated in the cell membrane structure with a fat-rich environment and harm the structure and function of the cell membrane. An essential oil that permeates a membrane could cause the cytoplasm to coagulate, disrupting fat and protein (Dorman *et al*., 2000). The method used to extract essential oils from plants, the concentration of essential oils used, the amount of inoculum used, the stage of microbial growth and the culture medium being used are all factors that influence the antimicrobial activity of the essential oil.

Antioxidant activity

Lipid oxidation, which is the main chemical change involved in the deterioration of food during processing and storage, is brought by free radicals and reactive oxygen species. Antioxidants are commonly used food additives to protect foods and oils against oxidative deterioration. When DPPH radical was scavenged by an antioxidant during hydrogen donation to form a stable DPPH molecule, the colour changed from purple to yellow and the absorbance at 517 nm was reduced (Arshath *et*

Table 2. Antimicrobial property of essential oil against indicator organisms

Indicator organisms	Zone of clearance (mm)
E coli	23±0.5
Staphylococcus aureus	16±0.5

al., 2022). The DPPH radical scavenging activity of nutmeg fruit flesh essential oil is 66.66%.

Assa *et al*. (2014) evaluated the antioxidant potential of nutmeg flesh, seed and mace (*Myristica fragrans Houtt*) with methods of 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), ferrous ion chelating activity and antioxidant activity assay in a linoleic acid system with ferrothiocyanate reagent. They found that, seed extract has the most powerful scavenging ability on free radicals based on tests of DPPH and FRAP, i.e., 154,55 (IC_{50}=µg/ml) and 82,33 (mg GAE/g extract) respectively. This is because of the large concentration of tannin, flavonoids and terpenoids components in seed extract, it has a strong antioxidant activity.

Due to the low concentrations of flavonoids and terpenoids, nutmeg flesh has the lowest antioxidant capacity as compared to nutmeg seed and mace. The two hydroxyl groups that are joined to the benzene ring form the foundation of flavonoids. The antioxidant activity may be increased by both hydroxyl groups, which function as electron donor groups (Zhang *et al*., 1999). According to Tomaino *et al.* (2005) reported that the antioxidant activity of nutmeg essential oil increases when heated at 180^{0}C for three hours. This is because the content of α-pinene, β -pinene and sabinene noticeably decreases while the content of safrole and myristicin simultaneously increases, indicating that these compounds are essential for the oil's antioxidant activity.

Sensory analysis of nutmeg pericarp essential oil coated paneer during storage

The results of the sensory evaluation given in Table 3 indicate that the scores for flavour, colour and appearance, body and texture, and overall acceptability for paneer with essential oil coating and control declined progressively during refrigerated storage (7±1°C). The flavour score for the control paneer stored at 7±1°C decreased from a mean value of 8.12 to 7.47 at the completion of the sixth day of storage. For paneer coated with essential oil, a decrease from the original mean value of 7.85 to 7.25 was observed only after 9 days of storage at 7 ±1°C. The flavour scores for control on 0th and 6th day differed significantly (p>0.05) while the flavour scores of paneer coated with essential oil differed significantly (p>0.05) between the scores of the 0th day and 9th day.

The scores for colour and appearance, given in Table 3 suggest that at the end of 6th day of refrigerated storage (7±1°C), the score for control declined from 8.57 to 7.67 and there was a significant difference (p<0.05) between the 0th day and 6th day of storage period. The initial sensory score of 8.62 declined to 7.75 in case of paneer with essential oil coating within 9 days of refrigerated storage and a significant difference (p<0.05) was observed between its colour and appearance scores on 0th day and 9th day of storage.

After sensory analysis, the control paneer's body and texture scores displayed a gradual decrease, ranging from an initial score of 8.45 to 7.75 within six days of refrigerated storage (7±1°C) and there was a significant difference (p<0.05) between the 0th and 6th day of storage period. In contrast, the scores for the body and texture of paneer with essential oil coating similarly showed a steady decline from the initial score of 8.42 to 7.62, but only after 9 days of refrigerated storage. The statistical analysis indicated that the time periods of refrigerated storage, 0th day and 9th day differed significantly (p<0.05).

The overall acceptability scores showed that the control paneer displayed a decrease in sensory scores from 8.05 to 7.57 with 6 days of refrigerated storage (7±1°C) and there was a significant difference (p<0.05) between time periods 0th and 6th day. Meanwhile, the scores for body and texture of paneer with essential oil coating also showed a decrease from an initial score of 8.0 to 7.1 but only within 9 days of refrigerated storage (7±1°C). The statistical analysis indicated that the time periods, 0th and 9th day showedsignificant differences (p<0.05) during refrigerated storage (7±1°C).

Table 3. Effect of refrigerated storage (7 ± 1°C) on the sensory quality of paneer coated with essential oil

Attributes		Days of storage					
		0	3	6	9	12	Chi square value
Flavour	Control	8.125± 0.075^{a}	7.625± 0.085^{ab}	7.475± 0.047^{b}	spoiled	spoiled	8.338*
	Paneer coated with essential oil	7.853± 0.064^{a}	7.702 ± 0.091^{ab}	7.453± 0.064^{ab}	7.251± 0.064^{b}	spoiled	11.662*
	U value	15^{ns}	10^{ns}	3^{ns}			
Colour and appearance	Control	8.575± 0.075^{a}	8.153± 0.064^{ab}	7.675± 0.085^{b}	spoiled	spoiled	9.881*
	Paneer coated with essential oil	8.625± 0.062^{a}	8.402± 0.040^{ab}	8.304± 0.108^{ab}	7.751± 0.119^{b}	spoiled	12.231*
	U value	9.5 ^{ns}	15.5*	16*			
Body and Texture	Control	8.453± 0.064^{a}	8.175± 0.062^{ab}	7.554± 0.064^{b}	spoiled	spoiled	9.615**
	Paneer coated with essential oil	8.425± 0.047^{a}	8.303± 0.129^{ab}	8.275± 0.047^{ab}	7.625± 0.047^{b}	spoiled	9.938*
	U value	7^{ns}	10^{ns}	16*			
Overall Acceptability	Control	8.054± 0.064^{a}	7.852± 0.064^{ab}	7.575± 0.047^{b}	spoiled	spoiled	8.552*
	Paneer coated with essential oil	8.014± 0.040^{a}	7.803± 0.108^{ab}	7.675± 0.085^{ab}	7.102± 0.040^{b}	spoiled	11.973**
	U value	6^{ns}	7.5^{ns}	11^{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.

It can be inferred from the sensory analysis of the paneer coated with the essential oil and the control paneer that the sensory scores for both samples decreased during refrigerated storage (7±1°C). However, the paneer coated with the essential oil had a slower rate of decline than the control. Khatkar *et al.* (2017) found that the sensory scores of cinnamon-treated paneer and control both decreased similarly during chilled storage (7±1°C) and concluded that when packed in LDPE and kept at 8±1°C, the cinnamon-treated sample had a shelf life of nine days. According to Jooyandeh (2011), the application of edible antimicrobial coatings would improve the sensory appeal and shelf life of coated products.

Conclusion

Paneer is susceptible to rapid deterioration because of its high fat and moisture content, which results in rancidity (fat breakdown) and a mouldy surface (mould growth). Therefore, it is necessary to develop a suitable method for extending paneer's shelf life. The demand for herbs and spices in food and dairy products is increasing compared to chemical preservatives due to the possibility of enhanced flavour and extended shelf life without any adverse side effects. Nutmeg pericarp is generally considered as agricultural waste that contains useful phytochemicals with good antimicrobial and antioxidant properties. The essential oil from the nutmeg pericarp was extracted by steam distillation method and physico-chemical properties of essential oil were evaluated. The essential oil is found to have excellent antimicrobial and antioxidant activity. The antimicrobial property of the essential oil was assessed against *Staphylococcus aureus* and *Escherichia coli* and exhibited inhibitory action against both the indicator organisms.

The essential oil was gently brushed on the surface of paneer cubes and packed in sterilized LDPE packages. Sensory analysis was conducted for paneer with essential oil coating over a period of 12 days at three days

intervals under refrigerated storage. Based on the observations of this study, it could be concluded that, coating essential oil extracted from nutmeg fruit flesh is an effective method for extending the shelf life of paneer.

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Conflict of interest

The authors declare that they have no conflict of interest.

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