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Image processing of chia seed mucilage film containing cinnamon essential oil nanoemulsion

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ABSTRACT

Imaging is a crucial tool for evaluating the structure of food materials. Image processing encompasses two-dimensional (2D) images of surfaces and cross-sections, such as those observed with microscopy, and three-dimensional (3D) internal structures obtained using confocal microscopy, computed tomography, and magnetic resonance imaging. This research utilized image processing to determine the hardness and compactness of edible biofilms infused with nanoemulsions. Chia seed gum edible films containing cinnamon essential oil were prepared at concentrations of 2%, 4%, and 6%. Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) images of the films were acquired. The AFM and image processing results demonstrated that increasing the essential oil concentration from 2% to 6% decreased compactness and increased surface roughness, consistent with the image processing findings. Therefore, this interpretive image processing offers a potential capability for determining the compactness of edible and non-edible films in the packaging industry.

Introduction

Plant-derived polysaccharide mucilages are used as sustainable biopolymers in food packaging films. The use of mucilage in edible packaging films is important due to its non-toxicity, biocompatibility, biodegradability, and selective permeability to oxygen/carbon dioxide [2-1]. Furthermore, the use of mucilage in edible films has shown significant health benefits such as cholesterol reduction, lowering high blood pressure, and improving the glycemic index. Edible films, due to their biodegradable nature, have become a substitute for plastic in food packaging, and the prevalent use of edible films can significantly reduce environmental pollution caused by plastics (approximately 7.8 billion tons worldwide) [3-4]. Several plant polysaccharides such as alginate, pectin, starch, basil, and xanthan gum are widely used to formulate edible films [5]. However, the resulting edible films have a brittle nature and weak mechanical strength. Such limitations are overcome by adding various polysaccharides, fillers, emulsifiers, and essential oils [6].

Chia seeds (*Salvia hispanica*) are an annual plant from the Lamiaceae family, native to Central America, and possess high nutritional value. Chia seeds contain 5-6% mucilage, which can absorb 27 times its weight in water. This substance is mainly composed of xylose, glucose, and methyl glucuronic acid, forming a branched high-molecular-weight polysaccharide [7-8]. It is recognized as a new polysaccharide gum by the FAO, due to its mucilaginous properties at low concentrations in aqueous solutions [9]. Chia seed mucilage is used as a stabilizer, thickener, and emulsifier in food systems, as well as in biodegradable packaging [10]. Due to its water holding capacity and suitable viscosity, chia seed mucilage is a potential material for producing edible films [9].

Essential oils (EOs) are volatile, natural compounds with a wide range of antibacterial, antifungal, antioxidant, and anti-inflammatory properties [11]. Among various EOs, cinnamon essential oil is a volatile compound with several biological properties, including antibacterial, antioxidant, and anti-inflammatory activities; its main components are cinnamaldehyde and eugenol. Although cinnamon essential oil is a flavor and fragrance additive in food products, it has poor solubility. This limitation is particularly significant in polysaccharide-based edible films [12]. Therefore, encapsulating cinnamon essential oil is crucial for its use in a film matrix.

Several strategies for preparing composite edible films with cross-linking and adding hydrophobic lipids and essential oils to increase the water resistance of edible films have been reported [13-14]. In one study, Luo et al. (2019) investigated the effect of chia seed mucilage on gelatin edible films. In this research, oregano essential oil was loaded into a chia seed mucilage/gelatin edible film, and then the antimicrobial activity of the film was investigated. According to the results, adding chia seed mucilage decreased tensile strength and water vapour permeability and increased elongation at break and contact angles. Also, due to the hydrogen bonds between the components of the film matrix, the retention rate of the essential oil in the film increased. Furthermore, the release of the essential oil, examined using gas chromatography, showed stable antimicrobial activity in the film, which resulted from the high remaining amounts of essential oil in the edible film [15]. Emir Çoban et al. (2020) investigated the effect of a chia seed mucilage edible coating containing different amounts of goji berry extract on the shelf life of rainbow trout fillets. The results showed that microbial growth in the sample coated with mucilage containing 2% goji berry extract was delayed compared to the control sample. The chia seed mucilage coating also prevented the increase in thiobarbituric acid index, pH, and volatile nitrogen [16]. In a study conducted by Mousavi et al. (2021), the effect of a chia seed mucilage/bacterial cellulose edible coating on the bioactive compounds and antioxidant activity of strawberries during cold storage was investigated. Strawberries were coated with chia seed mucilage containing 0.6% and 8% (w/w) bacterial cellulose nanofibers. The researchers analyzed the content of phenols, flavonoids, anthocyanins, ascorbic acid, protein, antioxidant activity, and the activity of polyphenol oxidase and peroxidase. The results showed that using chia seed mucilage led to preserving phenolic compounds, flavonoids, ascorbic acid, and antioxidant activity [17]. Semwal et al. (2022) added chia seed mucilage to protect a sodium caseinate-based probiotic film in another study. They investigated the improved viability of the encapsulated probiotic bacteria (*Limosilactobacillus fermentum* NKN51 and *Lactobacillus brevis* NKN52) stored at 25°C and 4°C. The results showed that the presence of chia seed mucAdding essential oils enhanced Antifungal activity significantly and significantly increased the viability of the probiotic bacteria. It also improved the film's flexibility and reduced its solubility, attributed to interactions between the chia seed mucilage and the protein matrix. SEM and AFM images showed healthy bacterial cells. The functionality of the probiotic, edible film was also evaluated by coating wheat bread, demonstrating probiotic viability for 3 weeks at 25°C, indicating the applicability of the probiotic, edible film as a carrier for probiotic bacteria in bakery products [18]. In a study by Muñoz-Tébar et al. (2023), edible films were produced from chia seed mucilage incorporating oregano (*Origanum vulgare*) and savory (*Satureja montana*) essential oils at various concentrations (0.1%, 1%, and 1.5% v/v). The researchers investigated these films' physical, optical (clarity), mechanical, antifungal, and structural properties. Results indicated that the type of essential oil significantly affected only the clarity, total color difference, and antifungal activity. However, the type of essential oil did not significantly influence the mechanical and physical properties. Increasing the essential oil concentration to 1.5% resulted in a decrease in tensile strength and an increase in elongation at break. Adding essential oils enhanced antifungal activity significantly; films with 0.1% essential oil showed no activity, while films with 1% and 1.5% effectively inhibited fungal growth (38-77%). SEM images revealed some heterogeneity in the film matrix and a surface lacking pores and cracks upon essential oil addition. A more compact and homogeneous structure was observed in the oregano essential oil-containing polymer. The authors concluded that incorporating savory and oregano essential oils as natural antimicrobial compounds holds potential for active packaging and mold growth control, thereby enhancing food safety [19].

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Despite comprehensive research on the microbial, mechanical, thermal, and physicochemical properties of various edible films, image processing has not yet been used to determine the compactness of edible films. This study utilized image processing of edible films, both with and without the presence of a cinnamon essential oil nanoemulsion, to analyze the degree of compactness. To this end, the pixel values of a film image were first considered as a spatial process. Using the periodically correlated spatial process characteristics and the spatial coherence function introduced by Ghasemi and Amiri (2019) [20], this was modeled as a periodically correlated spatial process. Based on the spatial coherence diagram, the periodicity of the film image was estimated, and the degree of film compactness was determined using the periodicity value. The R software was used for the analyses.

Materials and methods

1.1. Preparation of cinnamon essential oil- nanoemulsion

The cinnamon essential oil nanoemulsion (CEN) was prepared using the method described by Erfanifar et al. [36]. Cinnamon EO (8 % w/w), tween 80 (6 % w/w EO), and water were mixed and subjected to ultrasonic emulsification using a 29 kHz sonicator (Ultrasonic Honogenizer NL400, China) for 6 min [21].

1.2.Extraction of the chia seed mucilage

To extract CSM, chia seeds were hydrated in water at 1: 15 ratios at ambient for 2 h (pH was previously fixed at 8 with 0.1 M NaOH). Then, cheesecloth separated the mucilage from the mixture of seeds and water. After discarding the seeds, the mucilage was collected and dried at 50 °C for 16 h. Eventually, the dried mucilage was stored in a dryer for further use [22].

1.3. Preparation of CSM film

CSM film solution was prepared by dissolving the CSM powder (1 % w/v) into distilled water containing glycerol (30% w/w, based on CSM weight) using a magnetic stirring plate at 80 °C and 1200 rpm for 10 min and then cooled to room temperature [38]. For the fabrication of active CSM films, different amounts of prepared nanoemulsion in the 2.1 section (2, 4, and 6% w/w) were added into the CSM film solution and stirred. The solution was cast in a plate 8 mm and then dried for 72 h at ambient conditions. The prepared films were CSM (without CEN), CSM-2 (with 2% w/w CEN), CSM-4 (with 4% w/w CEN) and CSM-6 (with 6% w/w CEN). Then, the dried films were peeled and stored in a desiccator containing saturated magnesium nitrate solution at 25 °C until evaluation [22].

1.4.Scanning Electron Microscopy and Atomic Force Microscopy (AFM)

Images of the cross-sections of the films were obtained by SEM, using a Tescan-Vega 3, Czech Republic electron microscope. Film specimens were fractured by immersion in liquid nitrogen and mounted on an aluminum stub using double-sided tape, coated with thin film gold/palladium, and then examined at an accelerating voltage of 20 Kv [22]. The surface morphology of different CSM films was investigated by Atomic Force Microscopy (AFM) (Ara- Pajoohesh company, Iran) with a 125 x 125 µm scan size and a 6 µm vertical range. Measurements were taken from several areas of the film surface area (50 x 50 µm) [22].

1.5.Image processing of edible fils

In this study, R software was used to determine the film's compactness. The images of the films were called by the imager package in R, and the pixel values of the image were considered a spatial process. In image processing, each image can be considered a spatial process, and the property of the spatial process can be used in image analysis.

A collection $\boldsymbol{X} = \{X_{\boldsymbol{t}}: \boldsymbol{t} = (t_1, t_2) \in \boldsymbol{\mathcal{T}} \subseteq \mathbb{Z}^2\}$ is called a second-order spatial process; if any $\boldsymbol{t} \in \boldsymbol{\mathcal{T}}$, $X_{\boldsymbol{t}}$ is a random variable with zero mean ($E[X_{\boldsymbol{t}}] = 0$) and finite second moment ($E\left[{X_{\boldsymbol{t}}}^2\right] < \infty$). The autocovariance function of $\boldsymbol{X}$ is defined as the following

$$R_{\boldsymbol{X}}(\boldsymbol{t}, \boldsymbol{s}) = E[X_{\boldsymbol{t}} X_{\boldsymbol{s}}],\ \boldsymbol{t}, \boldsymbol{s} \in \boldsymbol{\mathcal{T}}.$$

A second-order spatial process $\boldsymbol{X} = \{\boldsymbol{X_t}: \boldsymbol{t} \in \boldsymbol{\mathcal{T}}\}$ is called a periodically correlated spatial process (PCSP) if there exists a $\boldsymbol{T} = (T_1, T_2) \in \mathbb{N}^2$, such that for any $\boldsymbol{t}, \boldsymbol{s} \in \boldsymbol{\mathcal{T}}$ and $\boldsymbol{n} \in \mathbb{N}^2$,

$$R_{\boldsymbol{X}}(\boldsymbol{t}, \boldsymbol{s}) = R_{\boldsymbol{X}}(\boldsymbol{t} + \boldsymbol{n} \odot \boldsymbol{T}, \boldsymbol{s} + \boldsymbol{n} \odot \boldsymbol{T}), \quad (1)$$

where $\boldsymbol{n} \odot \boldsymbol{T} = (n_1 T_1, n_2 T_2)$. If T_1 and T_2 are the smallest natural numbers that satisfy (1), $\boldsymbol{T} = (T_1, T_2)$ is called the period of the spatial process $\boldsymbol{X}$. Also, if $f_{\boldsymbol{X}}$ is the spectral density of $\boldsymbol{X}$, then support of $f_{\boldsymbol{X}}$, called $S_{f_{\boldsymbol{X}}}$, is

$$S_{f_{\boldsymbol{X}}} \coloneqq \{(\boldsymbol{\theta}, \boldsymbol{\eta}): \boldsymbol{\theta}, \boldsymbol{\eta} \in [0,2\pi)^2, f_{\boldsymbol{X}}(\boldsymbol{\theta}, \boldsymbol{\eta}) > 0)\} = \bigcup_{k_1=-T_1+1}^{T_1-1} \bigcup_{k_2=-T_2+1}^{T_2-1} \left\{(\boldsymbol{\theta}, \boldsymbol{\eta}): \boldsymbol{\theta}, \boldsymbol{\eta} \in [0,2\pi)^2, \theta_j - \eta_j = \frac{2\pi k_j}{T_j}, j = 1,2\right\} \quad (2)$$

Shishebor and Amiri (2019) presented a graphical method for detecting PCSP and determining the periodicity of a second-order spatial process in the form of $\boldsymbol{X} = \{X_{\boldsymbol{t}}: \boldsymbol{t} = (t_1, t_2),\ t_j = 1, \ldots, N_j, j = 1,2\}$ using (1) and (2). Their methodology is based on the following spatial coherence function, $\gamma_{\boldsymbol{X}}$,

$$\gamma_{\boldsymbol{X}}(p_1, p_2) = \frac{\left|\sum_{m_1=0}^{M_1-1} \sum_{m_2=0}^{M_2-1} d_X(m_1, m_2) \overline{d_X(m_1 + p_1, m_2 + p_2)}\right|^2}{\sum_{m_1=0}^{M_1-1} \sum_{m_2=0}^{M_2-1} |d_X(m_1, m_2)|^2 \sum_{m_1=0}^{M_1-1} \sum_{m_2=0}^{M_2-1} |d_X(m_1 + p_1, m_2 + p_2)|^2},$$

where $d_{\boldsymbol{X}}(m_1, m_2) = \frac{1}{\sqrt{N_1 N_2}} \sum_{t_1=1}^{N_1} \sum_{t_2=1}^{N_2} X_{\boldsymbol{t}} e^{i2\pi\left(\frac{t_1 m_1}{N_1} + \frac{t_2 m_2}{N_2}\right)}$, $m_j = 1, \ldots, N_j, j = 1,2$ and $\boldsymbol{M} = (M_1, M_2) \in \mathbb{N}^2$.

If the graph of the spatial coherence function for the spatial process $\boldsymbol{X} = \{X_{\boldsymbol{t}}: \boldsymbol{t} = (t_1, t_2),\ t_j = 1, \ldots, N_j, j = 1,2\}$ has a significant peak at the point $\boldsymbol{p}^* = (p_1^*, p_2^*)$, the process $\boldsymbol{X}$ is a PCSP, and its period equals to:

$$\boldsymbol{T} = \left(\frac{N_1}{p_1^*}, \frac{N_2}{p_2^*}\right). \quad (3)$$

According to (3), the farther the peak point is from the origin, the smaller the measure of the period is, and vice versa. Also, the peaks in t the spatial coherence graph are the main frequencies of a process. They have the greatest impact on the process. Therefore, if there are several peaks in the spatial coherence graph of a process, it means that the process is a combination of several periods. In addition, the highest peak determines the dominant period.

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Let $\boldsymbol{X} = \{X_{\boldsymbol{t}}: \boldsymbol{t} = (t_1, t_2),\ t_j = 1, \ldots, N_j\ , j = 1,2\ \}$ be a spatial process corresponding to an image with the number of pixels $N_1 \times N_2$, where $X_{\boldsymbol{t}}$ specifies the pixel value of the image at the position of $\boldsymbol{t} = (t_1, t_2)$. If the spatial process $\boldsymbol{X}$ is a PCSP, we can detect the periodicity of $\boldsymbol{X}$ (image) and determine the period of the image by using the properties of PCSP. Moreover, if the image of the film is modeled by a PCSP, then the compression rate can be obtained by this property. In this case, the smaller the period of the film is, the more compact the film will be.

In this study, to investigate the periodicity of a film and determine its compression, we use the spatial coherence graph and apply the following approach:

i. If the spatial coherence graph corresponding to a film has a significant peak, this film (image) is considered a PCSP.
ii. According to (3), in the spatial coherence graph, the farther the highest peak point is from the origin, the smaller the periodicity of the film is so that it will be more compact.
iii. If the spatial coherence graph of two films has a peak at the same point, it can be said that the compression of both is almost the same.
iv. If the peak of the spatial coherence graph corresponding to the film lies on the x or y-axis, it indicates the presence of periodicity in the same direction.

Results and discussion

Figure 1 shows the 2D and 3D surface topography of SEM and AFM images of chia seed gum films containing a cinnamon essential oil nanoemulsion. The surface of the films was relatively smooth and even. However, films with higher emulsion concentrations exhibited a relatively rough and uneven surface. The roughness of the edible films containing the emulsion is attributed to the distribution of particles on the film's surface during drying [23].

Our observations were similar to those of Almasi et al. (2020), who confirmed the uneven surface of pectin films loaded with Pickering emulsion [24]. Furthermore, Sun et al. (2020) observed the homogeneity of sodium starch octenyl succinate films incorporated with cinnamon Pickering emulsion, showing a uniform emulsion distribution [25]. However, droplet aggregation might occur after drying, leading to some clumping and coalescence. Similarly, Shen et al. (2021) showed that pullulan-gelatin films had a smooth surface, while adding Pickering emulsion caused a relatively rough and uneven surface. This observation might be due to the migration of globules to the surface during drying [26].

This study treated the images of films shown in Figure 2 as spatial processes, and their spatial coherence diagrams are depicted in Figure 3. The presence of peaks in the spatial coherence diagrams corresponding to the films indicates their periodicity. The peak's location determines the image's periodicity in one direction (along the y-axis). Based on the spatial coherence diagrams corresponding to the films, shown in Figure 3 (note: the text mentions Figure 1, but it is likely a typo and should refer to Figure 3), films f, e, g, and h exhibit the highest compactness, confirming the AFM results. It's important to note that since the peak in the spatial coherence diagram for both films e and f is at point p=(0,4), their compactness is approximately equal. However, the higher peak height in the coherence diagram for film f compared to e allows a more confident determination of its greater compactness.

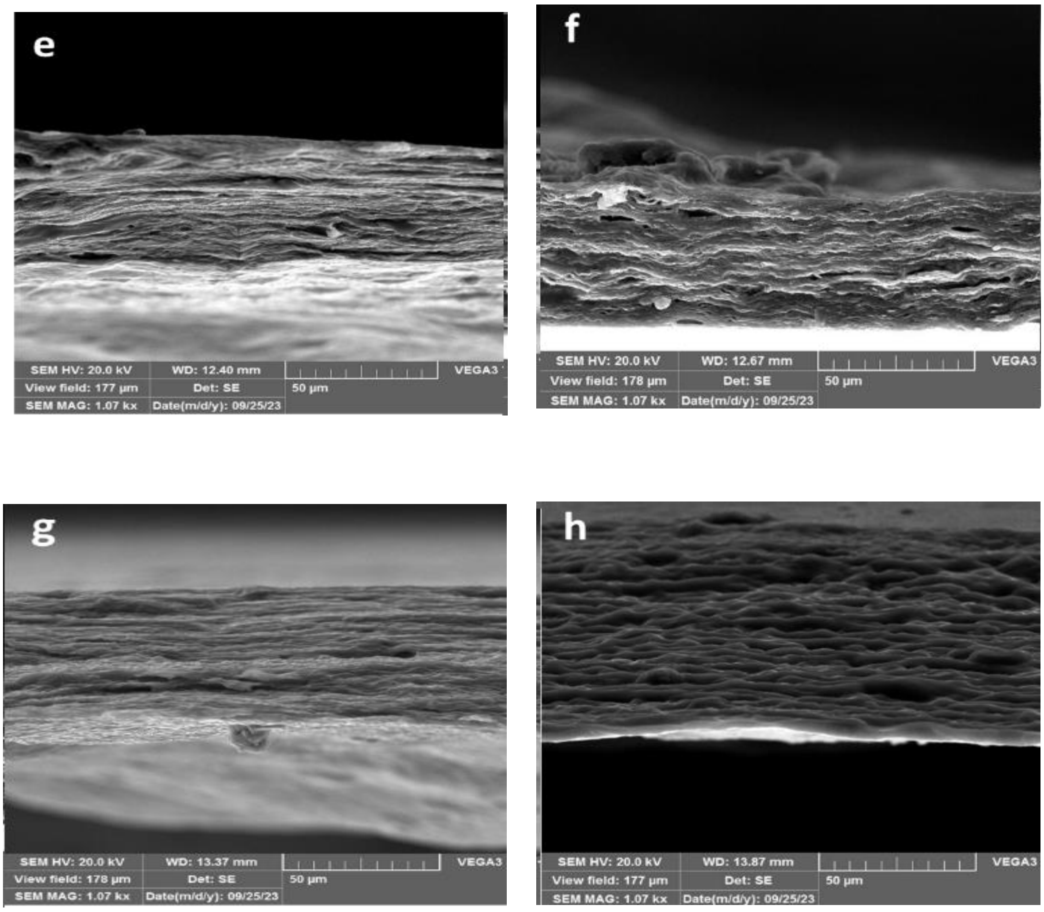

Fig. 1. SEM images of thecross-section of CSM film (e), CSM-2 (f), CSM-4 (g), CSM-6 (h).

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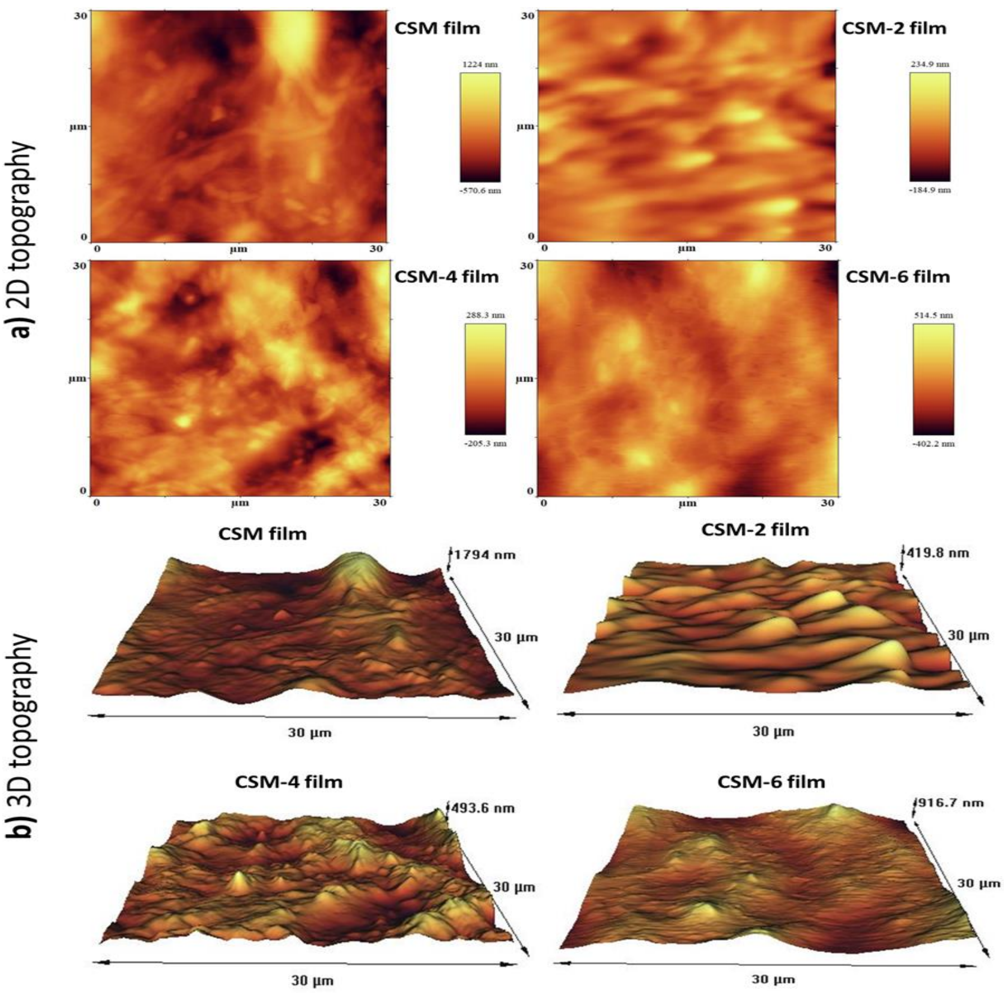

Fig. 2. AFM images of 2D surface (a) and 3D topography (b) of chia seed mucilage (CSM) films with different concentrations (2, 4, and 6%) of cinnamon essential oil nanoemulsion.

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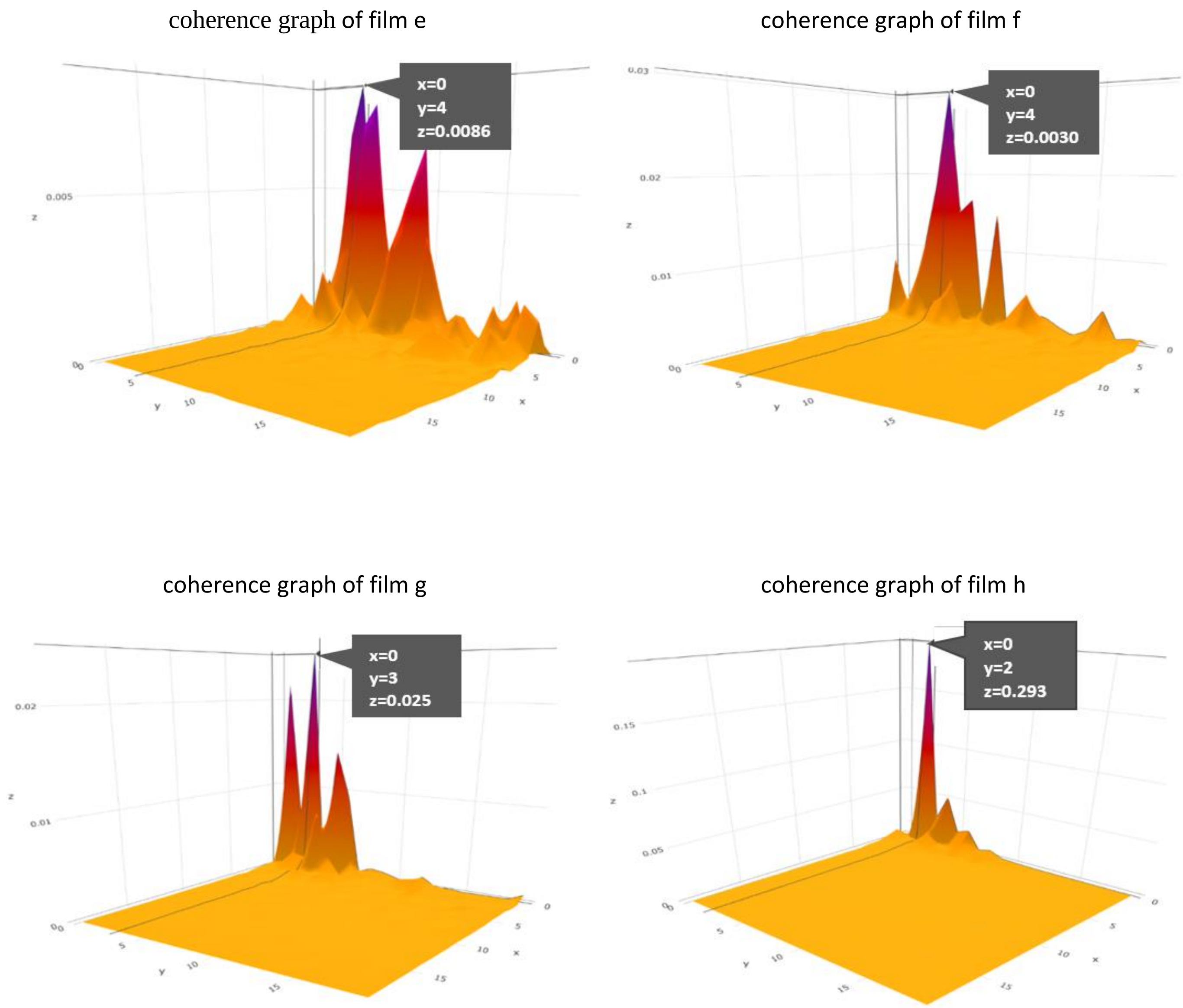

Fig. 3. Coherence graph of the films

Conclusion

In this research, chia gum films containing cinnamon essential oil nanoemulsion were prepared, and their structural and morphological properties were investigated using scanning electron microscopy (SEM) and atomic force microscopy (AFM). The SEM images of the films were then subjected to image processing to determine surface compactness, and the results were compared with those obtained from AFM. The image processing results, as a novel method, predicted the roughness and compactness of the films. The findings indicated that increasing the essential oil concentration in the nanoemulsion films decreased surface compactness.

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چکیده

تصویربرداری ابزاری مهم برای ارزیابی ساختار مواد غذایی است. پردازش تصویر شامل تصاویر دو بعدی (D۲) از سطوح و مقاطع، مانند آنچه در میکروسکوپ مشاهده می‌شود و همچنین تصاویر سه‌بعدی (D۳) از ساختار داخلی، مانند آنچه توسط میکروسکوپ کانفوکال، توموگرافی کامپیوتری و تصویربرداری تشدید مغناطیسی می‌باشد. در این تحقیق از پردازش تصاویر برای تعیین سختی و فشردگی بیو فیلم‌های خوراکی آغشته به نانوامولسیون استفاده شد. فیلم خوراکی صمغ دانه چیا حاوی اسانس دارچین با غلظت ۲٪، ٤٪ و ٦٪ تهیه شد. تصاویر میکروسکوپ الکترونی روبشی[1] و میکروسکوپ نیروی اتمی[2] از فیلم‌ها تهیه شد. نتایج AFM و پردازش تصویر نشان دادند که با افزایش میزان اسانس از ۲٪ به ٦٪ میزان فشردگی کاهش و زبری سطح افزایش پیدا کرد که با نتایج پردازش تصاویر مطابقت داشت. بنابراین پردازش تفسیر این امکان را فراهم می‌کند که قابلیت بالقوه‌ای برای تعیین فشردگی فیلم‌های خوراکی و غیر خوراکی در صنعت بسته‌بندی دارد.

1: Scanning electron microscopy (SEM)
2: Atomic Force Microscopy (AFM)

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