



## Scientific Research

# Production and investigation of properties of biodegradable antioxidant film based on zucchini flour containing chitosan nanoparticles loaded with Fennel essential oil

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ARTICLE INFO	ABSTRACT
<p><b>Article History:</b></p> <p>Received: 2025/1/9</p> <p>Accepted: 2025/2/16</p> <hr/> <p><b>Keywords:</b></p> <p>Courgette flour, Antioxidant film, Foeniculum vulgare essential oil, mechanical properties, morphology</p> <hr/> <p><b>DOI:</b> 10.22034/FSCT.22.165.196.</p> <p>*Corresponding Author E- mohtarami.f@gmail.com; f.mohtarami@urmia.ac.ir</p>	<p>The objective of this research was to produce biodegradable films based on zucchini flour and to investigate the effect of adding chitosan nanoparticles loaded with fennel essential oil (0, 3, 6, and 9% w/w) on the physicochemical and mechanical properties of the films. The results showed that adding chitosan nanoparticles significantly increased the tensile strength and elongation at the break of the films, indicating that the nanoparticles can act as a reinforcing agent and improve the film structure. Increasing the percentage of nanoparticles increased the water solubility and decreased the water contact angle of the films. The antioxidant activity of the films increased significantly with increasing nanoparticle concentration, which may be due to the presence of fennel essential oil in the nanoparticles and its antioxidant properties. The FTIR test results showed that the chitosan nanoparticles were well chemically bonded to the polymeric matrix of the zucchini flour film. The SEM images also showed that adding nanoparticles increased the surface roughness of the films and the uniformity of the film surface decreased with increasing chitosan nanoparticles. Based on the results of this study, the film containing 6% chitosan nanoparticles loaded with fennel essential oil showed the best mechanical and physical properties. In general, an edible film of zucchini flour activated with fennel essential oil and chitosan nanoparticles can be introduced as an active edible film with desirable properties for packaging food products sensitive to oxidative spoilage.</p>

## 1.Introduction

The widespread adoption of plastics in food packaging has increased globally owing to their advantageous properties, including low weight, cost efficiency, and favorable mechanical strength. Nevertheless, the extensive dependence on petroleum-based plastics presents considerable environmental challenges, such as persistent pollution and unsustainable resource consumption. To address these concerns, biodegradable plastics have been developed as a sustainable alternative, offering reduced ecological impact and enhanced biocompatibility. Recent progress in material science has facilitated the fabrication of biodegradable films from renewable resources, providing a promising pathway toward environmentally responsible packaging solutions. Edible films are primarily composed of three key elements: a base material (or blend of materials), a solvent, and plasticizers. The base materials commonly consist of biopolymers categorized into polysaccharides (e.g., starch, pectin, and various gums), lipids (e.g., oils, fats, waxes, and essential oils), and proteins (e.g., casein, whey, wheat gluten, and soy protein). These components can be utilized individually or in composite formulations to tailor the functional properties of the films [2]. Recent research has increasingly focused on the development and characterization of novel raw materials for film preparation, with particular emphasis on evaluating the film-forming potential of flours derived from diverse agricultural sources [3]. Flours derived from agricultural products are complex natural compositions of polysaccharides, proteins, and lipids, and in recent years, there has been a growing interest in utilizing flour as a film matrix [4]. Various studies investigated the production of the films from different sources of flour, such as pea, plantain, beet, bocaiuva, brazilian pine seed, chickpea, etc. [5-8]. Zucchini (*Cucurbita pepo* L.) is a popular and seasonal vegetable with high nutritional value, cultivated worldwide. The beneficial properties of this plant are attributed to the presence of bioactive

compounds, including lutein, beta-carotene, and folic acid. Zucchini is a nutritionally significant vegetable, contributing essential macro- and micronutrients to a balanced diet. It is a rich source of dietary fiber, carbohydrates, and plant-based proteins, along with key vitamins, including ascorbic acid (vitamin C), phyloquinone (vitamin K), and pyridoxine (vitamin B6). Additionally, zucchini provides essential minerals such as potassium and manganese, as well as folate, a critical B-vitamin involved in metabolic processes. Its high nutrient density and bioactive compound content underscore its potential as a functional food component [9]. Various studies have investigated the utilization of zucchini for food enrichment. For instance, Różyło et al. [10] examined zucchini-enriched wheat bread in their research and reported that zucchini significantly improved the properties of dietary bread. Additionally, Zhang et al. [11] explored the effect of zucchini polysaccharide on pasting properties, rheology, structural characteristics, and in vitro digestibility of potato starch, concluding that starch-based functional foods could be developed by incorporating zucchini polysaccharide with potato starch. To enhance the functional properties of these films, the incorporation of natural bioactive compounds, such as polyphenols, essential oils, or antimicrobial peptides, has been widely investigated. Fennel (*Foeniculum vulgare*) is a perennial plant with extensive applications in the pharmaceutical, food, and cosmetic industries. Numerous studies have validated the broad pharmacological potential of fennel (*Foeniculum vulgare*) essential oil, demonstrating hepatoprotective, antioxidant, anti-inflammatory, antidiabetic, antineoplastic, antifungal, and antibacterial properties. Chromatographic analyses have identified twenty-two bioactive compounds in fennel essential oil, with compositional variations observed across different phenological stages. The predominant constituents, which contribute significantly to its bioactivity, include trans-anethole, limonene, fenchone, estragole, and  $\alpha$ -pinene [12, 13]. Encapsulation

has emerged as an efficient and economically viable strategy for enhancing the stability of essential oils by mitigating oxidative degradation, photolysis, thermal decomposition, and moisture-induced deterioration. This technique not only preserves the volatile constituents but also enables controlled release kinetics, thereby optimizing their functional performance [14]. Various techniques have been explored for the nanoencapsulation of bioactive materials. Nanomaterials refer to substances with particles ranging in size from 1 to 100 nanometers. Encapsulated nanoparticles containing essential oils are typically prepared using various biopolymers, with chitosan often employed as a primary material in nanoparticle formation due to its properties such as safety, biocompatibility, and biodegradability [15]. Chitosan (CH) is a cationic polysaccharide that has widespread applications in nanotechnology due to its controllable and easy extraction, biocompatibility, biodegradability, non-toxicity, antifungal properties, facile chemical modification, and ability to form gels, films, and more [16]. Chitosan nanoparticles are synthesized through ionic gelation, a process driven by electrostatic interactions between protonated amino groups ( $-\text{NH}_3^+$ ) on the chitosan backbone and multivalent anionic groups ( $-\text{PO}_4^{3-}$ ) of sodium tripolyphosphate (TPP) under acidic conditions [17]. Chitosan nanoparticles have been widely utilized for the encapsulation of various bioactive compounds, such as chavire essential oil [18], clove [19], cinnamon [20], and mandarin [21].

To the best of our knowledge, no prior studies have investigated the development of edible films derived from zucchini flour. Furthermore, chitosan nanoparticles have not been employed for the encapsulation of fennel essential oil. The present study aims to (1) evaluate the technical feasibility of developing edible films utilizing zucchini flour as a biopolymer matrix, and (2) characterize the influence of chitosan nanoparticle-encapsulated fennel (*Foeniculum vulgare*) essential oil incorporation on the resultant films' physicochemical properties,

structural organization, and morphological features.

## 2. Materials and methods

### 2.1. Materials

Zucchini (*Cucurbita pepo*) used in this study were procured from the local market at Urmia, West Azerbaijan Province, Iran. Low molecular weight chitosan (MW = 50–90 kDa, deacetylation degree = 75–85%) and sodium tripolyphosphate (STPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DPPH (2,2-diphenylpicrylhydrazyl), glacial acetic acid, Tween 80, and glycerol were supplied from Merck (Darmstadt, Germany).

### Preparation of Zucchini flour

Zucchini flour (ZF) was prepared according to the method described by Kręcis et al. (2021) with slight modifications. The zucchinis were washed and cut into disk-shaped slices with the same thickness without peeling and were dried in a hot air oven at 65°C for 6 hours. The dried Zucchini were ground into powder form with a blender and sieved using a 60 mesh (250 µm) sieve. The resulting flour was packed in polyethylene bags and stored at 4 °C.

### Chemical analyses of zucchini flour

The moisture, ash, crude protein, fat, and crude fiber contents of the ZF were determined according to AOAC 2000 methods, and the carbohydrate content was calculated by subtracting the sum of the moisture, ash, protein, and fat content from 100.

### Preparation of FEO-loaded NPs

FEO-loaded-NPs (FEO-NPs) were prepared by an emulsification ion-gelation technique according to the method described by Hosseini et al. (2013) with slight modifications. CH (100 mg) was added to 10 mL acetic acid (1% v/v) and stirred at room temperature overnight until fully dissolved. The solution was centrifuged for 30 min at 9000 rpm; the supernatant was removed and the pH was adjusted to 5 using 10 M NaOH. Tween 80 was then added to the solution as a surfactant to achieve a CH:Tween

ratio of 1:1.10 (w/w) and stirred at 25 °C for 30 min to form a homogeneous emulsion. Preliminary experiments were conducted to find the optimal ratio of chitosan and EO, and finally, based on the highest encapsulation efficiency (96%) and the smallest particle size (115 nm), the ratio of chitosan to EO 1:1 (w/w) was selected. FEO was added to 1 mL of ethanol, and then this oil phase was gradually dropped into the CH emulsion by stirring at 12000 rpm for 30 min in an ice bath to prepare an O/W emulsion. Then, a 0.4% (w/v) STPP solution (STPP: emulsion ratio of 1:1 (v/v)) was added dropwise into the emulsion and stirred at 900 rpm for 40 min. The obtained solution was centrifuged at 10,000 rpm for 30 min, and deionized water was used to wash the collected wet pellets. Then, they were sonicated in an ice bath for 4 min. The obtained wet FEO-NPs were dispersed in 15 mL distilled water and kept at 4 °C for further analysis.

### Preparation of films

Film preparation was performed according to the method of Yazicioglu [25] with slight modifications. Briefly, 4 g of ZF was dispersed in 150 mL distilled water, heated to 85 °C under continuous stirring at 200 rpm for 30 min, and then cooled to 40 °C. Then the pH of the solution was adjusted to 10 with NaOH (0.1 N). Glycerol 36% (w/w, based on the content of dry materials) was added as a plasticizer, and the solution was kept under magnetic stirring for 30 minutes. The prepared FEO-NPs were first dispersed into 50 mL of distilled water and sonicated for 10 min. Then, the homogenous suspension of fresh nanoparticles was added to the FFS and stirred at 500 for 40 min. FEO-NPs were used at concentrations of 0 (blank), 3, 6, and 9% (w/w, biopolymer dry basis). The resulting FFS was subjected to an ultrasonic bath to remove air bubbles for 5 min. 30 mL of the film-forming solution was cast on polystyrene plates (10 cm in diameter) and dried at 35 °C for 24 h. The dried films were peeled off the plates and stored inside zip-lock plastic bags at 4 °C until the next characterization. The films containing 0, 3, 6, and 9% FEO-NPs were named blank, F3, F6, and F9, respectively.

## Characterization of FEO-NPs

### Particle size

The particle size, polydispersity index (PDI), and zeta potential of the FEO-NPs suspensions were measured by dynamic light scattering (DLS) technique using a Zetasizer Nano ZS device (model ZEN3600, Malvern Instruments, Worcestershire, UK). Prior to analysis, samples underwent a tenfold dilution with distilled water.

### Encapsulation efficiency

The encapsulation efficiency (EE) of FEO-NPs was determined by UV–Vis spectrophotometry (Shetta et al., 2019). Briefly, 0.5 mL nanoparticle suspension was mixed with 3.5 mL ethanol (50% v/v) and kept constant for 20 min. The mixture was centrifuged at 9000 rpm for 5 min at 25 °C. The obtained supernatant was analyzed for EO content with a UV/Vis spectrophotometer at 254 nm (the maximum absorption wavelength for FEO). A standard curve was used to calculate the amount of loaded FEO. EE was calculated by the following Eq:

$$EE (\%) = \frac{((\text{Total FEO} - \text{Free FEO}) / \text{Initial amount of FEO}) \times 100}{100} \quad (1)$$

### Morphology of the FEO-NPs

The morphology of the FEO-NPs was observed with field emission scanning electron microscopy (FE-SEM, MIRA III, TESCAN, Czech). About 1 mg of freeze-dried FEO-NPs was suspended in 20 mL of distilled water and sonicated for 4 min. One drop of this dispersion was spread on a glass substrate, air-dried, mounted on an aluminum column, and then coated with gold and observation was carried out with FESEM.

### Characterization of the films

#### Water solubility (WS)

To determine the water solubility, the films were cut into 2×2 cm dimensions and dried for 24 h at 105°C. The dried films were weighed ( $W_1$ ) and kept in 10 mL of distilled water for 24 h at room temperature. Then, the films were

passed through filter paper and dried at 105°C to reach constant weight (Ebrahimi et al., 2016). The final weight of the films was measured again ( $W_2$ ). Finally, the solubility of edible film was calculated using the following equation:

$$WS (\%) = ((W_1 - W_2) / W_1) \times 100 \quad (2)$$

### Water contact angle (WCA)

To determine the water contact angle, a drop of distilled water (5  $\mu$ L) was placed on the surface of the films and a photo was taken of the shape of the drop using a digital camera (Microsoft, 185 LifeCam, H5D-00013, zoom 24 $\times$ ). The angle of the droplets placed on the films was determined using Image J 1.48 software (Marand et al., 2021).

### Mechanical properties

The mechanical properties of the films, such as tensile strength (TS) (MPa) and percent elongation at break (EAB%) were analyzed by TA.TXT plus Texture Analyzer (Yuan et al., 2021). Three samples of the films were cut into dumbbell shapes (1 $\times$ 8 cm) and placed between the two grips with an initial distance of 40 mm with a test speed of 0.5 mm/s. The tensile strength (TS) and elongation at break (EAB) were calculated using the following Eqs.

$$TS = F_{\max} / A$$

$$EAB = (\Delta L / L) \times 100$$

Where A is the cross-section area ( $m^2$ ),  $F_{\max}$  represents the maximum tensile force (N) at the breaking point,  $\Delta L$  is the deformation or elongation ( $L_{\max} - L$ ), and L is the initial length of the film (m).

### Scanning electron microscopy (SEM)

A scanning electron microscope (MIRA3, TESCAN, Brno, Czech Republic) was used to examine the surface morphology of films. The samples were covered with a thin conductive layer of gold, and the observations were made at an accelerating voltage of 20 kV.

### Fourier-transformed infrared spectroscopy (FTIR)

FTIR analysis was used to investigate structural interactions in films. To this end, an FTIR spectrometer (Bruker-Tensor 27, Bremen, Germany) was used, and the spectra were recorded in the scanning range of 4000–400  $cm^{-1}$ .

### DPPH antioxidant activity

The antioxidant activity of the films was evaluated using the DPPH free radical inhibitory method described by Li et al. (2022). Briefly, 0.02 g of each film was dissolved in 4 mL of distilled water and shaken for 5 minutes. Then, 2 mL of the supernatant solution was mixed with 1 mL of 0.1 mM DPPH ethanol solution. After 30 minutes of storage in the dark at room temperature, the absorbance of the solution was read by a spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the films was measured using the following formula:

$$DPPH (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (5)$$

where  $A_c$  is the absorption of the control and  $A_s$  is the absorption of the film sample.

### Statistical analysis

Statistical analysis was performed using SPSS software in a completely randomized design. Duncan's multiple range test was used to determine significant differences between means at a 5% level of significance.

## 3. Results and discussion

### Chemical composition of Zucchini flour

Zucchini flour (ZF) contained 10.52% moisture, 7.58% ash, 52.03% carbohydrates, 11.7% crude fiber, 2.80% fat, and 26.65% protein. In comparison to plantain, pea, pumpkin, and beet flour, the carbohydrate content of ZF was relatively low. However, ZF exhibited the highest protein content among kiwi, pumpkin, bocaiuva, achira, plantain, beet, and pea flours [5, 7, 31–34]. The film-forming

properties of zucchini flour can be attributed to its protein and polysaccharide carbohydrate content, particularly starch. The high concentration of these two chemical components suggests that zucchini flour possesses the potential to form desirable edible films.

### Characterization of FEO-NPs

#### Particle size and zeta potential (DLS)

The particle size, dispersion index (PDI), and zeta potential of chitosan nanoparticles loaded with FEO are presented in Fig.1. The chitosan nanoparticles exhibited an average particle size of 260.8 nm, with a zeta potential of 52.3 mV. The nanoparticle size, which was below 500 nm, demonstrates the efficacy of the nanoparticle synthesis method employed in this study. Generally, high zeta potential values

(positive or negative) indicate a stronger surface electric charge, which creates electrostatic repulsive forces on the surface of the nanoparticles and causes their physical stability. Zeta potential values greater than +30 mV indicate high physical stability, values around +20 mV suggest short-term stability, and values below +5 mV indicate low stability and a tendency for rapid aggregation [18]. Chitosan has a net positive charge due to the presence of amine groups, and a high zeta potential indicates the desired purity of the produced nanoparticles. The PDI of chitosan nanoparticles was determined to be 0.28. A PDI value between 0.1 and 0.25 indicates a close size distribution, while a PDI value greater than 0.5 indicates a non-uniform particle size distribution [35].

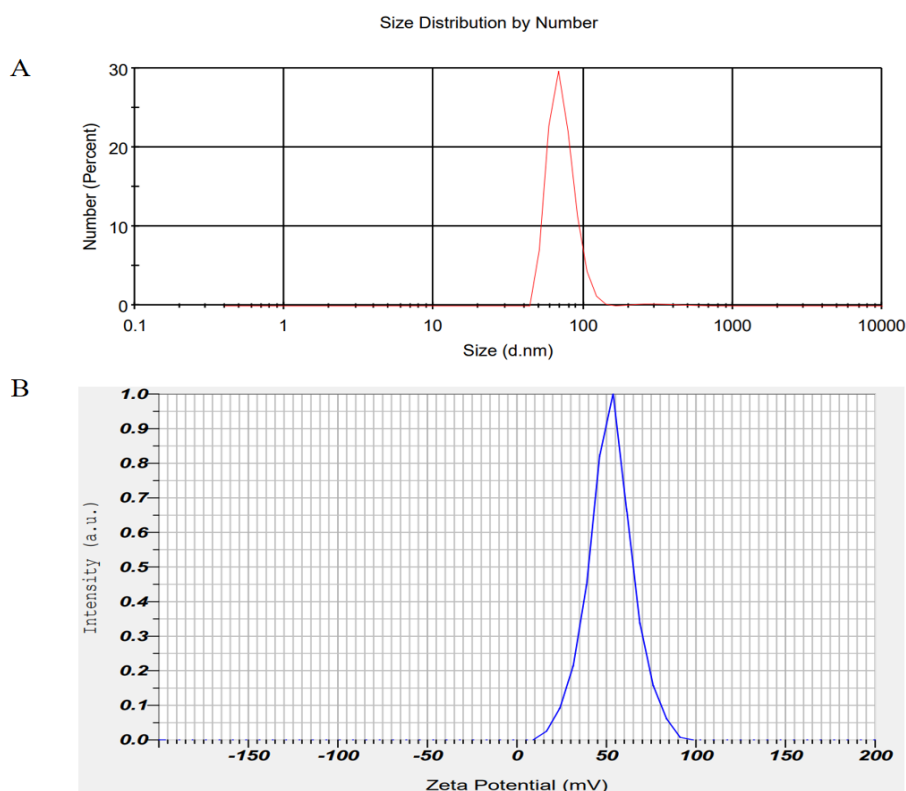


Fig. 1. (A) size distribution, (B)  $\zeta$ -potential of FEO-NPs.

#### Encapsulation Efficiency

Encapsulation efficiency (EE) refers to the proportion of the actual content of essential oil successfully incorporated into the

nanoparticles. In this study, the encapsulation efficiency of chitosan nanoparticles loaded with fennel essential oil was determined to be 96%, demonstrating the effective encapsulation of

the essential oil within the chitosan nanoparticles. Similar results have been reported in previous studies, such as the encapsulation efficiency of gallic acid in chitosan nanoparticles, which was found to be 89.63% [36]. Similarly, another study reported an encapsulation efficiency of 51.9% for grape extract loaded into chitosan nanoparticles. The molecular weight of the encapsulated compound plays a significant role in determining the encapsulation efficiency, as higher molecular weight compounds typically exhibit reduced mobility, resulting in lower encapsulation efficiency [37].

### The morphology of the FEO-NPs

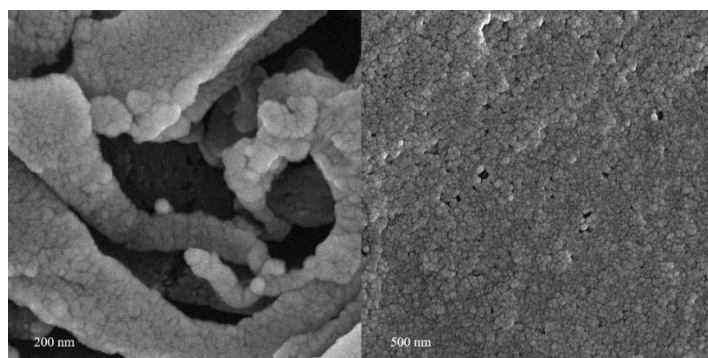


Fig. 2. SEM images of FEO-NP powders

### Characterization of the films

#### Water solubility (WS)

Water solubility is a critical parameter evaluated in the development of edible films. As shown in Table 1, the incorporation of nanoparticles reduced the water solubility of the films from 11% in the control sample to 9.5% in the film containing 3% nanoparticles. This reduction in water solubility can be attributed to the formation of hydrogen bonds between the film matrix and the chitosan nanoparticles, which enhances the structural integrity of the films. However, a further increase in nanoparticle concentration at 6 and 9% levels increased the solubility of the films. This phenomenon may be explained by the aggregation of nanoparticles at higher concentrations, as well as the inherent hydrophilic nature of chitosan, which promotes

water absorption through its surface hydrophilic groups. Similar findings were reported by de Moura et al [40], who observed a decrease in water solubility with the addition of chitosan nanoparticles in methylcellulose films. Also, Lee et al [41] reported that chitosan-p-coumaric acid nanoparticles slightly improved the solubility of polyvinyl alcohol/starch composite films due to their compact structure, which could prevent water diffusion. Additionally, Ediyilyam et al [42] demonstrated that the incorporation of silver nanoparticles into chitosan/gelatin composite films led to a slight increase in film solubility.

#### Water contact angle (WCA)

The degree of hydrophilicity and hydrophobicity of the packaging film surface is evaluated by measuring the water contact angle between the film surface and water droplets. A



material with a contact angle of less than  $65^\circ$  is considered hydrophilic, while a contact angle greater than  $65^\circ$  indicates a hydrophobic surface [43]. The incorporation of chitosan nanoparticles slightly affects the water contact angle of the films, and with increasing the concentration of chitosan nanoparticles, the contact angle decreases slightly. This reduction in contact angle suggests an enhancement in the hydrophilicity of the film surface. This phenomenon may be attributed to the tendency of nanoparticles to aggregate at higher concentrations, which reduces their effectiveness and limits the improvement of

surface hydrophobicity. Furthermore, the hydrophilic nature of the nanoparticles due to the presence of hydrophilic functional groups on the chitosan surface could be the reason for this increase in the hydrophilic nature of the film surface [44]. Consistent with these findings, Liu et al [45] reported a decrease in the contact angle of corn starch films when the concentration of chitosan nanoparticles was increased from 3% to 4.5%. Similarly, Hosseini et al [46] observed a reduction in the contact angle upon the addition of chitosan nanoparticles to chitosan-polyvinyl alcohol-fish gelatin composite films.

Table 1. Water solubility (WS), and water contact angle (WCA) of the films.

Samples	WS (%)	WCA ( $^\circ$ )
Blank	$11.5 \pm 0.005^b$	$30.81 \pm 2.09^a$
F <sub>3</sub>	$9.5 \pm 0.013^c$	$27.77 \pm 3.37^a$
F <sub>6</sub>	$17.9 \pm 0.002^a$	$21.69 \pm 0.75^b$
F <sub>9</sub>	$18.4 \pm 0.002^a$	$21.74 \pm 0.10^b$

Blank, F<sub>3</sub>, F<sub>6</sub>, and F<sub>9</sub> represent films containing different concentrations of nanoparticles (0, 3, 6, and 9%). The data are presented as mean  $\pm$  SD. Different letters in the same column indicate significantly different ( $p < 0.05$ ).

### Mechanical properties

The mechanical properties of the films, including tensile strength (TS) and elongation at break (EAB), were evaluated in this study, and the results are summarized in Table 2. As can be seen, the incorporation of chitosan nanoparticles led to an increase in both tensile strength (TS) and percent elongation at break (EAB) of the films, such that the TS of the control film was 0.577 MPa and increased significantly after the addition of chitosan nanoparticles. However, with further increase in the concentration of chitosan nanoparticles,

the TS decreased slightly but was still higher than the control film. This behavior can be attributed to the enhancement of intermolecular attractive forces resulting from the formation of hydrogen bonds between the chitosan nanoparticles and the polymer matrix, leading to the development of a denser network structure [47]. Similar findings have been reported for chitosan-based nanocomposite films [48] and hydroxypropyl methylcellulose/hydroxypropyl starch nanocomposite films reinforced with chitosan nanoparticles containing cinnamon essential oil [49].

Table 2. Tensile strength (TS), elongation-at-break (EAB), water vapor permeability (WVP), and DPPH radical scavenging activity of the films.

Samples	TS (MPa)	EAB (%)	DPPH scavenging (%)
Blank	$0.577 \pm 0.018^c$	$17.331 \pm 0.628^b$	$24.460 \pm 0.100^d$
F <sub>3</sub>	$0.745 \pm 0.006^a$	$19.820 \pm 2.760^{ab}$	$36.636 \pm 0.102^c$
F <sub>6</sub>	$0.752 \pm 0.0008^a$	$21.961 \pm 0.538^a$	$37.840 \pm 0.100^b$
F <sub>9</sub>	$0.682 \pm 0.003^b$	$21.145 \pm 1.715^a$	$43.823 \pm 0.080^a$

Blank, F<sub>3</sub>, F<sub>6</sub>, and F<sub>9</sub> represent films containing different concentrations of nanoparticles (0, 3, 6, and 9%). The data are presented as mean  $\pm$  SD. Different letters in the same column indicate significantly different ( $p < 0.05$ ).

### Scanning electron microscopy (SEM)



Scanning electron microscope images of different magnifications of the film surfaces are shown in Fig.3. As can be seen, the control film (Figure 3-A) exhibited a smooth, homogeneous, and uniform surface, indicating the excellent film-forming capability of zucchini flour as a base material, resulting in films with desirable properties and favorable morphology. However, with the incorporation of chitosan nanoparticles, the surface morphology of the films underwent noticeable changes, characterized by increased surface roughness and unevenness. Additionally,

nanoparticle aggregates were visible as protrusions on the film surfaces, which were uniformly dispersed within the film matrix, contributing to a more homogeneous structure. These findings align with those reported by Zeng et al [50], who observed uniform dispersion of nanoparticles in a lotus root starch matrix and noted increased surface roughness with higher nanoparticle content. Similar results were also documented by Al-Maqtari et al [51].

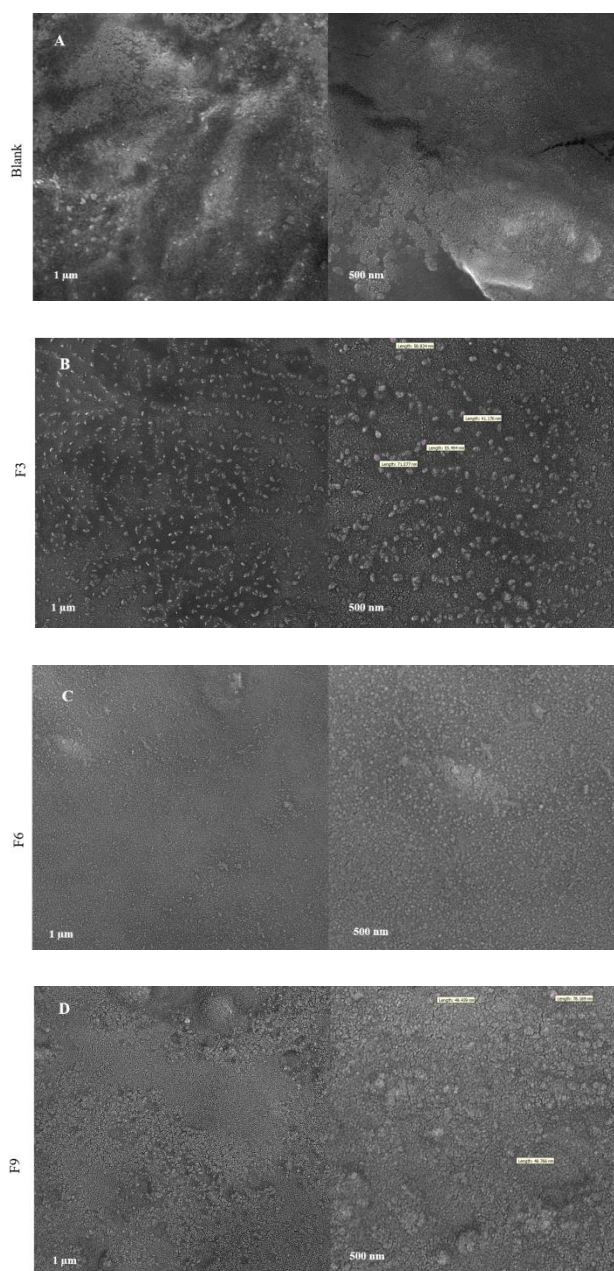


Fig. 3. Scanning electron microscopy (SEM) images of the surface of the (A) blank film and films containing (B) 3%, (C) 6%, and (D) 9% FEO-NP.

### Fourier transformed infrared spectroscopy (FTIR)

Fig.4. presents the results of the FTIR spectrum analysis for the various films studied. The FTIR spectrum of the blank film exhibited characteristic peaks at 3413, 2935, 1623, 1421, and 1051  $\text{cm}^{-1}$ , corresponding to the stretching vibrations of hydroxyl groups (O-H), CH stretching vibrations (likely from amylose and amylopectin content) [52], stretching of amide I groups (C=O, associated with proteins) [53], symmetric stretching of the carboxyl group (-COO) [33], and stretching vibrations of C-O (related to starch), respectively. According to the FTIR results of different flours, the band located between 3000 and 2700  $\text{cm}^{-1}$  was due to the presence of fat in the flour, and the band between 1700 and 1500  $\text{cm}^{-1}$  was related to the protein region [7]. Chitosan nanoparticles loaded with fennel essential oil showed spectra

at 3444  $\text{cm}^{-1}$  (chitosan -OH and NH stretching) [54], 2879  $\text{cm}^{-1}$  (alkane C-H stretching vibrations) [55], 1643  $\text{cm}^{-1}$  (alkane C=C stretching) [56], 1542  $\text{cm}^{-1}$  (Amide-II, N-H bending) [41], and 1072  $\text{cm}^{-1}$  (C-O-C asymmetric stretching) [57]. All films exhibited an absorption peak at 3413  $\text{cm}^{-1}$ , attributed to -OH stretching vibrations, with slight shifts observed as the percentage of nanoparticles in the film composition increased. The peaks in the films containing different amounts of chitosan nanoparticles were slightly different from the control film, and most of them were similar to the control film, only the intensity of the peaks decreased slightly. These findings suggest that the incorporation of chitosan nanoparticles influenced the chemical structure of the films, as evidenced by the subtle changes in the FTIR spectra.

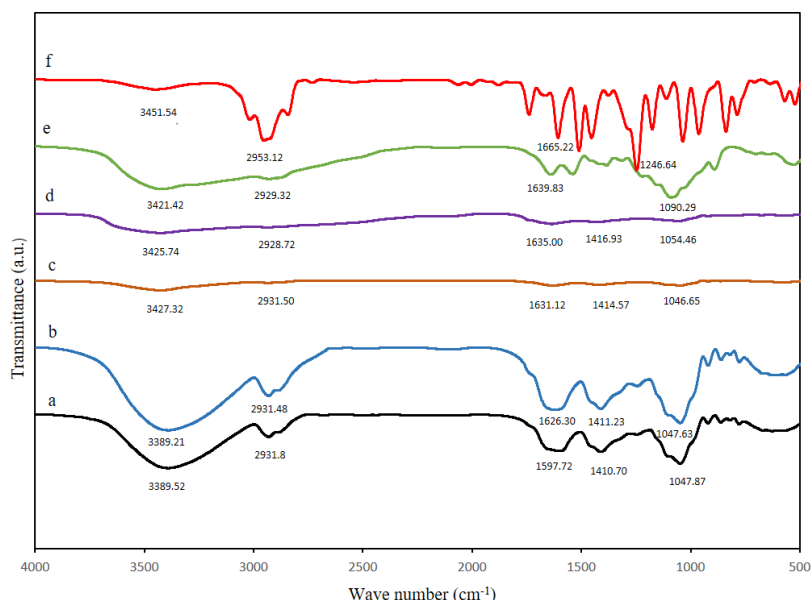


Fig. 4. FTIR spectra of (a) blank, (b) F3, (c) F6, (d) F9, (e) FEO-NP, and (f) FEO.

### DPPH antioxidant activity

Food packaging films are expected to exhibit strong antioxidant activity to enhance the

preservation and shelf life of food products. The antioxidant activity of the films was evaluated using the DPPH free radical

scavenging method and the results are presented in Table 2. The pure zucchini flour film (control) showed an antioxidant activity of 24.46%, which is due to the presence of compounds with antioxidant properties such as phenolics, carotenoids, chlorophylls, and vitamin C [58-59]. Films containing chitosan nanoparticles showed significant antioxidant activity, as the antioxidant activity of the films increased with increasing concentration of chitosan nanoparticles. The highest DPPH radical scavenging activity (43.82%) was observed in the film containing 9% chitosan nanoparticles, while films with 6% and 3% nanoparticles showed scavenging activities of 37.84% and 36.63%, respectively. This enhancement in antioxidant activity with increasing nanoparticle concentration is likely due to the potent antioxidant properties of both the encapsulated fennel essential oil (FEO) and chitosan itself. The free amino groups (NH<sub>2</sub>) in chitosan are known to contribute to radical scavenging [60]. Several studies have proven that the antioxidant activity of fennel essential oil is related to the abundant polyphenolic compounds present in it, which play a key role in scavenging free radicals [61-63]. Similar results to this study were obtained in the study of Soltanzadeh et al [64] reported that antioxidant activity increased significantly with increasing concentration of chitosan nanoparticles loaded with pomegranate peel extract in gelatin/cress seed gum composite film. Similarly, Fang et al [65] demonstrated that the antioxidant activity of carboxymethyl chitosan films increased with the incorporation of gliadin-carboxymethyl chitosan nanoparticles containing natamycin and theaflavin (Nata/TFs-GC). These results collectively highlight the effectiveness of chitosan nanoparticles in enhancing the antioxidant properties of food packaging films.

#### 4. Conclusion

This research explored the utilization of zucchini flour as an accessible, abundant, and cost-effective material for the production of edible films. Initially, chitosan nanoparticles containing fennel essential oil were synthesized via ionic

gelation, and their characteristics, including encapsulation efficiency, particle size, polydispersity index (PDI), zeta potential, and morphology, were evaluated. The results indicated that the encapsulation efficiency of the essential oil within the chitosan nanoparticles was 96%, with an average particle size of 260.8 nm, a PDI of 0.28, and a zeta potential of 52.3 mV. Scanning electron microscopy (SEM) images of the nanoparticles revealed nearly spherical particles with uniform distribution and a particle size of less than 500 nm. Subsequently, the chitosan nanoparticles loaded with fennel essential oil were incorporated into zucchini flour-based films at concentrations of 0%, 3%, 6%, and 9%, and the physicochemical and mechanical properties of the resulting films were investigated. The findings demonstrated that the addition of nanoparticles improved the film characteristics. Mechanical testing revealed that tensile strength and elongation at break of the films increased with increasing nanoparticle concentration. Furthermore, the solubility and contact angle of the films increased and decreased, respectively, with increasing nanoparticle concentration, attributed to the hydrophilic nature of chitosan nanoparticles. The antioxidant activity of the films significantly increased with increasing nanoparticle concentration. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed that chitosan nanoparticles effectively bonded with the polymer matrix. SEM images of the films showed a smooth surface for the control film, while the surface roughness increased with the addition of nanoparticles. Overall, based on the results of this study, the film containing 6% chitosan nanoparticles exhibited the most favorable mechanical and physical properties compared to other nanoparticle-containing films. This research suggests that edible films based on zucchini flour incorporating chitosan nanoparticles loaded with fennel essential oil possess desirable physicochemical and mechanical properties, potentially offering

a wide range of applications in the food and pharmaceutical industries.

## 5. Reference

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## تولید و بررسی خواص فیلم زیست تخریب پذیر آنتی اکسیدانی برپایه آرد کدوسبز حاوی نانوذرات کیتوزان بارگذاری شده با اسانس رازیانه

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اطلاعات مقاله	چکیده
<p>تاریخ های مقاله :</p> <p>تاریخ دریافت: ۱۴۰۳/۱۰/۲۰</p> <p>تاریخ پذیرش: ۱۴۰۳/۱۱/۲۸</p> <p>کلمات کلیدی:</p> <p>آرد کدوسبز، فیلم آنتی اکسیدانی، اسانس رازیانه، خواص مکانیکی، مورفولوژی</p> <p>DOI: 10.22034/FSCT.22.165.196.</p> <p>* مسئول مکاتبات:</p> <p>mohtarami.f@gmail.com; f.mohtarami@urmia.ac.ir</p>	<p>هدف این پژوهش تولید فیلم زیست تخریب پذیر برپایه آرد کدوسبز و بررسی تأثیر افزودن نانوذرات کیتوزان بارگذاری شده با اسانس رازیانه (۰، ۳، ۶ و ۹ درصد وزنی/وزنی) بر ویژگی های فیزیکوشیمیایی و مکانیکی فیلم ها بود. نتایج نشان داد که افزودن نانوذرات کیتوزان به طور قابل توجهی مقاومت کششی و ازدیاد طول تا نقطه شکست فیلم ها را افزایش داد که نشان می دهد نانوذرات می توانند به عنوان تقویت کننده عمل کرده و ساختار فیلم را بهبود بخشند. افزایش درصد نانوذرات باعث افزایش حلالیت در آب و کاهش زاویه تماس آب فیلم ها شد. فعالیت آنتی اکسیدانی فیلم ها با افزایش غلظت نانوذرات به طور قابل توجهی افزایش یافت که می تواند به دلیل وجود اسانس رازیانه در نانوذرات و بروز خواص آنتی اکسیدانی آن باشد. نتایج آزمون FTIR نشان داد که نانوذرات کیتوزان به خوبی با ماتریس پلیمری فیلم آرد کدوسبز پیوند شیمیایی برقرار کرده اند. تصاویر SEM نیز نشان داد که افزودن نانوذرات باعث افزایش زبری سطح فیلم ها شده و یکنواختی سطح فیلم با افزایش میزان نانوذرات کیتوزان کمتر شده است. بر اساس نتایج این پژوهش، فیلم حاوی ۶ درصد نانوذره کیتوزان حامل اسانس رازیانه، از نظر خواص مکانیکی و فیزیکی بهترین عملکرد را نشان داد. بطور کلی، فیلم خوراکی آرد کدوسبز فعال شده با اسانس رازیانه و نانوذرات کیتوزانی می تواند به عنوان فیلم خوراکی فعال با خواص مطلوب جهت استفاده در بسته بندی مواد غذایی حساس به فساد اکسایشی معرفی شود.</p>