Urmia University

Food Safety and Packaging

2026, 2 (3): In press

Journal Homepage: fsp.urmia.ac.ir

FOOD Safety and Packaging

Research Article

Physicochemical, antioxidant, and antibacterial properties of tragacanth gum/whey protein concentrate films containing fennel essential oil and chitosan nanoparticles

Hojjat Azad[1], Tooraj Mehdizadeh[1*], Shahin Zomorodi[2]

[1]Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; [2]Agricultural Engineering Research Department, West Azerbaijan Agricultural and Natural Resources Research and Education Center, AREEO, Urmia, Iran

Abstract

Active films based on tragacanth gum and whey protein concentrate (WPC) were developed with fennel essential oil (FEO) and chitosan nanoparticles (CNPs). Nine formulations were prepared using a 3 × 3 factorial design (FEO: 0, 3, and 4%; CNPs: 0, 5, and 8%). Gas chromatography–mass spectrometry identified ten compounds, accounting for 99.78% of the volatile fractions. Compared with the control film, FEO4–CNP8 showed significantly greater thickness (0.160 vs. 0.037 mm; $p < 0.05$) and lower moisture content (31.48 vs. 37.15%), water solubility (29.99 vs. 45.13%), and swelling capacity (154.15 vs. 244.44%). FEO concentration, CNP concentration, and their interaction significantly affected DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. FEO4–CNP8 (59.48%) did not differ from FEO4–CNP0 (59.05%), exceeded the control (21.42%; $p < 0.05$), and remained below BHT (Butylated hydroxytoluene) (92.36%; $p < 0.05$). The control film showed no antibacterial activity, whereas all FEO- and/or CNP-containing films inhibited the four tested bacteria, with FEO4–CNP8 producing zones of 28.33–33.33 mm. To our knowledge, this is the first study combining tragacanth gum and WPC with FEO and CNPs. These films demonstrated considerable potential for application in active food packaging, although further investigations are needed to determine whether the combined effects of FEO and CNPs are additive or synergistic.

Keywords: Active packaging; Chitosan nanoparticles; Fennel essential oil; Tragacanth gum; Whey protein concentrate.

Introduction

Reducing food loss and waste is essential for improving food security and the sustainability of food supply chains. Inefficiencies during production, distribution, storage, and consumption contribute to food losses, highlighting the need for effective preservation and packaging strategies (Sarangi et al., 2024; Yu et al., 2025). Synthetic polymers are widely used in food packaging because of their desirable mechanical properties, processability, durability, and relatively low cost. However, their limited biodegradability contributes to the persistent accumulation of plastic waste in terrestrial and aquatic environments (Omerović et al., 2021; Otto et

* **Correspondence:** T. Mehdizadeh (t.mehdizadeh@urmia.ac.ir).
https://doi.org/10.30466/fsp.2026.57764.1044
Received: 13 October 2026 **Accepted:** 13 November 2026 **Available online:** 15 November 2026
© 2026 Urmia University. All rights reserved.

al., 2021). Consequently, biodegradable packaging materials derived from renewable biopolymers have attracted increasing scientific and industrial interest. Tragacanth gum is a plant-derived hydrocolloid with thermal stability, acid resistance, emulsifying capacity, and relatively good storage stability. It consists mainly of two fractions: bassorin, which is water-insoluble, and tragacanthin, which is water-soluble. Because of its film-forming properties, tragacanth gum has attracted attention as a material for edible and biodegradable packaging. However, gum-based films may have limited mechanical strength and inadequate barrier properties. Blending tragacanth gum with other biopolymers and incorporating active compounds or nanoparticles into the film matrix may improve film functionality and its potential for food preservation (Ahmed et al., 2023; Emam-Djomeh et al., 2019; Featherstone, 2015). Whey, the liquid by-product of cheese manufacture, contains approximately 92% water, together with lactose, proteins, minerals, and B-group vitamins. Although whey is used in the food, pharmaceutical, and chemical industries, a considerable proportion remains underutilized or is discarded, potentially contributing to environmental pollution. Whey proteins are used in meat, dairy, and bakery products because they improve texture, retain moisture, and stabilize foams and emulsions. They can also form biodegradable packaging films; however, these films may be moisture-sensitive and mechanically weak. The use of plasticizers and composite structures can therefore improve their flexibility, strength, and functional performance (Avalos et al., 2020; Bacenetti et al., 2018).

Foeniculum vulgare Mill., commonly known as fennel, is an aromatic plant belonging to the *Apiaceae* family and is used as a flavoring ingredient and in traditional medicinal applications. Fennel essential oil (FEO) contains bioactive compounds with antioxidant and antibacterial properties. These properties support its use in active packaging, where it may help limit oxidative deterioration and inhibit spoilage or pathogenic microorganisms (Mandal et al., 2023; Noreen et al., 2023; Rather et al., 2016).

Chitosan is a biodegradable polysaccharide that has been extensively investigated for active packaging and edible films. Its solubility and functional behavior depend strongly on its degree of deacetylation, molecular weight, and environmental pH. Chitosan films exhibit film-forming ability and antibacterial activity. The positively charged amino groups of chitosan can interact with negatively charged microbial surfaces, potentially disrupting membrane integrity and essential cellular functions. Chitosan nanoparticles (CNPs) may provide a larger surface area and stronger interactions with microbial cells and the film matrix, thereby enhancing antibacterial efficacy and their potential application in active packaging (Akdaşçi et al., 2025; Aranaz et al., 2021; Eker et al., 2024; El-Araby et al., 2023; Muñoz-Tebar et al., 2023). Therefore, this study aimed to develop active biodegradable films based on a tragacanth gum/whey protein concentrate (WPC) composite matrix incorporating FEO and CNPs, and to evaluate the physicochemical, antioxidant, and antibacterial properties of the resulting films as a potential strategy for active food packaging.

Materials and Methods

Materials

Tragacanth gum and Fennel (*F. vulgare* Mill.) seeds were purchased from a local market in Urmia, Iran. Whey protein concentrate (approximately 80% protein) was obtained from Behtam Powder Iran Co. (Karaj, Iran), and CNPs (approximately 50 nm; purity > 99%) from Nanosany Corporation (Mashhad, Iran). Glycerol (Merck, Darmstadt, Germany) was used as a plasticizer at 25% (w/w) of the combined polymer dry weight. 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), acetic acid and anhydrous sodium sulfate were also purchased from Merck. *n*-hexane was obtained from Merck (Darmstadt, Germany), All other chemicals were of analytical grade.

Salmonella enterica serovar Enteritidis (ATCC 13076) and *Escherichia coli* O157:H7 (ATCC 43890) were obtained from the Razi Vaccine and Serum Research Institute (Karaj, Iran), while *Listeria monocytogenes* (ATCC 13932) and *Staphylococcus aureus* (ATCC 6538) were obtained from the Iranian Biological Resource Center. Mueller–Hinton broth and agar were purchased from Quelab Laboratories Inc. (Montréal, QC, Canada). Commercial gentamicin

discs (10 µg per disc; Faraz Med Co., Tehran, Iran) were used as the positive control.

Plant material and essential oil extraction

Fennel seeds were authenticated at the Herbarium of the Agricultural Research Center, where voucher specimen No. 11076 was deposited. The seeds were ground, and 100 g of powder was hydrodistilled with 900 mL of distilled water for 3 h using a Clevenger-type apparatus (Kazemi et al., 2026). The extracted oil was dried over anhydrous sodium sulfate, filtered through a 0.22 µm syringe filter (Merck, Darmstadt, Germany), stored in sealed amber vials at 4 °C, and used for gas chromatography-mass spectrometry (GC–MS) analysis and film preparation. The extraction yield was calculated as:

Extraction yield (%) = [Mass of extracted essential oil (g) / Mass of fennel seeds used (g)] × 100

Chemical characterization of the essential oil by GC–MS

The essential oil was analyzed using an Agilent 7890A GC coupled with a 7000 triple-quadrupole MS. The oil (10 µL) was diluted with 80 µL of *n*-hexane, and 1 µL was injected in splitless mode. Separation was performed on an HP-5MS column (30 m × 0.25 mm, 0.25 µm) with helium (99.99%) as the carrier gas at 1.00 mL min^{-1}. The injector temperature was 260 °C. The oven temperature was held at 40 °C for 2 min, increased to 150 °C at 20 °C min^{-1}, to 220 °C at 8 °C min^{-1}, and finally to 280 °C at 20 °C min^{-1}, where it was held for 20 min. The MS operated in electron-impact mode at 70 eV. Compounds were identified by comparison with the instrument spectral library, and their relative contents were calculated from the total chromatographic peak area (Veryer & Bozok, 2023).

Film treatments

Nine film formulations were prepared according to a 3 × 3 factorial design, with FEO incorporated at 0%, 3%, and 4% and CNPs at 0%, 5%, and 8%. The treatments were coded as FEO0–CNP0, FEO0–CNP5, FEO0–CNP8, FEO3–CNP0, FEO3–CNP5, FEO3–CNP8, FEO4–CNP0, FEO4–CNP5, and FEO4–CNP8. The FEO0–CNP0 film served as the base-polymer control. A Tween 80 control film, prepared without FEO or CNPs, contained Tween 80 at 60% of the FEO mass used in the 4% FEO formulation.

Film preparation

Films were prepared by casting a WPC–tragacanth gum matrix. Tragacanth gum (1.20 g) was hydrated in 150 mL of sterile distilled water at 50 °C. WPC (4.80 g) was dispersed separately in 150 mL of water and heated at 80 °C for 30 min. After cooling, the pH of the WPC/tragacanth dispersion was measured using a calibrated digital pH meter (Metrohm, Switzerland) and adjusted to 5.95 with 3.00% (v/v) acetic acid before adding CNPs, FEO, and Tween 80 (Amani et al., 2022; Kontogianni et al., 2022). The dispersions were combined at a dry-matter ratio of 4:1 and stirred at 500 rpm for 1 h. Glycerol was then added at 25% (w/w) of the total polymer dry weight, followed by stirring for 30 min.

CNPs were incorporated at 5 or 8% (w/w) of the polymer dry weight, corresponding to 0.30 or 0.48 g, respectively. Each amount was gradually dispersed in 8 mL of 2.00% acetic acid at 1,500 rpm for 30 min and added to the film-forming mixture. The pH of the CNP dispersion and final suspension was not measured; therefore, the reported CNP concentrations represent nominal added masses and were not analytically verified in the final suspension.

FEO was added at 3 or 4% (w/w) of the polymer dry weight and mixed with Tween 80 at 60% of the FEO mass. The mixture was stirred until optically clear, indicating complete solubilization of FEO by Tween 80, added to the film suspension, homogenized at 10,000 rpm for 3–5 min, and sonicated at 37 kHz for 20 min in an ice-water bath to prevent thermal degradation of FEO. Nine formulations were prepared using a 3 × 3 factorial design with FEO concentrations of 0, 3, and 4% and CNP concentrations of 0, 5, and 8%.

For casting, 50 mL of each suspension was transferred into a 16 cm diameter polytetrafluoroethylene (PTFE) mold (Pars Polymer, Tehran, Iran) and dried at 25 °C. The films were peeled and conditioned for at least 48 h at 25 °C in desiccators containing saturated magnesium nitrate solution (approximately 51–53% relative humidity).

Physicochemical characterization

Film thickness

Thickness was measured at ten randomly selected points on each film using a digital micrometer with 0.001-mm resolution, and the ten measurements were averaged per film (Khan et al., 2025).

Water solubility

Film strips (3 × 1 cm) were dried at 110 °C for 24 h and weighed (Wi). Each sample was immersed in 30 mL of distilled water and shaken at 25 °C for 24 h. The undissolved residue was filtered, dried at 110 °C to constant weight, and weighed (W_f) (Liu et al., 2020). Solubility was calculated as:

$$WS\ (\%) = [(W_i - W_f) / Wi] \times 100$$

Moisture content

Film pieces (3 × 3 cm) were weighed (m_{wet}), dried at 110 °C to constant weight, cooled in a desiccator, and reweighed (W_{dry}) (Karamali et al., 2023). Moisture content was calculated on a wet-weight basis:

$$MC\ (\%) = [(W_{wet} - W_{dry}) / W_{wet}] \times 100$$

Swelling degree

Film pieces (2 × 2 cm) were dried at 50 °C for 24 h and weighed (W_0). They were immersed in 30 mL of distilled water and shaken at 150 rpm and 25 °C. At 1-h intervals, samples were removed, gently blotted, and weighed (W_t) until equilibrium, defined as no appreciable change between two consecutive measurements (Anisimov et al., 2020). Swelling was calculated as

$$SD\ (\%) = [(W_t - W_0) / W_0] \times 100$$

Antioxidant activity

Film samples (100 mg) were extracted with 4 mL of methanol–distilled water (1:1, v/v) at 200 rpm for 30 min at room temperature. Extracts were centrifuged at 5,000 rpm for 10 min, and 1 mL of each supernatant was mixed with 1 mL of freshly prepared DPPH solution (0.01 mM in methanol). After incubation in the dark for 30 min, absorbance was measured at 517 nm. A mixture of DPPH solution and extraction solvent served as the control (Javid et al., 2024). Radical scavenging activity (RSA) was calculated as:

$$RSA\ (\%) = [(Ac - As) / Ac] \times 100$$

Where Ac and As represent the absorbance values of the control and the sample, respectively. BHT served as the reference antioxidant.

Antibacterial activity assay

For the agar disc-diffusion assay, 100 µL of the bacterial suspension at the experimentally used concentration was spread evenly over Mueller–Hinton agar plates. The tested organisms were S. enterica, *E. coli, L. monocytogenes*, and *S. aureus*. Circular film discs (15 mm in diameter) were placed on the inoculated agar surfaces, and the plates were incubated at 37 °C for 24 h. Circular film discs (15 mm) were placed on Mueller–Hinton agar, inoculated with *S. enterica*, *E. coli*, *L. monocytogenes*, or *S. aureus*. The FEO0–CNP0 film served as the base-polymer control. A Tween 80 control film was prepared using the same matrix and processing conditions, without FEO or CNPs, with Tween 80 corresponding to 60% of the FEO mass used in the 4% FEO formulation. A sterile water-loaded Whatman disc served as the negative procedural control, and a commercial gentamicin disc (10 µg) as the positive control. Plates were incubated at 37 °C for 24 h. The total inhibition-zone diameter, including the disc diameter, was measured at the widest point using a digital caliper. Absence of a zone beyond the disc edge was recorded as zero.

Statistical analysis

Data from the 3 × 3 factorial designs were analyzed using two-way ANOVA, with FEO and CNP concentrations as fixed factors. Main effects and their interaction were evaluated for thickness, moisture content, solubility, swelling, and DPPH activity. Significant effects were followed by Tukey's HSD test comparing the nine FEO–CNP combinations. Antibacterial data were analyzed separately by one-way ANOVA, including the nine film formulations, Tween 80 control, and gentamicin; the negative control was excluded from statistical comparisons. DPPH radical scavenging activity of the nine film formulations and BHT was

additionally compared by one-way ANOVA followed by Tukey's HSD test. Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., USA). Statistical significance was set at $p < 0.05$, and results with $p < 0.001$ are indicated accordingly. Unless otherwise stated, all physicochemical measurements were performed on three independently prepared film specimens per formulation, and results are expressed as mean ± standard deviation (SD).

Results and Discussion

Extraction yield and GC–MS profile of fennel EO

The extraction yield was 0.98% (w/w). GC–MS analysis identified ten compounds in FEO, representing 99.78% of the total detected composition **(Table 1**). The total ion chromatogram of the FEO is presented in **Figure 1**. Trans-Anethole was the predominant compound (68.11%), followed by fenchone (15.42%), estragole (7.21%), and eucalyptol (6.59%), which together accounted for 97.33% of the total composition. The remaining constituents (α-pinene, 2.20%; D-limonene, fenchyl acetate, p-anisaldehyde, carvone, and anisole, each ≤0.08%) accounted for 2.45% of the total composition, while the undetected 0.22% likely represents trace or unidentified constituents below the reporting threshold. The dominance of trans-anethole is consistent with previous reports on FEO composition (Abdou et al., 2025; Barrahi et al., 2020), with published values ranging from approximately 34.8–82.0%, placing the 68.11% recorded here within the documented range. The combined proportion of trans-anethole and estragole (75.32%) reflects the predominance of phenylpropanoid compounds in the oil profile, consistent with the findings of Marčac et al. (2023), who identified phenylpropanoids as the most abundant chemical group in FEO, regardless of the isolation procedure. Fenchone, eucalyptol, and α-pinene represent the hydrocarbon and oxygenated monoterpene fraction, and their proportions fall within the variability described in the literature (Barrahi et al., 2020; Koljančić et al., 2023). Differences in compound percentages across studies can be attributed to cultivar, geographical origin, plant part used, developmental stage, and extraction method (Koljančić et al., 2023; Šunić et al., 2023), and therefore do not necessarily reflect differences in oil quality. Estragole, identified at 7.21%, requires consideration in the context of food contact applications. Safety assessment for active films should not rely on relative composition alone; factors including the amount of essential oil incorporated, migration rate into food, and consumer exposure must also be evaluated, and a lower estragole content compared with some reports does not in itself guarantee safety (Abdelbaky et al., 2023) .GC–MS data describe the volatile composition of the oil but cannot establish which individual constituents were responsible for the antioxidant or antibacterial responses observed in the films. Accordingly, the reported antioxidant and antibacterial activities are attributed to the complete film formulation, rather than to individual components or potential synergistic interactions.

Table 1. Chemical composition of FEO identified by GC–MS.

No.	Compound	Relative content (%)	Retention time (min)
1	trans-Anethole	68.11	18.51
2	Fenchone	15.42	12.09
3	Estragole	7.21	15.52
4	Eucalyptol	6.59	10.25
5	α-Pinene	2.20	7.56
6	D-Limonene	0.08	5.39
7	Fenchyl acetate	0.08	7.07
8	p-Anisaldehyde	0.04	7.25
9	Carvone	0.03	7.15
10	Anisole	0.02	8.90
	Total	99.78	

Thickness of the films

The physicochemical properties of the tragacanth gum/WPC-based films were significantly influenced by the incorporation of FEO and CNP. Two-way ANOVA confirmed significant treatment effects on all four parameters: thickness ($p < 0.001$), moisture content ($p < 0.001$), water solubility ($p < 0.001$), and swelling capacity ($p < 0.001$) (**Table 2**).

Film thickness increased progressively with FEO and CNP addition, from 0.037 ± 0.006 mm in the control (FEO0–CNP0) to 0.160 ± 0.010 mm in FEO4–CNP8, a

value statistically comparable to FEO3–CNP8 (p > 0.05). Thickness governs mechanical performance, gas permeability, water vapor transmission, and the retention and release kinetics of active compounds (Dehankar et al., 2023).

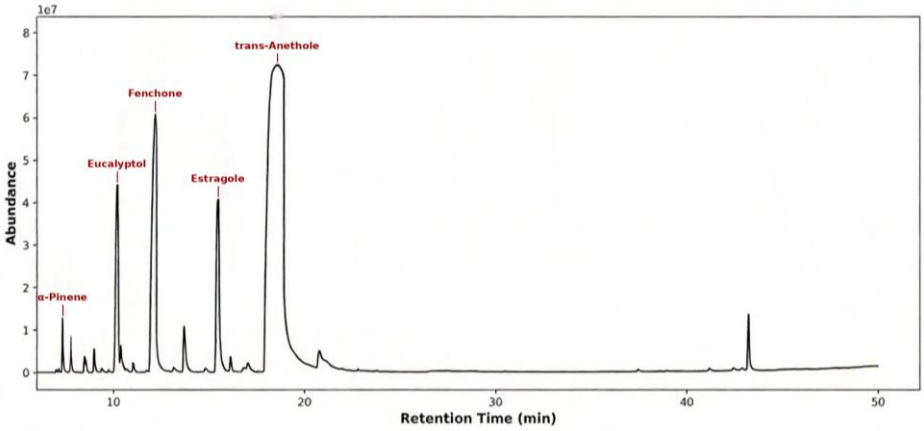


Figure 1. Chromatogram of fennel essential oil (FEO; *Foeniculum vulgare* Mill.) obtained by GC–MS. Labeled peaks correspond to α-pinene, eucalyptol, fenchone, estragole, and trans-anethole, respectively. Unlabeled peaks, including the peak at 43 min, correspond to minor components.

The observed increase can be attributed to two primary mechanisms. The first involves the contribution of CNP to the dry matter content and network architecture of the film. The present study yielded statistically significant thickness increases across all CNP-containing formulations, suggesting that within a tragacanth gum/WPC matrix, CNP at concentrations of 5% and particularly 8% engage in intermolecular interactions likely hydrogen bonds and electrostatic forces, which produce a denser three-dimensional network rather than acting solely as inert fillers. This active structural role has been documented in analogous protein- and polysaccharide-based composite films (Ahmad & Sarbon, 2021; Lorevice et al., 2016).

The second mechanism involves the steric effect of dispersed FEO droplets. Because FEO is predominantly hydrophobic consistent with the dominance of trans-anethole (68.11%) identified by GC–MS, the oil partitions as micro-droplets within the hydrophilic polymer matrix, increasing free volume and disrupting compact chain packing (Gao et al., 2024). This steric mechanism is well-supported: increased film thickness upon incorporation of hydrophobic essential oils into polysaccharide and protein matrices has been reported consistently (Ardebilchi Marand et al., 2023; Zhang et al., 2017). The maximum thickness in FEO4–CNP8 therefore reflects the cumulative contribution of both components. From an active packaging standpoint, greater thickness may enhance retention and sustained release of antimicrobial and antioxidant compounds; however, its practical benefit must be assessed alongside mechanical and barrier properties.

Moisture content, water solubility, and swelling capacity

Moisture content decreased from 37.15 ± 0.28% in FEO0–CNP0 to 31.48 ± 0.25% in FEO4–CNP8, water solubility from 45.13 ± 3.12% to 29.99 ± 2.11%, and swelling capacity from 244.44 ± 4.07% to 154.15 ± 1.45% (**Table 2**). FEO-only and CNP-only formulations produced intermediate reductions, while combined formulations achieved the greatest decreases. FEO4–CNP5 and FEO4–CNP8 did not differ significantly in moisture content (p > 0.05), and FEO3–CNP8 and FEO4–CNP8 were comparable in swelling (p > 0.05).

The high water sensitivity of the control film reflects the abundance of polar functional groups (hydroxyl, amino, and carboxyl groups) in tragacanth gum and WPC, which readily form hydrogen bonds with water and render the matrix highly susceptible to moisture

absorption, dissolution, and osmotic swelling (Azizi-Lalabadi et al., 2021; Dehankar et al., 2023). The reduction following FEO incorporation is consistent with the hydrophobic character of trans-anethole: lipophilic constituents interact with hydroxyl groups of polymer chains, reducing active sites for water-polymer hydrogen bonding, while dispersed oil droplets create a tortuous diffusion pathway that slows water ingress (Teymoorian et al., 2024). It is worth noting that this effect is not universal. Shirdel Marandi et al. (2022) reported higher swelling in essential oil-containing chitosan films relative to their control, attributing this to the plasticizing effect of volatiles within a strongly hydrophilic matrix, a contrasting result that underscores the dependence of this behavior on matrix composition.

Table 2. Physicochemical properties of the prepared composite films.

Formulations	Thickness (mm)	Moisture (%)	Solubility (%)	Swelling (%)
FEO0–CNP0	0.037 ± 0.006^{g}	37.15 ± 0.28^{a}	45.13 ± 3.12^{a}	244.44 ± 4.07^{a}
FEO3–CNP0	0.057 ± 0.012^{fg}	33.60 ± 0.26^{c}	40.90 ± 2.78^{ab}	226.82 ± 3.09^{b}
FEO4–CNP0	0.073 ± 0.006^{ef}	33.00 ± 0.25^{c}	40.05 ± 2.74^{ab}	217.30 ± 3.62^{c}
FEO0–CNP5	0.093 ± 0.006^{de}	36.23 ± 0.33^{b}	37.58 ± 2.58^{bc}	182.95 ± 3.04^{d}
FEO0–CNP8	0.103 ± 0.006^{cd}	35.99 ± 0.26^{b}	35.21 ± 2.46^{bcd}	175.60 ± 3.87^{de}
FEO3–CNP5	0.113 ± 0.006^{cd}	33.51 ± 0.33^{c}	34.70 ± 2.37^{bcd}	169.00 ± 2.86^{e}
FEO3–CNP8	0.143 ± 0.006^{ab}	33.29 ± 0.24^{c}	32.50 ± 2.20^{cd}	156.22 ± 2.63^{f}
FEO4–CNP5	0.123 ± 0.011^{bc}	31.70 ± 0.29^{d}	32.02 ± 2.16^{cd}	168.45 ± 2.88^{e}
FEO4–CNP8	0.160 ± 0.010^{a}	31.48 ± 0.25^{d}	29.99 ± 2.11^{d}	154.15 ± 1.45^{f}

Values are presented as mean ± SD (n = 3). Within each column, different lowercase superscript letters indicate statistically significant differences among the nine film formulations (Tukey's honestly significant difference (HSD) test, $p < 0.05$). FEO: fennel essential oil; CNP: chitosan nanoparticles; WPC: whey protein concentrate.

CNP contributed through a distinct but complementary mechanism: by occupying interchain spaces and forming hydrogen bonds with the polymer backbone, CNP restricts chain mobility and reduces the accessibility of free hydroxyl and amino groups to water molecules (Mohamed & Ramasswamy, 2022). In a directly relevant study on tragacanth-based nanocomposites, Janani et al. (2020) demonstrated that nanoparticle incorporation acts as physical cross-linking, reducing solubility and improving structural integrity in humid environments, a finding that aligns closely with the behavior observed here. Comparable network-densification effects reducing moisture uptake and swelling have been reported for CNPs in gelatin films (Khodaman et al., 2022), and for other insoluble particulate fillers (Mirpoor et al., 2025).

The combined FEO–CNP formulations outperformed the single-component films for several parameters, suggesting a possible interaction between FEO and CNP, although additivity was not formally tested. CNP may densify the polymer network and restrict chain mobility, whereas FEO may reduce the matrix's intrinsic hydrophilicity and limit water access to hydrophilic sites. Such modifications could improve the suitability of these bionanocomposites for humid environments (Fakraoui et al., 2024). Further studies on barrier, mechanical, and migration properties are needed to confirm their practical utility.

Antioxidant activity

DPPH radical scavenging activity was significantly influenced by FEO concentration, CNP concentration, and their interaction (FEO: $F(2,18) = 1986.94$, $p < 0.001$; CNP: $F(2,18) = 1068.42$, $p < 0.001$; FEO × CNP: $F(4,18) = 725.05$, $p < 0.001$).

Packaging materials capable of releasing antioxidant compounds can slow lipid oxidation and extend the shelf life of oxidation-sensitive food products such as fish fillets. The antioxidant properties of active films are generally evaluated based on their capacity to

transfer hydrogen atoms or electrons to free radicals, thereby converting them into more stable, non-reactive species (Omar et al., 2022). In the present study, the DPPH radical scavenging capacity of all prepared films was evaluated (**Table 3**). The base film, composed of tragacanth gum and whey protein concentrate, exhibited the lowest free radical scavenging activity (21.42 ± 0.44%), which is likely attributable to the intrinsic protein structure of whey protein concentrate. BHT, used as a synthetic reference antioxidant, exhibited significantly greater radical scavenging activity (92.36 ± 1.02%) than all film formulations. Nevertheless, the incorporation of natural bioactive compounds into the polymer matrix substantially enhanced antioxidant activity relative to the base film.

Table 3. DPPH radical scavenging activity of the prepared composite films.

Treatments	DPPH radical scavenging activity (%)
BHT	92.36 ± 1.02 [a]
FEO4–CNP8	59.48 ± 0.46 [b]
FEO4–CNP0	59.05 ± 0.71 [b]
FEO3–CNP0	53.81 ± 0.55 [c]
FEO3–CNP8	51.98 ± 0.71 [c]
FEO0–CNP8	45.41 ± 0.94 [d]
FEO4–CNP5	40.72 ± 0.39 [e]
FEO3–CNP5	37.38 ± 0.36 [f]
FEO0–CNP5	36.97 ± 0.90 [f]
FEO0–CNP0 (base-polymer film)	21.42 ± 0.44 [g]

Values are presented as mean ± SD (n = 3). Different lowercase superscript letters indicate statistically significant differences among treatments based on one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). BHT was used as a synthetic reference antioxidant and excluded from the 3 × 3 factorial analysis. FEO: fennel essential oil; CNPs: chitosan nanoparticles; BHT: butylated hydroxytoluene.

The addition of FEO alone increased antioxidant capacity in a concentration-dependent manner: films containing 3% and 4% FEO demonstrated radical scavenging activities of 53.81 ± 0.55% and 59.05 ± 0.71%, respectively. The antioxidant properties of plant essential oils depend on the type and abundance of their bioactive constituents, particularly phenolic and terpenoid compounds, which contribute to free-radical scavenging and the inhibition of oxidative chain reactions through hydrogen atom or electron donation (Omar et al., 2022). These findings are consistent with those of Nabgan et al. (2025), who reported that addition of marjoram essential oil to whey protein films increased antioxidant activity by 65%.

CNP alone also enhanced antioxidant activity relative to the base film, reaching 36.97 ± 0.90% and 45.41 ± 0.94% at concentrations of 5% and 8%, respectively. The antioxidant activity of chitosan is attributed to its free amino ($-NH_2$) and hydroxyl (–OH) groups; reducing particle size to the nanoscale increases specific surface area, thereby improving accessibility of these functional groups to free radicals (Meshkini & Khoshsokhan, 2025).

Analysis of films containing both FEO and CNP revealed that the combined effect did not follow a uniform pattern across all formulations (**Table 3**). FEO4–CNP8 exhibited the highest activity among the composite films (59.48 ± 0.46%), comparable to FEO4–CNP0 (59.05 ± 0.71%), and FEO3–CNP8 (51.98 ± 0.71%) did not differ significantly from FEO3–CNP0 (53.81 ± 0.55%). In contrast, the addition of 5% CNP reduced activity in FEO3–CNP5 (37.38 ± 0.36%) and FEO4–CNP5 (40.72 ± 0.39%) compared with their respective FEO-only counterparts. These results indicate that the antioxidant effect of CNP was concentration-dependent and not consistently synergistic with FEO.

One plausible explanation for this non-uniform behavior is the restricted accessibility of FEO's active compounds to DPPH radicals in the presence of CNP and the polymer matrix. Interaction of the essential oil with CNPs and the tragacanth gum–whey protein concentrate network may limit the release of bioactive compounds during the assay period. Nabgan et al. (2025) similarly attributed a reduction in initial antioxidant activity of nanoparticle-encapsulated essential oil to the physical barrier effect of the nanocarriers. It should be noted, however, that a reduction in DPPH assay activity alone is insufficient to confirm encapsulation or controlled release within the films investigated in this study.

Incorporating essential oils into CNPs can protect bioactive compounds against thermal and photodegradation while enabling their gradual release, limiting undesirable flavor migration and sustaining antioxidant activity during storage (Hadidi et al., 2020). Collectively, these findings demonstrate the potential of combining FEO and CNP to enhance the antioxidant functionality of the base film (Moosavy et al., 2023).

Antibacterial activity of the films

The antibacterial activity of the prepared films was evaluated by measuring inhibition zone diameters against four foodborne pathogens: *E. coli*, *L. monocytogenes*, *S. aureus*, and *S. enterica* (**Table 4**). Inhibition zones differed significantly among treatments within each species ($p < 0.05$). The base-polymer film (FEO0–CNP0), Tween 80, and sterile distilled water produced no detectable inhibition zones against any of the tested organisms, consistent with previous reports of non-inhibitory behavior in biopolymer matrices lacking active components (Iamareerat et al., 2018; Ma et al., 2024).

Incorporation of FEO into the film matrix produced significant inhibitory activity against all four species ($p < 0.05$), and this effect was concentration-dependent: increasing FEO from 3% to 4% consistently increased inhibition-zone diameters across all tested organisms. This dose–response pattern aligns with previous reports of concentration-dependent antibacterial activity for plant essential oils incorporated into biopolymer films (Iamareerat et al., 2018).

Table 4. Antibacterial activity of the composite films against foodborne pathogens.

Formulations	*E. coli* (mm)	*L. monocytogenes* (mm)	*S. aureus* (mm)	*S. enterica* (mm)
FEO0–CNP0	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}
FEO3–CNP0	23.67 ± 0.60^{b}	24.83 ± 0.21^{c}	25.70 ± 0.31^{d}	22.63 ± 0.30^{c}
FEO4–CNP0	24.80 ± 0.92^{b}	26.13 ± 0.31^{b}	27.01 ± 0.24^{c}	23.70 ± 0.30^{b}
FEO0–CNP5	18.67 ± 0.15^{c}	19.07 ± 0.12^{d}	19.40 ± 0.10^{e}	18.03 ± 0.15^{d}
FEO0–CNP8	18.77 ± 0.15^{c}	19.20 ± 0.10^{d}	19.53 ± 0.06^{e}	18.13 ± 0.15^{d}
FEO3–CNP5	29.93 ± 0.67^{a}	31.18 ± 0.39^{a}	32.63 ± 0.21^{b}	27.84 ± 0.39^{a}
FEO3–CNP8	30.27 ± 0.72^{a}	31.49 ± 0.37^{a}	32.93 ± 0.21^{ab}	28.10 ± 0.40^{a}
FEO4–CNP5	30.10 ± 0.69^{a}	31.30 ± 0.36^{a}	32.83 ± 0.21^{ab}	27.97 ± 0.45^{a}
FEO4–CNP8	30.57 ± 0.72^{a}	31.73 ± 0.33^{a}	33.33 ± 0.21^{a}	28.33 ± 0.45^{a}
Tween 80	0.00 ± 0.00†	0.00 ± 0.00†	0.00 ± 0.00†	0.00 ± 0.00†
Gentamicin	31.33 ± 0.58	27.00 ± 1.00	32.33 ± 0.58	30.00 ± 0.00
Negative control (sterile distilled water)	0.00 ± 0.00†	0.00 ± 0.00†	0.00 ± 0.00†	0.00 ± 0.00†

Values are expressed as mean ± SD (n = 3) of inhibition-zone diameters. Within each column, different lowercase superscript letters indicate significant differences among the film formulations based on one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Gentamicin (positive control) is reported descriptively without superscripts. † Values marked with a dagger (Tween 80 and sterile distilled water negative control) showed no inhibition zone and were excluded from statistical analysis. FEO: fennel essential oil; CNP: chitosan nanoparticles.

The GC–MS data identifying *trans*-anethole as the dominant constituent of the FEO used in this study are relevant to the observed antibacterial activity. The lipophilic nature of anethole is consistent with its reported capacity to partition into the hydrophobic core of the phospholipid bilayer, altering membrane integrity and fluidity and inducing leakage of intracellular ions including potassium (K^+) and other cytoplasmic constituents (Tariq et al., 2019). These membrane-level

disruptions, together with interference in ATP production, may contribute to growth inhibition or cell death (Elahi et al., 2024).

Films containing CNP alone also produced measurable inhibition zones, though smaller than those of the corresponding FEO-only films. One proposed mechanism for chitosan's antibacterial action is electrostatic attraction between protonated amino groups ($-NH_3^+$) and negatively charged components of the microbial cell surface, leading to altered membrane permeability and disrupted cellular function (Hadidi et al., 2020). Reduction of chitosan to the nanoscale increases the surface-area-to-volume ratio, potentially enhancing contact with bacterial cell surfaces (Shahbazi & Moosavy, 2019). The relatively small inhibition zones produced by CNP-containing films are not necessarily indicative of limited efficacy, because the diffusion of nanoparticles through agar is physically restricted; accordingly, the inhibition-zone diameter may underestimate the activity observed under direct-contact conditions (Li et al., 2022).

Combined FEO–CNP films produced the largest inhibition zones across all four species against *E. coli*, FEO–CNP films yielded zones of 29.93–30.57 mm, compared with 23.67–24.80 mm for FEO-only and 18.67–18.77 mm for CNP-only films, and were not significantly different from gentamicin (31.33 ± 0.58 mm). Against *L. monocytogenes*, combined films produced zones of 31.18–31.73 mm, significantly exceeding both single-component films and gentamicin (27.00 ± 1.00 mm). Against *S. aureus*, combined formulations yielded zones of 32.63–33.33 mm; FEO4–CNP8 produced the largest zone (33.33 ± 0.21 mm), which was numerically greater than gentamicin (32.33 ± 0.58 mm), while the other combined formulations were statistically comparable to the positive control. Against *S. enterica*, combined films produced zones of 27.84–28.33 mm, significantly exceeding single-component films but remaining below gentamicin (30.00 ± 0.00 mm). Among the combined formulations, no statistically significant differences were observed within any species (**Table 4**).

One mechanism proposed for the enhanced activity of combined FEO–CNP films is that chitosan interacts with the outer membrane of Gram-negative bacteria, potentially facilitating access of essential oil constituents to the inner cell membrane (Khodaman et al., 2022). Whether the observed increase over single-component films constitutes true synergy requires direct comparison of combined and individual effect sizes; the present data are consistent with additive or potentiated activity but do not establish a mechanistic synergy. An important finding was the relatively greater susceptibility of Gram-positive organisms. *L. monocytogenes* and *S. aureus* consistently produced larger inhibition zones than *E. coli* and *S. enterica* at comparable concentrations, attributable at least in part to the LPS-containing outer membrane of Gram-negative bacteria, which acts as a permeability barrier to hydrophobic compounds (Moghimi et al., 2017).

The greater resistance of *S. enterica* is consistent with findings by Saberi et al. (2017), who identified *S.* Typhimurium as among the most resistant organisms against active packaging films. Khodaman et al. (2022) similarly reported higher activity against Gram-positive organisms, with inhibition zones reaching 21.66 mm for *S. aureus* versus a maximum of 12.33 mm for Gram-negative species. Activity against *E. coli* observed in the present study nonetheless confirms that FEO constituents can penetrate or disrupt the Gram-negative cell envelope under the conditions tested. It is important to note that inhibition zone diameters reflect diffusion-dependent activity; comparisons with gentamicin indicate a difference in active-agent loading and diffusion rather than equivalent potency. Collectively, these findings demonstrate that combining FEO and CNP in a tragacanth gum–whey protein concentrate matrix produces films with broad-spectrum antibacterial activity against relevant foodborne pathogens (Moosavy et al., 2023).

Conclusion

This study demonstrated that incorporating FEO and CNPs into tragacanth gum/whey protein concentrate films was associated with changes in their physicochemical, antioxidant, and antibacterial properties. GC–MS analysis showed that the FEO was dominated by trans-anethole (68.11%), fenchone (15.42%), estragole (7.21%), and eucalyptol (6.59%). FEO4–CNP8 yielded the greatest film

thickness (0.160 mm) and the lowest moisture content (31.48%), water solubility (29.99%), and swelling capacity (154.15%) among the nine formulations, compared with the base-polymer film. DPPH radical scavenging activity increased from 21.42% in the base-polymer film to 59.48% in FEO4–CNP8, and this increase was associated mainly with FEO content, while the effect of CNPs was concentration-dependent and not consistently additive. Combined FEO–CNP films produced inhibition zones against all four pathogens, with the largest zone against S. aureus (33.33 mm for FEO4–CNP8). These findings suggest that tragacanth gum/WPC films containing FEO and CNPs may be suitable for further evaluation as active packaging materials. Future research should evaluate these films under real food-contact conditions, assess their mechanical and barrier properties, and examine their safety for direct food applications, including migration of estragole and other volatile constituents.

Acknowledgments

This article is derived from the first author's PhD dissertation at Urmia University. The authors gratefully acknowledge the West Azerbaijan Agricultural and Natural Resources Research and Education Center and the Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, for their support and assistance throughout this research.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this study.

Disclaimer

During the preparation of this manuscript, the authors used an artificial intelligence-based language tool only for language editing, grammar correction, and improvement of textual clarity. The authors reviewed and verified all outputs and take full responsibility for the accuracy, originality, interpretation, and integrity of the submitted work.

References

Abdelbaky, A. S., Mohamed, A. M. H. A., Abd El-Mageed, T. A., Rady, M. M., Alshehri, F., El-Saadony, M. T., AbuQamar, S. F., El-Tarabily, K. A., & Al-Elwany, O. A. A. (2023). Bio-organic fertilizers promote yield, chemical composition, and antioxidant and antimicrobial activities of essential oil in fennel (*Foeniculum vulgare*) seeds. *Scientific Reports*, *13*(1), 1–15. https://doi.org/10.1038/s41598-023-40579-7

Abdou, A., Maaghloud, F. E., Kamal, F. Z., Rammali, S., & Ciobica, A. (2025). Chemical composition of clove and fennel seed essential oils and a comparison of their *In Silico* and *In Vitro* antibacterial activity with that of their main compounds. *Current Issues in Molecular Biology*, *47*(9), 694. https://doi.org/10.3390/cimb47090694

Ahmad, A. A., & Sarbon, N. M. (2021). A comparative study: Physical, mechanical and antibacterial properties of bio-composite gelatin films as influenced by chitosan and zinc oxide nanoparticles incorporation. *Food Bioscience*, *43*, 101250. https://doi.org/10.1016/j.fbio.2021.101250

Ahmed, J., Santhosh, R., Thakur, R., Mulla, M., & Sarkar, P. (2023). Thermo-mechanical, rheological, microstructural, and barrier properties of gum-based edible packaging: A review. *Food Packaging and Shelf Life*, *38*(101117), 101117. https://doi.org/10.1016/j.fpsl.2023.101117

Akdaşçi, E., Duman, H., Eker, F., Bechelany, M., & Karav, S. (2025). Chitosan and its nanoparticles: a multifaceted approach to antibacterial applications. *Nanomaterials*, *15*(2). 126.. https://doi.org/10.3390/nano15020126

Amani, F., Rezaei, A., Akbari, H., Dima, C., & Jafari, S. M. (2022). Active packaging films made by complex coacervation of tragacanth gum and gelatin loaded with curcumin; characterization and antioxidant activity. *Foods, 11*(20),3168. https://doi.org/10.3390/foods11203168

Anisimov, Y. A., Cree, D. E., & Wilson, L. D. (2020). Preparation of multicomponent biocomposites and characterization of their physicochemical and mechanical properties. *Journal of Composites Science*, *4*(1), 18. https://doi.org/10.3390/jcs4010018

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Heras Caballero, A., Acosta, N., Alc, R., & Concepci, M. (2021). Chitosan: An overview of its properties and applications. *Polymers*, *13*(19), 3256–3265. https://doi.org/10.3390/polym13193256

Ardebilchi Marand, S., Almasi, H., Amjadi, S., Ghadiri Alamdari, N., & Salmasi, S. (2023). Ixiolirion tataricum mucilage/chitosan based antioxidant films activated by free and nanoliposomal fennel essential oil. *International Journal of Biological Macromolecules, 230*, 123119. https://doi.org/10.1016/j.ijbiomac.2022.123119

Avalos, J. M. M.-, Altamura, D., Herrera, M. L., Huck-Iriart, C., Scattarella, F., Siliqi, D., Giannini, C., & Candal, R. J. (2020). Physical and structural properties of whey protein concentrate - Corn oil - TiO2 nanocomposite films for edible food-packaging. *Food Packaging and Shelf Life*, *26*, 1–12. https://doi.org/10.1016/j.fpsl.2020.100590

Azizi-Lalabadi, M., Rahimzade-sani, Z., Feng, J., Hosseini, H., & Jafari, S. M. (2021). The impact of essential oils on the qualitative properties, release profile, and stimuli- responsiveness of active food packaging nanocomposites. *Critical Reviews in Food Science and Nutrition,*

Bacenetti, J., Bava, L., Schievano, A., & Zucali, M. (2018). Whey protein concentrate (WPC) production: Environmental impact assessment. *Journal of Food Engineering, 224,* 139–147.https://doi.org/10.1016/j.jfoodeng.2017.12.018

Barrahi, M., Esmail, A., Elhartiti, H., Chahboun, N., Benali, A., Amiyare, R., Lakhrissi, B., Rhaiem, N., Zarrouk, A., & Ouhssine, M. (2020). Chemical composition and evaluation of antibacterial activity of fennel (*Foeniculum vulgare* mill) seed essential oil against some pathogenic bacterial strains. *Caspian Journal of Environmental Sciences*, *18*(4), 295–307. https://doi.org/10.22124/cjes.2020.4276

Dehankar, H. B., Mali, P. S., & Kumar, P. (2023). Edible composite films based on chitosan/guar gum with ZnO NPs and roselle calyx extract for active food packaging. *Applied Food Research*, *3*(1), 100276. https://doi.org/10.1016/j.afres.2023.100276

Eker, F., Duman, H., Akdaşçi, E., Bolat, E., Sarıtaş, S., Karav, S., Witkowska, A. M., Akdasçi, E., Witkowska, A. M., Bolat, E., Sarıta¸s, S., & Karav, S. (2024). A comprehensive review of nanoparticles: from classification to application and toxicity. *Molecules*, *29*(15), 3482. https://doi.org/10.3390/molecules29153482

El-Araby, A., Janati, W., Ullah, R., Ercisli, S., & Errachidi, F. (2023). Chitosan, chitosan derivatives, and chitosan-based nanocomposites: eco-friendly materials for advanced applications (a review). *Frontiers in Chemistry*, *11* 1327426. https://doi.org/10.3389/fchem.2023.1327426

Elahi, R., Jamshidi, A., & Fallah, A. A. (2024). Effect of active composite coating based on nanochitosan-whey protein isolate on the microbial safety of chilled rainbow trout fillets packed with oxygen absorber. *International Journal of Biological Macromolecules*, *277*, 133756. https://doi.org/10.1016/j.ijbiomac.2024.133756

Emam-Djomeh, Z., Fathi, M., & Askari, G. (2019). Gum tragacanth (*Astragalus gummifer* Labillardiere). In S. M. A. Razavi (Ed.), *Emerging Natural Hydrocolloids: Rheology and Function* (pp. 299–326). USA: John Wiley & Sons. https://doi.org/10.1002/9781119418511.ch12

Fakraoui, O., Atanase, L. I., Salhi, S., Arous, M., & Ayadi, Z. (2024). Investigation of lemon peel extract as a natural additive in polyvinyl alcohol / chitosan blend for advanced bioactive food packaging. *Journal of Polymer Science*, *63*(24), 5328-5341.. https://doi.org/10.1002/pol.20240268

Featherstone, S. (2015). Ingredients used in the preparation of canned foods. In S. Featherstone (Ed.), *A Complete Course in Canning and Related Processes* (pp. 147–211). Cambridge, UK: Woodhead Publishing. https://doi.org/10.1016/b978-0-85709-678-4.00008-7

Gao, Q., Feng, Z., Wang, J., Zhao, F., & Li, C. (2024). Application of nano-ZnO in the food preservation industry: antibacterial mechanisms, influencing factors, intelligent packaging, preservation film and safety. *Critical Reviews in Food Science and Nutrition*, *65*(22), 4327-4353. https://doi.org/10.1080/10408398.2024.2387327

Hadidi, M., Pouramin, S., Adinepour, F., Haghani, S., & Jafari, S. M. (2020). Chitosan nanoparticles loaded with clove essential oil: Characterization, antioxidant and antibacterial activities. *Carbohydrate Polymers*, *236*, 116075. https://doi.org/10.1016/j.carbpol.2020.116075

Iamareerat, B., Singh, M. S., Sadiq, M. B., & Anal, A. K. (2018). Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material. *Journal of Food Science and Technology*. *55*(5), 1953-1959.https://doi.org/doi.org/10.1007/s13197-018-3100-7

Janani, N., Zare, E. N., Salimi, F., & Makvandi, P. (2020). Antibacterial tragacanth gum-based nanocomposite films carrying ascorbic acid antioxidant for bioactive food packaging. *Carbohydrate Polymers*, *247*, 116678. https://doi.org/10.1016/j.carbpol.2020.116678

Javid, A. L., Almasi, H., Molaei, R., & Moradi, M. (2024). Basil essential oil-incorporated bacterial nanocellulose foam: Fabrication, characterization, and application in extending ground beef shelf life. *Journal of Agriculture and Food Research*, *18*, 101453. https://doi.org/10.1016/j.jafr.2024.101453

Karamali, F., Asnaashari, M., & Farahmandfar, R. (2023). Biodegradable packaging based on chitosan-potato starch biopolymer containing *Apium graveolens* seed extract for chicken fillets. *Journal of Food Science and Technology (Iran)*, *20*(137), 111–128. https://doi.org/10.22034/FSCT.20.137.111

Kazemi, M., Aliakbarlu, J., & Lin, L. (2026). Development of bilayer film of Persian acorn starch and zein electrospun nanofibers loaded with cinnamon essential oil for shelf - life extension of rainbow trout fillet. *Food Production, Processing and Nutrition*. *(2026)* 8:12. https://doi.org/10.1186/s43014-025-00361-w

Khan, N., Sudhakar, K., & Mamat, R. (2025). Biodegradable plastics from marine biomass: A solution to marine plastic pollution. *Journal of Hazardous Materials Advances*, *17*, 100559. https://doi.org/10.1016/j.hazadv.2024.100559

Khodaman, E., Barzegar, H., Jokar, A., & Jooyandeh, H. (2022). Production and evaluation of physicochemical, mechanical and antimicrobial properties of chia (*Salvia hispanica* L.) mucilage-gelatin based edible films incorporated with chitosan nanoparticles. *Journal of Food Measurement and Characterization*, *16*(5), 3547–3556. https://doi.org/10.1007/s11694-022-01470-7

Koljančić, N., Vyviurska, O., & Špánik, I. (2023). Aroma compounds in essential oils: Analyzing chemical composition using two-dimensional gas chromatography–high resolution time-of-flight mass spectrometry combined with chemometrics. *Plants, 12*(12), 2362. https://doi.org/10.3390/plants12122362

Kontogianni, V. G., Kasapidou, E., Mitlianga, P., Mataragas, M., Pappa, E., Kondyli, E., & Bosnea, L. (2022). Production, characteristics and application of whey protein films activated with rosemary and sage extract in preserving soft cheese. *LWT-*

Food Science and Technology, *155*, 112996. https://doi.org/10.1016/j.lwt.2021.112996

Li, Z., Jiang, X., Huang, H., Liu, A., Liu, H., Abid, N., & ming, lianshan. (2022). Chitosan/zein films incorporated with essential oil nanoparticles and nanoemulsions: Similarities and differences. *International Journal of Biological Macromolecules*, *208*, 983–994. https://doi.org/10.1016/j.ijbiomac.2022.03.149

Liu, T. T., Wang, J. J., Chi, F., Tan, Z., & liu, L. (2020). Development and characterization of novel active chitosan films containing fennel and peppermint essential oils. *Coatings*, *10*(10), 936. https://doi.org/10.3390/coatings10100936

Lorevice, M. V., Otoni, C. G., de Moura, M. R., Mattoso, L. H. C., Mattoso, H. C., Lorevice, M. V., Otoni, C. G., de Moura, M. R., Mattoso, L. H. C., Moura, M. R. de, & Mattoso, L. H. C. (2016). Chitosan nanoparticles on the improvement of thermal, barrier, and mechanical properties of high- and low-methyl pectin films. *Food Hydrocolloids*, *52*, 732–740. https://doi.org/10.1016/j.foodhyd.2015.08.003

Ma, Y., Cao, Y., Zhang, L., & Yu, Q. (2024). Preservation of chilled beef using active films based on bacterial cellulose and polyvinyl alcohol with the incorporation of Perilla essential oil Pickering emulsion. *International Journal of Biological Macromolecules*, *271*(P1), 132118. https://doi.org/10.1016/j.ijbiomac.2024.132118

Mandal, D., Sarkar, T., & Chakraborty, R. (2023). Critical review on nutritional, bioactive, and medicinal potential of spices and herbs and their application in food fortification and nanotechnology. *Applied Biochemistry and Biotechnology*, *195*(2), 1319-1513.https://doi.org/10.1007/s12010-022-04132-y

Marčac, N., Balbino, S., Tonković, P., Medved, A. M., Cegledi, E., Dragović, S., Dragović-Uzelac, V., & Repajić, M. (2023). Hydrodistillation and steam distillation of fennel seeds essential oil: Parameter optimization and application of cryomilling pretreatment. Processes, *11*(8), 2354. https://doi.org/10.3390/pr11082354

Meshkini, A., & Khoshsokhan, F. (2025). Transformative nanoantioxidant chitosan films: High-performance active packaging with minimal cytotoxicity. *International Journal of Biological Macromolecules, 304*, 140983. https://doi.org/10.1016/j.ijbiomac.2025.140983

Mirpoor, S. F., Massironi, A., Winning, D., Lignou, S., Ghawi, S. K., Trotta, F., & Charalampopoulos, D. (2025). Agar/carboxymethyl cellulose blended films with green-synthesised silver nanoparticles as a sustainable alternative for food packaging applications. *Polymers*, 17(23), 3126. https://doi.org/10.3390/polym17233126

Moghimi, R., Aliahmadi, A., & Rafati, H. (2017). Antibacterial hydroxypropyl methyl cellulose edible films containing nanoemulsions of *Thymus daenensis* essential oil for food packaging. *Carbohydrate Polymers*, *175*, 241–248. https://doi.org/10.1016/j.carbpol.2017.07.086 Mohamed, A., & Ramaswamy, H. S. (2022). Characterization of caseinate–carboxymethyl chitosan-based edible films formulated with and without transglutaminase enzyme. *Journal of Composites Science, 6*(7), 216. https://doi.org/10.3390/jcs6070216

Moosavy, M. H., de la Guardia, M., Mokhtarzadeh, A., Khatibi, S. A., Hosseinzadeh, N., & Hajipour, N. (2023). Green synthesis, characterization, and biological evaluation of gold and silver nanoparticles using *Mentha spicata* essential oil. *Scientific Reports, 13*(1), 7230. https://doi.org/10.1038/s41598-023-33632-y

Muñoz-Tebar, N., Pérez-Álvarez, J. A., Fernández-López, J., & Viuda-Martos, M. (2023). Chitosan edible films and coatings with added bioactive compounds: Antibacterial and antioxidant properties and their application to food products: A review. *Polymers*, *15*(2), 396. https://doi.org/10.3390/polym15020396

Nabgan, M., Shariatifar, N., Jahed-Khaniki, G., Molaee-Aghaee, E., & Sani, M. A. (2025). Development and characterization of whey protein packaging film loaded with Pickering emulsion stabilized marjoram (*Origanum majorana* L.) essential oil. *International Journal of Biological Macromolecules, 334*, 149178. https://doi.org/10.1016/j.ijbiomac.2025.149178

Noreen, S., Tufail, T., Badar Ul Ain, H., & Awuchi, C. G. (2023). Pharmacological, nutraceutical, functional and therapeutic properties of fennel (*Foeniculum vulgare*). *International Journal of Food Properties*, *26*(1), 915–927. https://doi.org/10.1080/10942912.2023.2192436.

Omar, Z. A., Abduljabar, R. S., Sajadi, S. M., Mahmud, S. A., & Yahya, R. O. (2022). Recent progress in eco-synthesis of essential oil-based nanoparticles and their possible mechanisms. *Industrial Crops and Products*, *187*, 115322. https://doi.org/10.1016/j.indcrop.2022.115322

Omerović, N., Djisalov, M., Živojević, K., Mladenović, M., Vunduk, J., Milenković, I., Knežević, N. Ž., Gadjanski, I., & Vidić, J. (2021). Antimicrobial nanoparticles and biodegradable polymer composites for active food packaging applications. *Comprehensive Reviews in Food Science and Food Safety*, 20(3), 2428–2454. https://doi.org/10.1111/1541-4337.12727

Otto, S., Strenger, M., Maier-Nöth, A., & Schmid, M. (2021). Food packaging and sustainability – Consumer perception vs. correlated scientific facts: A review. *Journal of Cleaner Production*, *298,* 126733. https://doi.org/10.1016/j.jclepro.2021.126733

Rather, M. A., Dar, B. A., Sofi, S. N., Bhat, B. A., & Qurishi, M. A. (2016). Foeniculum vulgare: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arabian Journal of Chemistry*, *9*, S1574–S1583. https://doi.org/10.1016/j.arabjc.2012.04.011

Saberi, B., Chockchaisawasdee, S., Golding, J. B., Scarlett, C. J., & Stathopoulos, C. E. (2017). Characterization of pea starch-guar gum biocomposite edible films enriched by natural antimicrobial agents for active food packaging. *Food and Bioproducts Processing*. *105*, 51-63. https://doi.org/10.1016/j.fbp.2017.06.003

Sarangi, P. K., Pal, P., Singh, A. K., & Sahoo, U. K. (2024). Food Waste to Food Security: Transition from Bioresources to Sustainability. *Resources*, *13*(12), 164. https://doi.org/10.3390/resources13120164

Shahbazi, Y., & Moosavy, M.-H. (2019). Quince seed mucilage supplemented with titanium dioxide and Silicon dioxide nanoparticles. *Nanomedicine Research Journal*, *4*(3), 157–163. https://doi.org/10.22034/nmrj.2019.03.004

Shirdel Marandi, M., Pirsa, S., Amiri, S., & Fazeli, M. (2022). Production of biodegradable films of zein containing *Mentha asiatica* essential oil and copper oxide nanoparticles: Investigation of physicochemical, antimicrobial, and antioxidant properties. *Journal of Polymers and the Environment, 30*(10), 4114–4129. https://doi.org/10.1007/s10924-022-02486-2

Šunić, L., Ilić, Z. S., Stanojević, L., Milenković, L., Stanojević, J., Kovač, R., Milenković, A., & Cvetković, D. (2023). Comparison of the essential oil content, constituents and antioxidant activity from different plant parts during development stages of wild fennel (*Foeniculum vulgare* Mill.). *Horticulturae*, *9*(3),364. https://doi.org/10.3390/horticulturae9030364

Tariq, S., Wani, S., Rasool, W., Shafi, K., Ahmad Bhat, M., Prabhakar, A., Shalla, A. H., & Rather, M. A. (2019). A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug- resistant microbial pathogens. *Microbial Pathogenesis*, *134*, 103580. https://doi.org/10.1016/j.micpath.2019.103580

Teymoorian, M., Moghimi, R., Hosseinzadeh, R., Zandi, F., & Lakouraj, M. M. (2024). Fabrication the emulsion-based edible film containing *Dracocephalum kotschyi* Boiss essential oil using chitosan–gelatin composite for grape preservation. *Carbohydrate Polymer Technologies and Applications*, *7*, 100444. https://doi.org/10.1016/j.carpta.2024.100444

Veryer, K., & Bozok, F. (2023). Chemical constituents of *Dysphania botrys* (L.) Mosyakin & Clemants essential oil: herbicidal and antimicrobial activities. *Journal of Essential Oil Bearing Plants*, *26*(2), 411–419. https://doi.org/10.1080/0972060X.2023.2189531.

Yu, K., Yang, L., & Zhang, S. (2025). Multifunctional sodium alginate/nanocellulose fibers/chitosan edible coating: A sustainable strategy for prolonged fruit preservation and reduced cold-chain dependency. *Food Chemistry*,*50*, 147535. https://doi.org/10.1016/j.foodchem.2025.147535

Zhang, L., Liu, A., Wang, W., Ye, R., Liu, Y., Xiao, J., & Wang, K. (2017). Characterisation of microemulsion nanofilms based on Tilapia fish skin gelatine and ZnO nanoparticles incorporated with ginger essential oil: meat packaging application. *International Journal of Food Science and Technology, 52(7), 1670-1679*. https://doi.org/10.1111/ijfs.13441