Heliyon 10 (2024) e29036

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Insect protein-based composite film incorporated with *E. purpurea*-based nanoparticles augmented the storage stability of parmesan cheese

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ARTICLE INFO

Keywords:
Edible packaging
Insect protein
Parmesan cheese
Gastrointestinal digestion
Storage study

ABSTRACT

The objective of this study was to prepare an insect protein-based composite film containing plant extract-based nanoparticles to augment the lipid and microbial stability of cheese. An ultrasonication-mediated green method of synthesis was followed to develop the nanoparticles using *E. purpurea* flower extract (EP-NPs). The film was developed using locust protein (Loc-Pro) and different levels of EP-NPs [2.0% (T_3), 1.5% (T_2), 1.0% (T_1), and 0.0% (T_0)]. It was characterised and evaluated for efficacy using parmesan cheese (Par-Che) as a model system stored for 90 days (4 ± 1 °C). The addition of EP-NPs markedly enhanced the antioxidant and antimicrobial activities of the Loc-Pro-based film as indicated by the results of radical-scavenging activity (ABTS and DPPH), total-flavonoid and total-phenolic contents, ion-reducing potential (FRAP), and inhibitory halos (mm). It also increased ($P < 0.05$) the density (g/ml), redness (a*), and yellowness (b*) and reduced ($P < 0.05$) the WVTR (mg/m^{2}t), transparency (%) and lightness (L*) of the Loc-Pro-based film. The film incorporated with EP-NPs showed a marked desirable impact on protein oxidation, lipid stability, microbial quality and antioxidant potential of Par-Che during 90 days of storage. While cheese samples without any film showed mean values of 2.24 mg malondialdehyde/kg, 0.79% oleic acid, 1.22 nm/mg protein, 2.52 log CFU/g and 1.24 log CFU/g on day 90 for TBARS, FFA, total carbonyl content, total plate count and psychrophilic count, samples within T_3 films showed significantly lower values of 1.82, 0.67, 0.81, 2.15, and 0.81, respectively. A positive impact of the Loc-Pro-based film was found on the sensory characteristics of Par-Che. Both the Loc-Pro-based film and the digestion simulation improved the radical-scavenging activity and ion-reducing potential of the Par-Che. Our results indicate the potential of Loc-Pro-based film as a means to enhance the storage quality of cheese.

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https://doi.org/10.1016/j.heliyon.2024.e29036
Received 11 December 2023; Received in revised form 12 March 2024; Accepted 28 March 2024
Available online 3 April 2024

1. Introduction

Plastic packaging is a major source of pollution that is affecting the environment and human health on a global scale [1]. The food and beverage industry is responsible for producing a large proportion of single-use plastic packaging which contributes a large percentage to plastic waste leakage globally [1]. Recently, plastic packaging alternatives have been developed using materials from biological and sustainable sources, such as proteins and carbohydrates from plants and fungi e.g., cellulose and zein protein [2]. Although these new sources of packaging are biodegradable and environmentally friendly, most of them are costly and could be diverted to the human food supply chain. Recently, insect proteins have caught the attention of food scientists and processors. While several research works have investigated the use of insect proteins and flours to develop fancy and novel food products [3–6], the use of proteins harvested from insects for the preparation of packaging materials has received little to no attention. The development of food packaging from insects is of particular importance to those countries where insects do not constitute a part of a regular diet and have limited social acceptance as a food ingredient. Commercial farming of insects for the development of packaging materials is a novel idea that can provide a sustainable, biodegradable, and cheaper alternative for plastic packaging for food and other industries and needs immediate scientific attention.

The designing of edible and biodegradable packaging films for augmenting the storage quality of agricultural and animal food products has received increasing attention during the last decade and numerous food and non-food materials, by-products, and side streams have been used for the preparation of the films [7–10]. However, studies have not evaluated the utilization of proteins from insects for the preparation of edible and biodegradable packaging for food products. Therefore, a study was planned to prepare a Loc-Pro-based film to augment the storage quality of Par-Che. The bioactive properties of the film were improved using *E. purpurea* flower extract-based nanoparticles (EP-NPs). The flower extract of *E. purpurea* (EP-E) is a rich source of flavonoids and polyphenolics and has been associated with several medicinal properties such as immunomodulatory, antimicrobial, anticancer, and antioxidant [11]. Studies have not documented the use of EP-NPs for the preparation of edible films for augmenting the storage quality of food products to the best of our knowledge. The main aim of the research was to prepare a bioactive edible film based on insect protein and to evaluate its efficacy using Par-Che stored for 90 days at 4 ± 1 °C. The film was prepared and characterised for various physicomechanical and colour parameters, radical scavenging and ion-reducing abilities, and antimicrobial characteristics. The Par-Che packaged within the film were evaluated for physicochemical parameters, lipid-stability, protein oxidation, antioxidant potential, and microbiological and sensory quality for 90 days of storage. The Par-Che was also subjected to gastrointestinal simulation and the samples were evaluated for ion-reducing and radical scavenging potential.

2. Material and methods

2.1. Chemicals and reagents

Different chemicals, reagents and enzymes of food or analytical grade were supplied by standard firms and used during the study. The media used during microbiological assays such as violet-red bile agar, potato-dextrose agar, and total plate agar and the enzymes used during digestion simulation viz. pepsin (1000 NF U/mg) and pancreatin (protease activity of ≥75 USP U/mg, amylase activity of ≥75 USP U/mg and lipase activity of ≥6 USP U/mg) were bought from the HI-Media Company (India, Mumbai). The chemicals such as dinitrophenylhydrazine (DNPH), aluminium chloride ($AlCl_3$), 1,1,3,3 tetra-ethoxypropane, ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), quercetin, Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), Folin-Ciocalteu's phenol reagent, DPPH (2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl), silver nitrate, and gallic acid were purchased from Sigma-Aldrich (Bangalore, India).

2.2. Locust protein (Loc-Pro) flour and its defatting

The Loc-Pro flour containing 71% crude protein (DMB) was supplied by JR Unique Foods Ltd. and was prepared from the locusts (*L. migratoria*) raised on various vegetables and grasses. The manufacturer from Thailand (Thailand Unique Brand, Udon Thani) prepared the flour without adding any artificial colours, preservatives or flavours and used simple processing steps viz. cleaning, drying, grinding, and packaging under vacuum. The Loc-Pro flour was defatted following the method described by Lone et al. [12] using hexane (1:5 ratio, w/v). The centrifugation (7 min at 2000 rpm) was performed to remove the oil after stirring the mix for 30 min. After filtration (Whatman filter paper No. 1) and removal of residual hexane in a dry oven (50 °C), the defatted Loc-Pro flour was vacuum packed and kept at 4 ± 1 °C.

2.3. E. Purpurea-based nanoparticles (EP-NPs) and their characterization

Healthyhay Nutrition supplied the *E. purpurea* flower extract available in powder form (Healthyhay Foods LLP., Mumbai, India) and was dissolved in the aqueous solution of ethanol (85% v/v) with occasional stirring (45 °C) for 72 h. After filtration through Whatman No. 42 and 1.0 filter papers, the volume of the extract was reduced by employing a rotary vacuum evaporator (40 ± 5 °C) and the concentrated extract was lyophilized (INNOVA Bio Meditech Inc., INOFD-12S: Freeze Dryer, the USA) for 32 h and the freeze-dried powder was stored at −20 °C after vacuum packaging. This flower extract powder was used to prepare the nanoparticles (EP-NPs) following a green method of synthesis (with minor modifications) using silver nitrate ($AgNO_3$) as described by Fierascu et al. [13] and Singh et al. [14]. An aqueous solution of silver nitrate (1 mM strength) was used to prepare the EP-NPs using the extract prepared

above (extract: metallic salt = 1:1, v/v). An ultrasonication-mediated green method of synthesis was followed to develop the EP-NPs and the reaction mixture was sonicated (10 min at 750 W, 220 V and 20 kHz) and stirred for 2 h at 70 °C and was monitored for colour change and UV–Vis spectrum (800-300 nm). The appearance of a reddish-brown colour was an indication of the reduction of silver ions (Ag^{+} to Ag^{0}) by the extract and the formation of silver nanoparticles. The EP-NPs were obtained by centrifugation (12,000 rpm, 30 min) and freeze-dried to a powder form after washing with distilled H_2O and vacuum packaged and stored at −20 °C. The formation of EP-NPs was supported by the maximum absorption peak that was found at 471 nm. The EP-NPs were characterised by the scanning electron microscopy (SEM, data is not presented here) and dynamic light scattering (DLS) method using a Malvern Zetasizer Nano-ZS instrument (Malvern, UK) to measure the diameter range, average particle size (nm), zeta potential (mV), polydispersity index (PdI), and particle morphology.

2.4. Preparation of the Loc-Pro-based film

The Loc-Pro-based composite film was developed using the casting method elaborated by Kouser et al. [15]. The preliminary trials were carried out to optimise the ingredients and standardize the formulation for the development of film with desirable characteristics. The film was made using a film-forming solution containing 0.9 g (w/v) of carrageenan and 0.6 g (w/v) of defatted Loc-Pro flour in distilled water (100 ml). This solution was heated at 80 °C for 20 min while stirring it using a magnetic stirrer and was followed by the addition of 14 % (w/v) glycerol (plasticizer) and again mixed on the stirrer (10 min). This solution was cooled (60 °C) and casted on the flat glass plate (10 × 20 cm) in the form of a uniform layer and was thereafter dried for 5 h at 55 °C in an oven (dry air). The preservative characteristics were imparted to the film using increasing levels of EP-NPs (0.0, 1.0, 1.5, and 2.0%, w/v) in glycerol and the films developed were marked as T_0 (film containing 0% EP-NPs), T_1 (film containing 1.0% EP-NPs), T_2 (film containing 1.5% EP-NPs), and T_3 (film containing 2.0% EP-NPs). These films were characterized and evaluated for various physicomechanical properties, colour characteristics (L*, a*, b*), and antioxidant and antimicrobial activities (FRAP, ABTS, DPPH, total phenolic and total flavonoid contents, antioxidant release %, and Inhibitory halos). The only difference between the control (T_0) and treated films (T_1, T_2 and T_3) was that the control film contained no EP-NPs (0%) indicating that the higher preservative potential of treated films might be attributed to the presence of EP-NPs.

2.5. Parmesan cheese (Par-Che)

Par-Che of the 'Amul' brand was bought locally and 100 g of the sample contained 30 g of total fat (0 g trans fatty acids, 20 g saturated fatty acids, and 100 mg cholesterol), 40 g of protein, 0 g of carbohydrates and 400 kcal of energy. The cheese samples of uniform size (~50 g cubes) were packaged within the Loc-Pro-based films (T_3, T_2, T_1, and T_0) and stored at 4 ± 1 °C for 90 days together with control samples without any film. The samples from stored cheese were collected on days 0, 30, 60, and 90 and analysed for quality (lipid stability, protein oxidation, antioxidant potential, microbial quality, physicochemical properties, texture profile analysis, and sensory analysis). The influence of digestion simulation (gastrointestinal) was ass on the antioxidant activities of the cheese samples.

2.6. Physicomechanical and colour characteristics

The Loc-Pro-based film was characterized for different physicomechanical characteristics, such as moisture content (%), thickness (mm), transmittance (%), density (g/ml), solubility (%), elongation at break (%) and water vapour transmission rate (WVTR, mg/m^2t) using the methods elaborated in detail in our previous studies [15,16]. A colorflex colourimeter of the make Hunter Associated Laboratory Inc. (VA, USA) was used to measure the lightness (L*), yellowness (b*) and redness (a*) of the developed film [15,17].

2.7. Antioxidant and antimicrobial properties

2.7.1. Total phenolic and total flavonoid contents

The total phenolic and total flavonoid contents of the Loc-Pro-based film and the extract (EP-NPs) were evaluated using the FC (Folin-Ciocalteu) method and $AlCl_3$ (aluminium chloride) calorimetric assay, respectively, as elaborated in detail in our previous studies [15,16]. The results were presented as mg GAE (Gallic acid equivalents)/g and mg QE (Quercetin equivalents)/g for total phenolics and total flavonoids, respectively.

2.7.2. Antioxidant capacity (ABTS, DPPH, FRAP and release rate of polyphenols)

Both the Loc-Pro-based film and the extract (EP-NPs) were evaluated for antioxidant potential by determining their capacity to reduce ferric ions [FRAP, μM TE (Trolox equivalents)/100 g] and ability to scavenge ABTS and DPPH radicals (% inhibition) following the calorimetric methods described in detail in our recently published papers [15,16]. The release rate of phenolic compounds from the films was evaluated following the method elaborated by Cui et al. [18] and the release rate was expressed as a percentage. 10 ml deionized water (4 °C) was poured into a Petri plate kept on a rocker and a fixed weight of a film (1 g) was added to it. When the released phenolic compounds attained equilibrium in the deionized water [samples (0.1 ml) tested after every 48 h], the rate of release (%) was determined following the DPPH method described above.

Table 1
Characteristics of Loc-Pro-based film and EP-NPs.

Parameters		Loc-pro-based film			
		T_0 (0.0%)	T_1 (1.0%)	T_2 (1.5%)	T_3 (2.0%)
Total phenolic content (μg GAE/mg)		0.78 ± 0.04^{d}	3.05 ± 0.39^{c}	6.53 ± 0.43^{b}	9.81 ± 0.37^{a}
Total flavonoid content (μg QE/mg)		1.04 ± 0.05^{d}	5.59 ± 0.53^{c}	12.31 ± 0.39^{b}	16.92 ± 0.63^{a}
DPPH (% inhibition)		1.34 ± 0.04^{d}	30.28 ± 0.73^{c}	33.50 ± 0.33^{b}	37.25 ± 0.45^{a}
ABTS (% inhibition)		2.25 ± 0.05^{d}	34.51 ± 0.34^{c}	38.29 ± 0.45^{b}	44.08 ± 0.83^{a}
FRAP (μM TE/100g)		0.93 ± 0.04^{d}	12.86 ± 0.57^{c}	14.86 ± 0.63^{b}	17.86 ± 0.86^{a}
Antioxidant release (%)		–	80.14 ± 1.21^{c}	83.01 ± 1.36^{b}	85.93 ± 0.98^{a}
Inhibitory halos (mm)	*S. aureus*	0	11 ± 0.07^{c}	13 ± 0.05^{b}	14 ± 0.08^{a}
	E. coli	0	0	0	0

EP-NPs (freeze-dried powder)

Parameters			**Mean±S.E**
Yield of the extract (%)			27.40 ± 1.6
pH			4.80 ± 0.03
Total phenolic content (μg GAE/mg)			21.30 ± 1.0
Total flavonoid content (μg QE/mg)			85.0 ± 4.6
DPPH (% inhibition)			69.65 ± 0.75
ABTS (% inhibition)			76.95 ± 0.53
FRAP (μM TE/100g)			24.35 ± 0.26
Average size (nm)			58.13 ± 4.40
Diameter range (nm)			25–80
Zeta potential (mV)			-14.12 ± 0.62
Polydispersity index (PdI)			0.389
Maximum absorption peak (nm)			471
MIC (mg/ml)	*S. aureus* ATCC6538P		1.0
	E. coli ATCC9637		28
Inhibitory halos (mm)	*S. aureus* ATCC6538P	1.0%	11 ± 0.01^{C}
		1.5%	13 ± 0.15^{B}
		2.0%	14 ± 0.04^{A}
	E. coli ATCC9637	1.0–2.0%	0 (resistant)

Mean ± SE with different superscripts in a row (lower case alphabet) or column (upper case alphabet) differ significantly ($P < 0.05$).
n = 6 for each treatment.
WVTR = water vapour transmission rate.
MIC = minimum inhibitory concentration.
T_0 = film without any EP-NPs.
T_1 = film containing 1.0% EP-NPs.
T_2 = film containing 1.5% EP-NPs.
T_3 = film containing 2.0% EP-NPs.

2.8. Microbiological analysis and gastrointestinal simulation

The EPNs were evaluated for antimicrobial activity using MIC (minimum inhibitory concentration, mg/ml, a 96-well plate was used following the microdilution technique) and disk agar diffusion tests [10 mm filter paper discs infused with EPNs, inhibitory halos were determined (mm)] using Muller Hinton agar (37 °C, 24 h) against a Gram -ve and a Gram + ve bacteria (*S. aureus* and *E. coli*) (10^6 cfu/ml) [19]. The films were examined for disk agar diffusion test and the Par-Che samples packed within the films were examined for microbial quality during 90 days of storage by enumerating various microbial counts viz. coliform (violet-red bile agar), yeast/moulds (potato dextrose agar), psychrophilic (total plate agar), and total plate (total plate agar) counts ($\log_{10}$ cfu/g) considering the plates with 300-30 colonies.

The influence of digestion (gastrointestinal) on the antioxidant properties of Par-Che was examined on days 0 and 90. The static digestion simulation was performed using a protocol described by Lone et al. [19] for cheese using pepsin (1 h, pH 1.9) and pancreatin (2 h, pH 8.0). The enzyme: substrate ratio of 1: 100 (w/w) was used and the digestion was maintained at 37 °C. The digested aliquots were evaluated for ABTS, FRAP, and DPPH.

2.9. Physicochemical analysis, lipid stability and protein oxidation

The moisture content (%) of the Par-Che was evaluated using the oven drying method and pH using a digital pH meter as described by Lone et al. [19] for cheese. The lipid stability of stored Par-Che was assessed by determining FFA (free fatty acids) and TBARS (thiobarbituric acid reacting substances) values and the protein oxidation by total carbonyl content as elaborated by Lone et al. [12]. The results are presented as % oleic acid, mg malondialdehyde/kg of the sample, and nmol of carbonyl/mg protein, respectively.

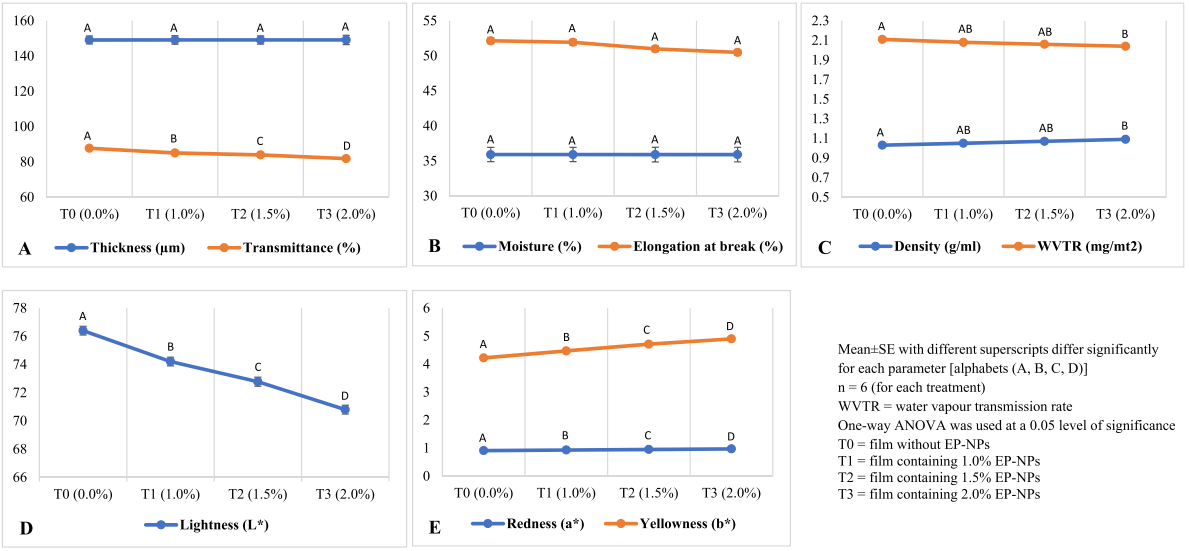


Fig. 1. Characteristics (physicomechanical and colour) of Loc-Pro-based film

2.10. Sensory evaluation and texture profile analysis

The faculty members and postgraduate students of the age group of 30–50 years who had years of experience in evaluating food products and have received training for routine sensory tests, such as recognition, threshold, hedonic, and descriptive tests, were engaged voluntarily in the sensory evaluation (prior informed consent was obtained). A mixed team (5 females and 5 males) performed the evaluation (03 replications) of the stored Par-Che on each storage point (0^{th}, 30th, 60th, and 90th day) for four sensory characteristics (texture, flavour, overall acceptability, and colour and appearance) using an 8-point scale (1–8, 1 anchored 'disliked extremely' and 8 anchored 'liked extremely') [12]. Separate samples (Par-Che cubes) identified by three-digit codes (blind labelled) were presented randomly to the panellists along with water to cleanse the palate. The evaluation complied with established regulations and ethical guidelines.

The texture analyser Shimadzu EZ-SX from Japan was employed to perform the texture profile analysis of Par-Che samples on days 0 and 90 using a compression platform (probe of 5 cm diameter) and a 25 kg load cell [12]. Two compression cycles (5 s time gap and 2 mm/s crosshead speed) were programmed allowing a 50% compression of the original height of Par-Che samples (20 × 20 mm) to evaluate textural characteristics (springiness, firmness, adhesiveness, cohesiveness, chewiness and resilience).

2.11. Statistical analysis

After performing the study's six replications (n = 6), the data was collected and analysed by 2-way or 1-way ANOVA using SPSS-21. Normal distribution and homogeneity tests of the data were checked before analysis. Duncan's multiple range test (DMRT) was used to examine the significance ($P \leq 0.05$, 5% level of significance) between the means. The results are illustrated as means along with standard errors in the figures and tables.

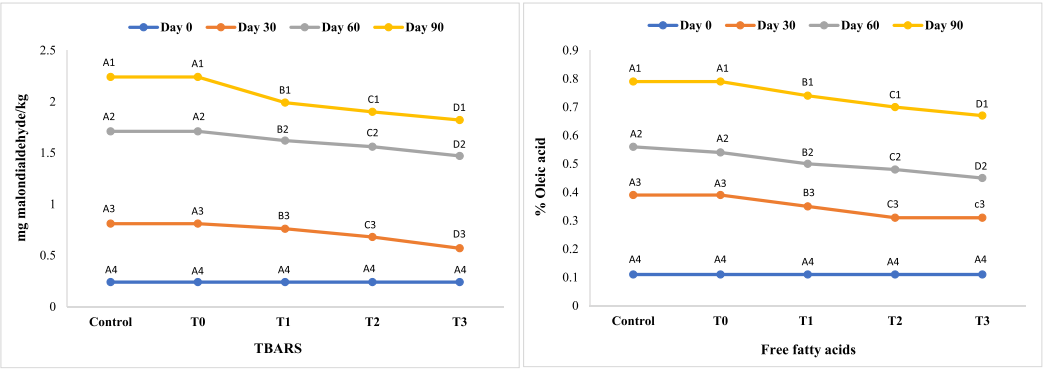
3. Results and discussion

3.1. Characteristics of Loc-Pro-based film and EP-NPs

The mean values of various characteristics of Loc-Pro-based films (physicochemical, antioxidant, antimicrobial and colour) and EP-NPs (physicochemical, antioxidant, and particle characteristics) are presented in Table 1 and Fig. 1.

3.1.1. Antioxidant, antimicrobial and particle characteristics

The incorporation of the EP-NPs significantly enhanced the antioxidant properties of the Loc-Pro-based film and all the related parameters viz. total phenolic and flavonoid contents, ABTS and DPPH scavenging activities (% inhibition), antioxidant release rate (%), and iron-reducing potential (µM TE/100g) showed a significant ($P < 0.05$) inclining trend with increasing incorporation levels [T_3(2.0%) > T_2(1.5%) > T_1(1.0%) > T_0(0.0%)]. This might be ascribed to the high antioxidant potential of EP-E which contains a high level of antioxidant phytochemicals and is also indicated by the high total flavonoid and phenolic contents of EP-NPs. This explains the high DPPH, ABTS and FRAP activities of the EP-NPs. The *E. purpurea* flower is a good source of phenolics, flavonoids, and organic acids such as chicoric and caftaric acids, catechin, caffeic acid derivatives, coumaric acid, epicatechin, hyperoside, malvidin, naringin, rutin, genistein, gallic and chlorogenic acid, naringenin, and chlorogenic and phenolic acids [11,20]. These compounds can reduce metal



Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D,⟶) for each treatment (T0, T1, T2, T3 or control)] and numerals (1, 2, 3, 4, ❙) for each time point (days 0, 30, 60 or 90)]
n = 6 (for each treatment)
Two-way ANOVA was used at a 0.05 level of significance
Control = cheese samples stored without any film
T0 = cheese samples with a film containing 0.0% EP-NPs
T1 = cheese samples with a film containing 1.0% EP-NPs
T2 = cheese samples with a film containing 1.5% EP-NPs
T3 = cheese samples with a film containing 2.0% EP-NPs

Fig. 2. Effect of Loc-Pro-based film incorporated with EP-NPs on the lipid stability of the parmesan cheese.

ions and scavenge free radicals. Mohamed Sharif et al. [11] reported a value of 14.0–45.60 mg GAE/g and 8.0–15.0 mg rutin equivalents/g for total phenolic and total flavonoid contents, respectively, for EP-E. While Kumar [21] reported strong antioxidant activity for EP-NPs, both Gecer and Erenler [22] and Fierascu et al. [13] found significantly higher radical scavenging and reducing power activities for EP-NPs compared to EP-E. Studies have documented a significant increase in the antioxidant capacity of edible films with the addition of plant extract-based nanoparticles. For example, the addition of ZnO nanoparticles significantly improved the antioxidant properties of the films prepared from agar and gelatin [23]. Similarly, Rasul et al. [24] improved the antioxidant potential of a chickpea protein and gelatin-based film using *Nigella sativa* EO (0–0.5%) and Cu-NPs (0–0.03%).

The addition of the EP-NPs also enhanced the antimicrobial activities of the Loc-Pro-based film and a significant ($P < 0.05$) increasing pattern was recorded for the size (mm) of inhibitory halos as the concentration of EP-NPs increased against *S. aureus*. The Loc-Pro-based film containing 1.0–2.0% EP-NPs did not show any effect against *E. coli* and halos were not found. The MIC (minimum inhibitory concentration) of EP-NPs was recorded to be 1 mg/ml and 28 mg/ml against *S. aureus* and *E. coli*, respectively, which explains the results of the inhibitory halos for both EP-NPs and the Loc-Pro-based film. The EP-E has been reported to contain several phytochemicals with antimicrobial properties with demonstrated activity against several microbes including *S. aureus* and *E. coli* [25]. Previous studies have reported the MIC value of 62.50–500 μg/ml for EP-E against various strains of *S. aureus* and 125–250 μg/ml against *E. coli* [11]. Satheesh et al. [21] demonstrated the antimicrobial properties of EP-NPs against several microorganisms such as *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae,* and *Proteus vulgaricus*. Fierascu et al. [13] reported significantly higher antimicrobial activity for EP-NPs compared to the simple flower extract. The antimicrobial nanoparticles have been utilized for the development of packaging films with antimicrobial activities. The incorporation of ZnO nanoparticles (1–3%) significantly improved the antimicrobial activities of carboxymethyl cellulose-based film [26].

The average particle size of EP-NPs was observed to be 58.13 ± 4.40 nm with an average zeta potential of −14.12 ± 0.62 and a polydispersity index of 0.389. The maximum absorption peak was documented at 471 nm which supported the formation of EP-NPs. Satheesh et al. [21] reported an average particle size of 50–70 nm for spherical-shaped EP-NPs. Our results were also in a similar range (25–80 nm). Small-sized particles make it possible to incorporate higher levels of the extract in the film matrices to impart stronger antioxidant and antimicrobial activities.

3.1.2. Physico-mechanical and colour properties

A significant impact ($P < 0.05$) of EP-NPs was found on the density of the Loc-Pro-based film and the samples containing 2% EP-NPs (T_3) showed significantly higher density in comparison to the film containing 0% EP-NPs and others [T_1 (1.0%) and T_2 (1.5%)]. This increase in density was reflected in the WVTR (mg/m^2t) and transmittance (%) which exhibited a significant decrease ($P < 0.05$) as the concentration of EP-NPs increased. While the films showed a significant decreasing trend in transmittance (%) with increasing concentration of EP-NPs, only T_3 films showed a significant decrease in WVTR in comparison to the control film containing 0% EP-NPs. A significant change in the composition affects the film density which in turn affects the transmittance of light and the passage of water vapours through it [15]. The use of silver for the preparation of nanoparticles which is a heavy metal may be the reason for an increase in the density of the film with increasing concentration of EP-NPs. The small size of nanoparticles allows them to occupy narrow spaces in the porous structure of protein films. Rahmasari and Yemis [27] reported a similar decrease in WVTR and transparency (transmittance %) of starch-based films as the levels of bioactive agents were increased. The incorporation of ZnO nanoparticles has been reported to decrease the transparency of edible films prepared from carboxymethylcellulose [26]. The WVTR is an important parameter from a storage quality point of view and helps to retain the moisture content and sensorial quality of the stored samples. It is also important to control the degradation of food structure that can promote microbial growth and adverse food reactions such as browning, vitamin reactions, and oxidation [28]. The transparency of a film is important from the consumer's point of view and can

Table 2
Effect of Loc-Pro-based composite film incorporated with EP-NPs on protein oxidation and physicochemical parameters of parmesan cheese.

Treatments	Storage period (days)			
	0	30	60	90
Total carbonyl content (nmol/mg protein)				
Control	0.42 ± 0.01^{Aa}	0.82 ± 0.01^{Ab}	1.01 ± 0.01^{Ac}	1.22 ± 0.01^{Ad}
T_0	0.41 ± 0.01^{Aa}	0.79 ± 0.01^{Ab}	0.97 ± 0.01^{Ac}	1.21 ± 0.01^{Ad}
T_1	0.41 ± 0.03^{Aa}	0.63 ± 0.01^{Bb}	0.85 ± 0.01^{Bc}	1.09 ± 0.02^{Bd}
T_2	0.40 ± 0.02^{Aa}	0.57 ± 0.01^{Cb}	0.76 ± 0.01^{Cc}	0.93 ± 0.01^{Cd}
T_3	0.40 ± 0.04^{Aa}	0.51 ± 0.01^{Db}	0.66 ± 0.01^{Dc}	0.81 ± 0.02^{Dd}
pH				
Control	5.40 ± 0.01^{Aa}	5.34 ± 0.009^{Ab}	6.05 ± 0.007^{Ac}	6.61 ± 0.02^{Ad}
T_0	5.40 ± 0.005^{Aa}	5.34 ± 0.02^{Ab}	6.01 ± 0.006^{ABc}	6.57 ± 0.009^{ABd}
T_1	5.39 ± 0.007^{Aa}	5.28 ± 0.006^{Ba}	5.96 ± 0.002^{ABb}	6.56 ± 0.02^{ABc}
T_2	5.39 ± 0.007^{Aa}	5.24 ± 0.01^{Cb}	5.90 ± 0.01^{Bc}	6.55 ± 0.01^{Bd}
T_3	5.39 ± 0.005^{Aa}	5.21 ± 0.01^{Cb}	5.86 ± 0.01^{Bc}	6.55 ± 0.01^{Bd}
Moisture (%)				
Control	24.18 ± 0.31^{Aa}	23.12 ± 0.51^{Ab}	22.33 ± 0.41^{Ac}	21.38 ± 0.71^{Ad}
T_0	24.17 ± 0.11^{Aa}	23.92 ± 0.20^{Bb}	22.87 ± 0.21^{Bc}	22.12 ± 0.30^{Bd}
T_1	24.17 ± 0.41^{Aa}	23.91 ± 0.31^{Bb}	22.87 ± 0.50^{Bc}	22.12 ± 0.51^{Bd}
T_2	24.16 ± 0.51^{Aa}	23.92 ± 0.50^{Bb}	22.88 ± 0.32^{Bc}	22.13 ± 0.41^{Bd}
T_3	24.15 ± 0.70^{Aa}	23.93 ± 0.60^{Bb}	22.89 ± 0.61^{Bc}	22.14 ± 0.11^{Bd}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P < 0.05$).
$n = 6$ for each treatment.
Control = cheese samples stored without a film.
T_0 = cheese samples with a film containing 0.0% EP-NPs.
T_1 = cheese samples with a film containing 1.0% EP-NPs.
T_2 = cheese samples with a film containing 1.5% EP-NPs.
T_3 = cheese samples with a film containing 2.0% EP-NPs.

affect the acceptability of a product. No significant effect ($P > 0.05$) of the incorporation of EP-NPs was observed on other physicomechanical parameters of the film such as thickness (μm), elongation at break (%), and moisture content (%). The yield of the extract was found to be 27.40% and the pH was 4.80. Studies, such as Silva et al. [28], have reported no significant change in the film characteristics, such as moisture content and elongation at break, on the addition of bioactive ingredients.

The incorporation of EP-NPs significantly affected ($P < 0.05$) the colour of the Loc-Pro film. While yellowness (b*) and redness (a*) showed a significant increase, the whiteness or luminosity (L*) of the films decreased with increasing concentration of EP-NPs which means the films became darker with increasing concentration of EP-NPs. A similar decrease in whiteness (L*) and an increase in redness (a*) and yellowness (b*) has been reported on the incorporation of ZnO nanoparticles into the CMC-based edible films [26]. Other studies, such as Rahmasari and Yemiş [27] and Ribeiro Sanches et al. [29], have reported a similar decrease in lightness and an increase in redness and yellowness values of starch-based films as the levels of bioactive agents increased.

3.2. Effect of Loc-Pro-based film on lipid stability and protein oxidation of Par-Che

The mean values for parameters related to lipid stability [free-fatty acids (FFA) and thiobarbituric acid reacting substances (TBARS)] and protein oxidation (total carbonyl content) of stored Par-Che are presented in Fig. 2 and Table 2. The Loc-Pro-based films enriched with EP-NPs significantly reduced the lipid oxidation (TBARS), lipolysis (FFA) and protein oxidation (nmol/mg) of the Par-Che from days 30–90 in comparison to the control and T_0 cheese. A significant ($P < 0.05$) impact of the concentration was also recorded and the lowest values were exhibited by the Par-Che samples wrapped with Loc-Pro-based films enriched with 2% EP-NPs and showed a particular pattern viz. $T_3 < T_2 < T_1 < T_0$ for all these parameters from days 30–90 of the storage. These results manifested a positive correlation with the antioxidant activities and release rate of the phenolics from the Loc-Pro-based films enriched with EP-NPs. The significantly higher release rate (%) and antioxidant properties (FRAP, DPPH, and ABTS) of the T_3 films were reflected in these results. The EP-E has been reported to contain several phenolic acids and flavonoids with antioxidant properties such as caftaric acid, chlorogenic acid, chicoric acid, meso-chicoric acid, coumaroylcaffeoyltartaric acid, feruloylcaffeoyltartaric acid, dicaffeoylquinic acid, quercetin-*O*-hex, rutin, isorhamnetin-*O*-rut, and kaempferol-*O*-rut [25]. It is an established phenomenon that plant-origin phenolics and flavonoids can reduce lipid and protein oxidation by neutralising the free radicals and inhibiting the chain reactions responsible for oxidation and lipolysis. Both lipid oxidation and protein oxidation catalyse the processes of each other and affect the sensory and nutritional quality of foods rich in fat such as cheese. Recently a similar reduction in the protein and lipid oxidation of *Karish* cheese has been reported by Kutlu et al. [30] using polyvinylalcohol-based nanomats loaded with pomegranate seed oil during 20 days of chilled storage.

3.3. Physicochemical and microbiological characteristics

The Loc-Pro-based film enriched with EP-NPs significantly lowered the pH of the stored Par-Che from 30 to 90 days in comparison

Table 3
Effect of Loc-Pro-based composite film incorporated with EP-NPs on the microbiological quality of parmesan cheese.

Treatments	Storage period (days)			
	0	30	60	90
Total plate count (log_{10} cfu/g)				
Control	1.10 ± 0.02^{Aa}	1.76 ± 0.01^{Ab}	2.15 ± 0.01^{Ac}	2.52 ± 0.01^{Ad}
T_0	1.07 ± 0.04^{Aa}	1.76 ± 0.007^{Ab}	2.14 ± 0.01^{Ac}	2.52 ± 0.01^{Ad}
T_1	1.06 ± 0.01^{Aa}	1.64 ± 0.02^{BCb}	2.00 ± 0.03^{Bc}	2.36 ± 0.01^{Bd}
T_2	1.05 ± 0.01^{Aa}	1.63 ± 0.007^{Cb}	1.94 ± 0.01^{Cc}	2.22 ± 0.01^{Cd}
T_3	1.04 ± 0.01^{Aa}	1.62 ± 0.01^{Cb}	1.90 ± 0.009^{Cc}	2.15 ± 0.03^{Dd}
Psychrophilic count (log_{10} cfu/g)				
Control	Not detected	Not detected	0.85 ± 0.01^{aA}	1.24 ± 0.008^{bA}
T_0	Not detected	Not detected	0.85 ± 0.02^{aA}	1.24 ± 0.007^{bA}
T_1	Not detected	Not detected	0.71 ± 0.03^{aB}	1.01 ± 0.03^{bB}
T_2	Not detected	Not detected	0.61 ± 0.02^{aC}	0.91 ± 0.01^{bC}
T_3	Not detected	Not detected	0.51 ± 0.01^{aD}	0.81 ± 0.006^{bD}
Coliform count (log_{10} cfu/g)				
Not detected in any sample during the entire storage period				
Yeast and mould count (log_{10} cfu/g)				
Control	Not detected	Not detected	Not detected	1.25 ± 0.01^{A}
T_0	Not detected	Not detected	Not detected	1.20 ± 0.02^{A}
T_1	Not detected	Not detected	Not detected	1.08 ± 0.03^{B}
T_2	Not detected	Not detected	Not detected	0.99 ± 0.01^{C}
T_3	Not detected	Not detected	Not detected	0.89 ± 0.02^{D}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P < 0.05$).
n = 6 for each treatment.
Control = cheese samples stored without a film.
T_0 = cheese samples with a film containing 0.0% EP-NPs.
T_1 = cheese samples with a film containing 1.0% EP-NPs.
T_2 = cheese samples with a film containing 1.5% EP-NPs.
T_3 = cheese samples with a film containing 2.0% EP-NPs.

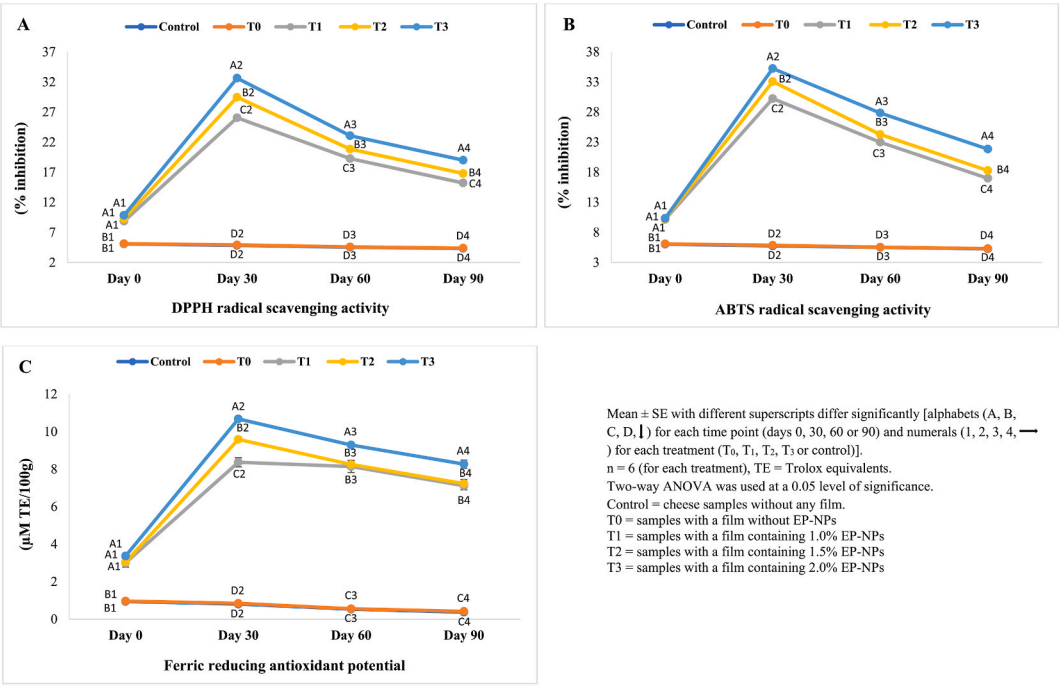
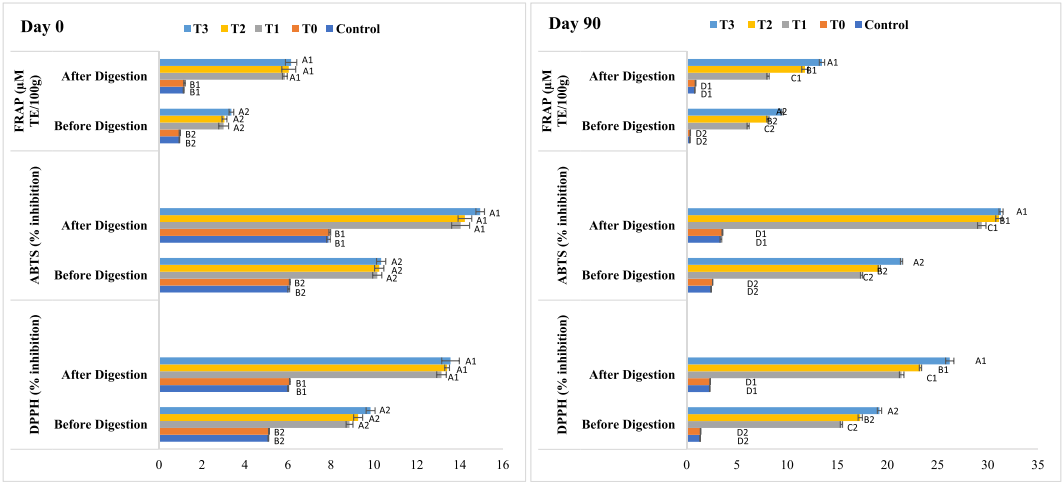


Fig. 3. Effect of Loc-Pro-based film incorporated with EP-NPs on the antioxidant potential of the parmesan cheese.

to the T_0 and control cheese (Table 2). This might be ascribed to the acidic pH (4.80 ± 0.03) of EP-NPs and the phytochemical constituents of *E. purpurea* which contains a good concentration of phenolic compounds, flavonoids, and organic acids such as chicoric and caftaric acids, caffeic acid derivatives, phenolic acids, and chlorogenic acid [11,20]. These acidic compounds can leach out of the



Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D) for each treatment (T_0, T_1, T_2, and control) and numerals (1, 2) for digestion (before or after).
n = 6 (for each treatment), TE = Trolox equivalents
Two-way ANOVA was used at a 0.05 level of significance
Control = cheese samples stored without any film
T_0 = cheese samples with a film containing 0% EP-NPs
T_1 = cheese samples with a film containing 1.0% EP-NPs
T_2 = cheese samples with a film containing 1.5% EP-NPs
T_3 = cheese samples with a film containing 2.0% EP-NPs

Fig. 4. Effect of *in vitro* gastrointestinal digestion on the antioxidant potential of the parmesan cheese.

film matrices and reduce the pH of the stored cheese. Previous studies have documented a decline in the pH of stored food products including cheese wrapped within edible films incorporated with phytochemicals and plant extracts [15,16]. The Loc-Pro-based film manifested a significant influence on the moisture content of the stored Par-Che from days 30–90 (Table 2) and provided a physical barrier that successfully reduced the rate of evaporation of moisture from the Par-Che surface. Recent studies have documented the use of edible packaging for retaining the moisture content and enhancing the sensorial quality of stored foods including cheese [15,16].

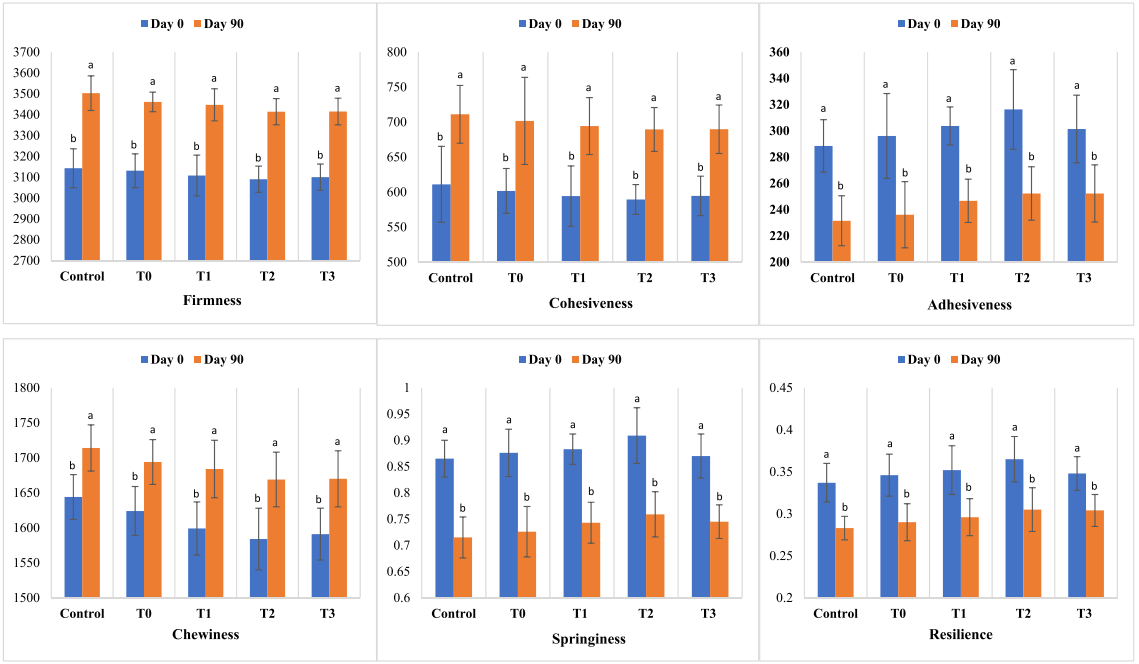
The Loc-Pro-based films enriched with EP-NPs significantly stabilized the microbial quality of stored Par-Che (Table 3). The Par-Che samples wrapped within the films enriched with EP-NPs manifested significantly lower total plate counts from 30 to 90 days in comparison to T_0 and control cheese. The psychrophiles were detected on day 60 and yeasts/moulds on day 90 and both showed similar results. The coliforms remained undetected during storage. A significant impact ($P < 0.05$) of the concentration was monitored and the lowest counts were observed for the Par-Che wrapped with Loc-Pro-based films enriched with 2% EP-NPs. The Par-Che samples showed a particular pattern viz. $T_3<T_2<T_1<T_0$ for all the microbial counts from days 30–90 of the storage. These results can be correlated with the antimicrobial properties and release rate of the phenolics from the Loc-Pro-based films enriched with EP-NPs. The significantly higher antimicrobial activities and release rate (%) of the T_3 films were reflected in these results. The *E. purpurea* extract has been reported to contain several phytochemicals with antimicrobial properties with demonstrated activity against several microorganisms such as *Streptococcus pyogenes, E. coli, Bacillus subtilis, Mycobacterium smegmatis, Pseudomonas aeruginosa, Enterococcus faecalis,* and *Staphylococcus aureus* [25]. Similar results have been reported by Chen et al. [31] who documented a significant enhancement in microbial stability of Mongolian cheese enclosed within polylactic acid nanofibrous membranes incorporated with lemon EO (10%) during 8 days of storage at 25 °C. The nanofibrous membranes prolonged the shelf-life of Mongolian cheese till day 8 at 25 °C compared to the control cheese which exhibited excessive microbial growth on and after day 6.

3.4. Antioxidant potential of Par-Che

The Par-Che samples wrapped within the films enriched with EP-NPs manifested significantly higher values for parameters related to ion-reducing [FRAP (μM TE/100 g)] and antioxidant [DPPH and ABTS radical scavenging activity (% inhibition)] potential from 0 to 90 days in comparison to T_0 and control cheese (Fig. 3). This was in full agreement with the results of antioxidant potential (ABTS, DPPH, and FRAP) and release rate (%) of polyphenols from the films which exhibited a similar pattern. These results indicate a significant enhancement in the product's functional value which can lead to a positive influence on the health of the consumers. Previous workers have recorded an enhancement in the antioxidant properties of stored cheese wrapped in edible packaging. Kouser et al. [32] documented a significant enhancement in antioxidant properties (ABTS, DPPH and FRAP) of stored *kalari* cheese wrapped within the edible films enriched with *A. vera* extract. A controlled release of flavonoids and polyphenols from the film matrices to the surface of Par-Che might be the cause for this enhancement in the antioxidant characteristics of Par-Che.

3.5. Digestion (gastrointestinal) simulation

The Par-Che wrapped within the films enriched with EP-NPs were subjected to *in vitro* gastrointestinal digestion (Fig. 4) on days



Mean ± SE with different superscripts differ significantly (a, b,), n = 10 (for each treatment), Two-way ANOVA was used at a 0.05 level of significance, Control = cheese samples stored without any film, T0 = cheese samples with a film containing 0% EP-NPs, T1 = cheese samples with a film containing 1.0% EP-NPs, T2 = cheese samples with a film containing 1.5% EP-NPs, T3 = cheese samples with a film containing 2.0% EP-NPs

Fig. 5. Effect of Loc-Pro-based film incorporated with EP-NPs on texture profile analysis of the parmesan cheese.

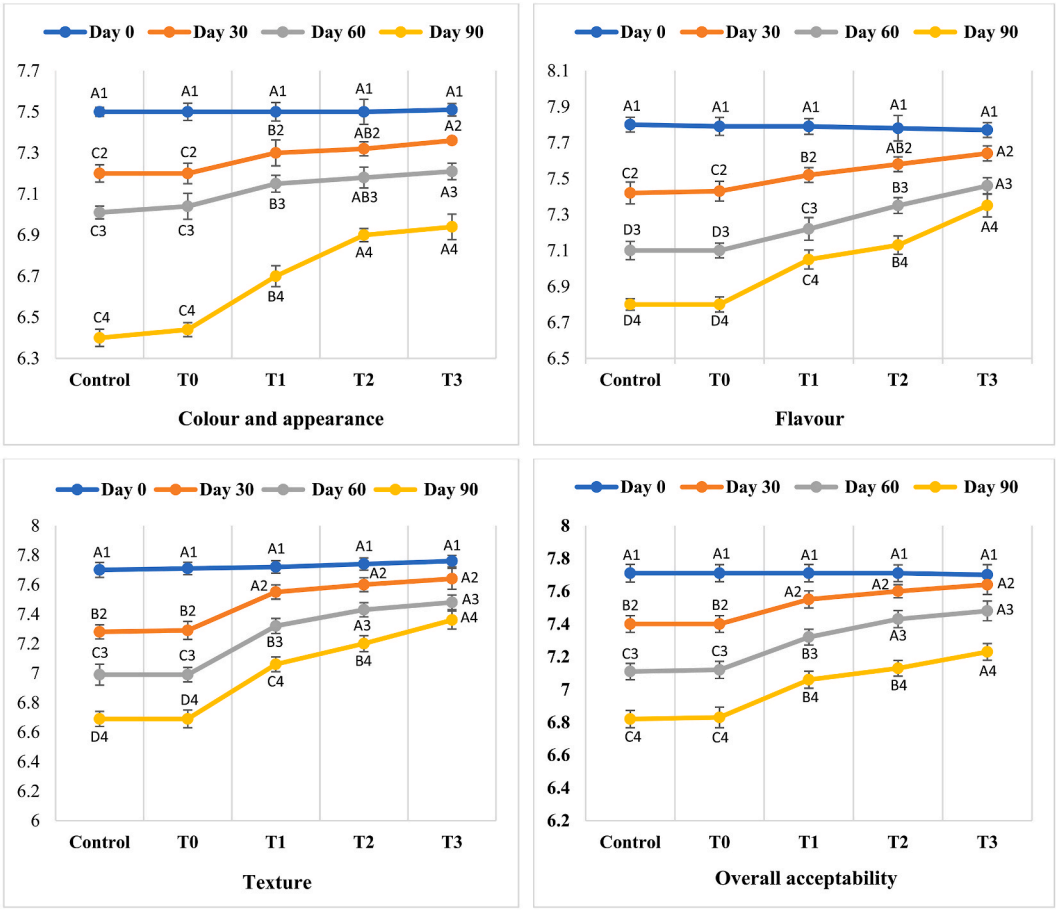
0 and 90 to decipher the effect of the human digestion process on antioxidant characteristics (FRAP, ABTS, and DPPH) and functional properties of Par-Che. The digestion process exhibited a positive impact and significantly augmented the antioxidant potential (FRAP, ABTS, and DPPH values) of the Par-Che wrapped within the ENP-enriched films on both days 0 and 90 in comparison to the control and T_0 samples. This may be due to the further hydrolysis of cheese proteins by pancreatin and pepsin resulting in the generation of amino acids and peptides of antioxidant nature. Recently a study by Lone et al. [12] found a favourable influence of digestion simulation on antioxidant characteristics (FRAP, ABTS, and DPPH values) of cheddar cheese. This demonstrates the product's functional value which can have a favourable influence on consumer health.

3.6. Sensory and texture profile analysis

The mean texture profile analysis and sensory scores of the stored Par-Che wrapped within the Loc-Pro-based films are presented in Figs. 5 and 6. The Loc-Pro-based films enriched with EP-NPs significantly enhanced the sensorial characteristics of stored Par-Che. The Par-Che samples wrapped within the films enriched with EP-NPs manifested significantly ($P < 0.05$) higher scores for all the four evaluated sensory characteristics (flavour, overall acceptability, texture, and colour) from days 30–90 in contrast to T_0 and control cheese. A significant impact ($P < 0.05$) of the concentration was documented and the highest means were found for the Par-Che samples wrapped with Loc-Pro-based films enriched with 2% EP-NPs. This might be ascribed to the results of lipid oxidation and microbial quality which were significantly reduced in the Par-Che samples wrapped with Loc-Pro-based films enriched with EP-NPs. Both these spoilage processes affect the food product's sensory quality by inducing lipolysis and proteolysis and by producing off-flavour and bitter compounds during storage. Recent studies have observed a positive influence of edible packaging systems on the sensory properties of stored cheese. Polat Yemis et al. [33] used myrtle (*Myrtus communis* L.) EO (0.5–2.0%) loaded nanoemulsion-based coating to enhance the colour and appearance of fresh Kasar cheese stored in a refrigerator (4 °C) for 24 days. A significant positive impact of nanofibrous membranes made of polylactic acid and incorporated with lemon EO has been documented by Ref. [31] on the colour and appearance and overall sensory score of Mongolian cheese during storage. The Par-Che samples wrapped with Loc-Pro-based films were also evaluated for texture profile analysis and no impact ($P > 0.05$) of the films was observed on the characteristics of the cheese. The reason for this might be that all the samples with or without edible films were packed within the pouches made of polyethylene. Mezhoudi et al. [34] reported no effect of an edible film developed from gelatin and leaf extract of *Moringa oleifera* (20 μg/ml) on the cohesiveness of ricotta cheese stored for 6 days in a refrigerator (4 °C). As expected a significant influence ($P < 0.05$) of the storage time was seen on the texture profile of the Par-Che.

3.7. Shortcomings and future suggestions

Our study did not evaluate the cost-effectiveness of the developed packaging film. Currently, insect flours are expensive due to the



Mean ± SE with different superscripts differ significantly [alphabets (A, B, C, D, ⟶) for each time point (day 0, 30, 60 or 90) and numerals (1, 2, 3, 4, ⟶) for each treatment (T_0, T_1, T_2, T_3 or control)].
Repeated measurements ANOVA was used at a 0.05 level of significance.
10 semi-trained panellists performed the sensory evaluation thrice for each treatment at each time point (days 0, 30, 60, and 90) using an 8-point descriptive scale (1 denoted 'disliked extremely' and 8 denoted 'liked extremely').
Control = cheese samples stored without any film
T_0 = cheese samples with a film containing 0% EP-NPs
T_1 = cheese samples with a film containing 1.0% EP-NPs
T_2 = cheese samples with a film containing 1.5% EP-NPs
T_3 = cheese samples with a film containing 2.0% EP-NPs

Fig. 6. Effect of Loc-Pro-based film incorporated with EP-NPs on sensory quality of the parmesan cheese.

small scale and small number of players involved in the production. With increasing demand, this will change in future with large-scale industrial production and will therefore reduce the cost of production of insect protein-based packaging materials. While this was the first attempt to develop a locust protein-based film for food packaging, our study aimed at producing the films at an experimental scale using the casting method. Future studies should evaluate the feasibility and economics of large-scale commercial production. Technology can be commercialized and become globally available only if it can be scaled up to the industrial level and there are favourable regulations. The international marketing strategies for insect-based products are complicated due to the differences in the regulatory demands between countries. There are different regulations in different countries about the use of insect protein-based ingredients for the development of food products or food packaging materials. Insects are traditionally consumed in many cultures in several countries including China, Mexico, and Australia without a specific regulatory framework. Further, the use of all potential insects as a novel protein source is not allowed by the regulations in most countries.

The film developed in this study was mainly targeted at cheese and parmesan was used as a model system for cheese. However, the potential of the film can be evaluated in future for other food products. Future studies may evaluate the suitability of the developed film for food products with high moisture content or with different fat profiles. Studies in future should examine the possibility of using other insect proteins to develop edible and biodegradable films for the packaging and preservation of food products.

4. Conclusions

The Loc-Pro-based films were successfully developed with strong antioxidant and antimicrobial characteristics using *E. purpurea flower*-based nanoparticles. The EP-NPs-enriched films significantly enhanced the antioxidant characteristics, lipid stability and microbiological quality of Par-Che stored for 3 months in a refrigerator. The films also enhanced the sensory characteristics of Par-Che from days 30–90. A positive influence of digestion was revealed on the antioxidant characteristics of Par-Che indicating health-promoting properties for the consumer. Overall, the results of our study indicate the preservative potential of Loc-Pro-based film which enhanced the storage quality of cheese.

CRediT authorship contribution statement

Shubam Singh: Methodology, Investigation. **Hina F. Bhat:** Writing – review & editing, Resources, Methodology. **Sunil Kumar:** Writing – review & editing, Visualization, Software. **Rana Muhammad Aadil:** Writing – review & editing, Visualization, Software. **Gholamreza Abdi:** Writing – review & editing, Validation, Resources, Methodology. **Zuhaib F. Bhat:** Writing – original draft, Validation, Supervision, Resources, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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