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Extended Abstract

Evaluation of Antimicrobial Properties of Gliadin Nanofibers Containing *Zataria multiflora* Boiss Essential Oil and its Effect on Shelf-life Extension of Smoked Salmon Fish Fillet

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Abstract

The aim of this study was to produce electrospun gliadin nanofibers containing *Zataria multiflora Boiss* essential oil (ZMEO) (5, 10 and 15%, w/w) to create active antimicrobial mats. The minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) of essential oil were measured. The ZMEO loaded gliadin nanofibers were characterized for physicochemical, antioxidant and antibacterial activity. Results according to the MIC and MBC revealed that the ZMEO showed the most remarkable bactericidal effect. The nanofibers showed an encapsulation efficiency close to 95% and the presence of ZMEO led to increased contact angle and opaque. ZMEO increased the absorption of color in the visible region, which in turn led to increase of the b^* parameter but reduced a^* and L^* parameters. The nanofibers' antimicrobial activities were induced by incorporating ZMEO, and *Bacillus subtilis* was the most sensitive bacterium to ZMEO-containing nanofibers, while *Salmonella tiphi* was the most resistant. The nanofibers incorporating ZMEO showed good antioxidant properties; this effect was greatly improved when the proportion of added ZMEO was 15%. Then, efficacy of bioactive nanofibers included 5, 10 and 15% (w/w) ZMEO to reduce microbial growth (*Listeria monocytogenes*) of smoked salmon fish fillet during chilled storage was evaluated; the results indicated that its final population was reduced to about 2.5-3 Log cycles after 16 days of storage at 4 °C in presence of ZMEO, compared with negative control mats produced without the ZMEO. These results suggest that the developed gliadin nanofibers with active substance could be used in designing antimicrobial packaging materials.

Keywords: Dairy product, Essential oil, Gliadin, Nanofibers, Packaging

Introduction

In recent years, the importance of medicinal plants as one of the most important sources of antimicrobials has been well understood (Heydari-Majd *et al.*, 2019). Among the medicinal plants, *Zataria multiflora Boiss* is an aromatic plant with medicinal properties and a member of the *Labiaceae* family. The use of nanofibers as carriers of these compounds causes the controlled release of these compounds in food products. Nanofibers are short strands with a diameter of less than 1000 nm, which are produced as a layer on a sheet. Several food grade polymers are used in electrospinning technique to encapsulate active pharmaceutical and food compounds. Among the polymers, gliadin is the stored protein in wheat grain and constitutes about 40-50% of the weight of the stored protein of wheat (Rezaeinia *et al.*, 2020).

Due to having high amounts of polyunsaturated fatty acids, protein and moisture, aquatic products are classified as food products with a high degree of spoilage. Active packaging can be a promising solution to increase the shelf life of food products (Heydari-Majd *et al.*, 2019). Therefore, in this research, gliadin has been used to form nanofibers using electrospinning technique.

Materials and methods

Gliadin extraction

Extraction of gliadin was done according to the method of Sharif *et al.* (2018) using 70% ethanol as a solvent and finally it was dried by a freeze dryer (TDS-00209-A, USA).

Preparation of gliadin nanofibers containing essential oil

Gliadin extracted from gluten with a concentration of 30% (w/v) was dissolved in 70% ethanol. Then essential oil was added to the gliadin solution at three different levels (5, 10 and 15%, w/w). The electrospinning process was performed using a syringe pump with a flow rate of 1.5 mL/h.

Encapsulation efficiency and loading capacity

Encapsulation efficiency and loading capacity of gliadin nanofibers containing different concentrations of essential oil were calculated using the method (Heydari-Majd *et al.*, 2019).

Water contact angle

Hydrophobicity of the surface of gliadin nanofibers containing essential oil was measured using the contact angle measurement (Rezaeinia *et al.*, 2020).

Color characteristics

A Minolta colorimeter (Minolta Camera Osaka, Japan) was used to check the color characteristics of gliadin included luminance or *L*, *a,* and *b*.

Antibacterial properties of nanofibers

In this test, MIC and MBC of the essential oil as well as the halo of inhibition were measured.

Antioxidant activity

The phenolic content and antioxidant activity of nanofibers was calculated by foolin method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, respectively.

Coating of salmon fillet

In this test, the effect of nanofiber coating on fish fillet was investigated over a period of 16 days (Yean *et al.*, 1998).

Statistical analysis

The statistical analysis was performed using analysis of variance (ANOVA) and SPSS statistical software (IBM SPSS-V.18, USA).

Results and discussion

Encapsulation efficiency and loading capacity

The results of this test are shown in Table (1). By increasing the concentration of essential oil from 5 to 15%, the percentage of EE and LC of essential oil in gliadin nanofibers increased from 80 to 95% and 16 to 23%, respectively. In similar studies, Yang *et al.* (2017), while loading essential oil in electrospun nanofibers by coaxial method stated that LC and EE values of loaded essential oil were about 14.5 and 99.6%, respectively.

Table 1. Physicochemical properties of gliadin nanofibers containing different concentrations of *Zataria multiflora* Boiss essential oil

Nanofibers	EE (%)	LC (%)	Contact angle (°)
Gliadin	-	-	50.60 ± 2.01^{d}
Gliadin/5%-ZMEO	80.10 ± 2.10^{c}	16.11 ± 1.30^{c}	59.20 ± 2.10^{c}
Gliadin/5%-ZMEO	89.20 ± 2.22^{b}	20.10 ± 1.22^{b}	66.00 ± 3.10^{b}
Gliadin/5%-ZMEO	95.15 ± 1.11^{a}	23.08 ± 0.09^{a}	73.11 ± 2.20^{a}

Data is the average of three replicates. Different letters in a column indicate a significant difference (*P*<0.05) between the means.

Surface hydrophobicity

Pure gliadin nanofibers showed a contact angle of 50 (Table 1). Adding three different concentrations (5, 10 and 15%, w/w) of essential oil increased the contact angle of nanofibers from 59 to 73 °.

Color characteristics

The results of color parameters (L^*, a^* and b^*) of gliadin nanofibers are shown in Table (2). The highest transparency (higher L^*) was observed in pure gliadin nanofibers. Addition of essential oil up to a concentration of 15% to nanofibers decreased the transparency from 86.97 to 79.63. Also, the addition of essential oil caused the color parameter a to decrease from -1.72 to -5.73, but on the other hand, an increasing trend was observed in parameter b from 6.50 to 24.46.

Table 2. Colorimetric and turbidity values of gliadin nanofibers containing *Zataria multiflora* Boiss essential oil

Nanofibers	*L*	*a*	*b*	Turbidity
gliadin	86.97 ± 0.003^{a}	-0.62 ± 0.13^{a}	8.00 ± 0.16^{d}	55.0 ± 0.90
Gliadin/5%-ZMEO	85.21 ± 0.002^{b}	-2.34 ± 0.23^{b}	14.80 ± 0.65^{c}	60.1 ± 0.30
Gliadin/5%-ZMEO	82.81 ± 0.002^{c}	-4.28 ± 0.11^{c}	23.22 ± 1.11^{b}	69.1 ± 0.80
Gliadin/5%-ZMEO	79.63 ± 0.002^{d}	-4.63 ± 0.20^{c}	30.32 ± 0.66^{a}	86.0 ± 0.80

Data is the average of three replicates. Different letters in a column indicate a significant difference (*P*<0.05) between the means.

Antibacterial properties of nanofibers

The results showed that the pathogenic bacteria *staphylococcus aureus* (MIC, 0.125 µ/mL and MBC, 0.2 µ/mL) is the most sensitive and *pseudomonas aeruginosa* (MIC, 1.25 µ/mL and MBC, 0.5 µ/mL) is the most resistant bacteria against the antimicrobial effects of essential oil. Pure gliadin nanofibers did not show any growth halo against pathogenic bacteria. Gram-negative pathogenic bacteria had more resistance to essential oil (Tables 3 and 4).

Table 3. MIC and MBC values of *Zataria multiflora Boiss* essential oil against four pathogenic bacteria

Microorganism	MIC (µ/mL)	MBC (µl/mL)
S.aureus	0.125	0.2
Ecoli	0.2	0.4
B. subtilis	0.2	0.4
P. aeruginosa	0.5	1.25

Table 4. Antimicrobial activity of active gliadin nanofibers

Nanofibers	Inhibition area (mm^2)	
	S. typhi	*B. subtilis*
gliadin	ND^c	ND^d
Gliadin/5%-ZMEO	ND^c	89.88 ± 6.70^c
Gliadin/5%-ZMEO	61.31 ± 15.39^b	177.26 ± 4.90^b
Gliadin/5%-ZMEO	195.59 ± 9.41^a	297.68 ± 5.70^a

ND: Not detected, the listed values for inhibition halo are the mean and standard deviation of three replicates. Different letters in a column indicate a significant difference ($P<0.05$) between the means.

Antioxidant properties of nanofibers

The total phenolic content of gliadin nanofibers increased with increasing concentration (Fig. 1). The highest rate of radical inhibition (56.5%) was observed in gliadin nanofibers containing 15% essential oil (Fig. 1).

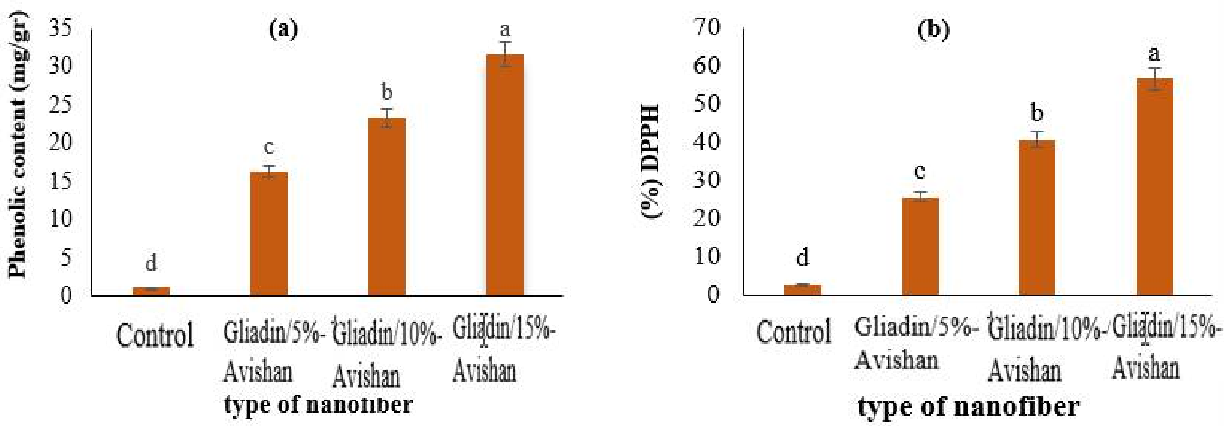

Fig. 1. Gliadin nanofibers containing *Zataria multiflora Boiss* essential oil (5, 10 and 15%, w/w), (a) phenolic content and (b) antioxidant activity. The error bars represent the standard deviation. Non-identical Latin letters indicate a significant difference ($P<0.05$) between the means.

Microbial evaluation of coated fillet

The initial amount of *listeria* bacteria in smoked fillet was 3 log. Nanofibers containing 15% essential oil caused a decrease of about 3 logs in the listeria population compared to the uncoated sample on the 16th day of storage (Fig. 2).

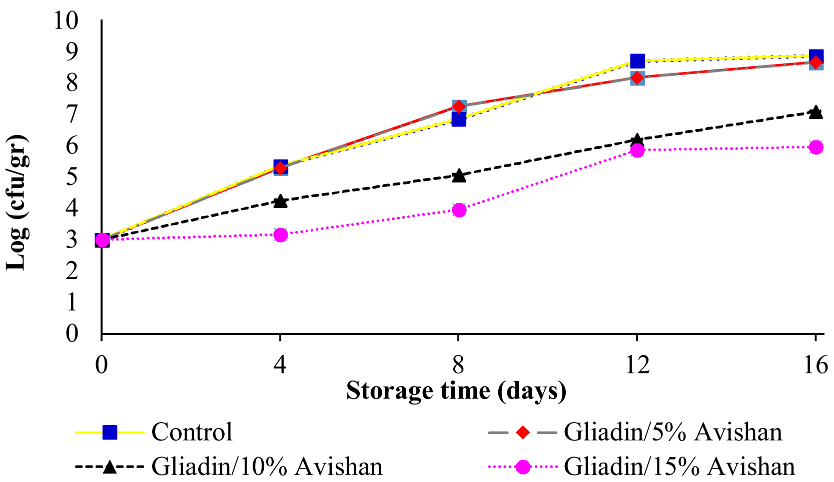

Fig. 2. The logarithmic average of *Listeria monocytogenes* bacteria in smoked salmon fillet during 16 days of storage at refrigerator temperature

Conclusions

In this research, the effect of different concentrations of Shirazi thyme essential oil on the properties of active gliadin nanofibers was investigated. In general, the results of this study showed that this nanofiber can be used as an active packaging in covering meat and agricultural products in order to increase their shelf life.

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