



Protective effect of grape seed oil-loaded nanofibers: Limitation of microbial growth and lipid oxidation in kashar cheese and fish meat samples

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ABSTRACT

Grape seed oil-loaded nanofibers (gsN) having 414.8 ± 58.7 nm diameter were fabricated using the electrospinning technique. Scanning electron microscopy images, encapsulation efficiency (92.4%), and molecular characterization analysis (FTIR) proved successful production of electrospun gsN. Limitation in total mesophilic aerobic bacteria count (TMABC) of kashar and fish meat samples coated with grape seed oil-loaded nanofibers and then stored at cold storage conditions was found in the range of 1.40 and 1.53 log during the experimental period. Coating with gsN as compared to the control group samples for each sample delayed rapid total yeast and mold growth in kashar (28%) and fish meat samples (20%) ($p < 0.05$). TBA value of fish control samples was increased from 1.38 to 2.06 mg MDA/kg in fish control samples and 1.65 mg MDA/kg in fish meat coated with gsN. In addition to fish samples, at the end of the 20th day, while the initial TBA value was determined as 1.32 mg MDA/kg, it reached 2.45 in kashar control samples and 2.18 mg MDA/kg in kashar coated with gsN. In this respect, besides microbiological limitation, grape seed oil nanofiber coating was found to be highly effective against the rapid oxidation in fish and kashar samples stored at 4 ± 1 °C ($p < 0.05$). The use of grape seed oil within nanoformulation effectively provided obtaining higher quality products having two different matrices in the food industry. Therefore, this nano methodology provided with the electrospinning technique could play a guiding role for different food products in the industry as well.

1. Introduction

Food is a vital source to be able to sustain human life. It is known to the consumer that food safety is as important as food sustainability. In this respect, after harvesting or following some processes, some food preservation methods called conventional are already used to improve food safety. Among them, food additives, irradiation technology, different kinds of food packaging treatments, edible coating are utilized to improve food stability. On the other hand, today, instead of food additives, edible coating, and packaging applications in food science, nano-based applications such as coating, wrapping have been successfully used.

There are a few ways in order to obtain nano-scale materials. In this regard, spray drying and freeze-drying are widely known (Drusch et al.,

2007; Ratti, 2013). Especially, the uses of electrospinning and electrospinning, which evaporate the solvents under high voltage and provide nano-scale material, have risen steadily during the past decade (Ceylan, 2019; Isik et al., 2018; Lim et al., 2019; Yilmaz et al., 2020). With electrospinning methods, different types of biopolymers like chitosan, oils, protein-based materials, bioactive materials like thyme could be electrospun, and then obtained nano-scale material could be effectively used in order to delay the microbiological spoilage, chemical and sensory deterioration in foods (Ceylan, Unal Sengor, Basahel et al., 2018; Ceylan, Meral, Alav, et al., 2020). This is a highly novel application and it can be evaluated as a promising technology for food science and the food industry. Actually, nanoscience applications within the food can present different types of materials having antimicrobial properties. For example, nanoemulsions that could be obtained from different essential,

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commercial, and citrus essential oils such as rosemary, laurel, thyme, sage, sunflower, canola, corn, olive, orange, and lemon, could be effectively used to improve the shelf life of fish meat (Uçak et al., 2011; Ozogul et al., 2017, 2020; Yazgan et al., 2017; Durmus, 2020; Uçar, 2020). Besides nanoemulsions, nanoparticles were successfully limited the rapid microbiological spoilage of different sorts of food materials (chicken and fish) stored at cold storage conditions (Galocchio et al., 2016; Ceylan, 2018).

As known, the consumption of meat products is important for the consumers. But, particularly, fish meat is highly perishable because of the fact that it contains high-level moisture and weak connective tissue etc as compared to the connective tissue of land animals (Petricorena, 2015). Besides meat, chicken, fish meat, the consumption of dairy products play a key role within a well-balanced diet as well. In this sense, cheese is widely consumed in daily life by consumers. Although it consists of highly important nutrients, kashar samples can be easily spoiled. Especially, besides the total aerobic bacteria, total aerobic psychrophilic bacteria, the rapid yeast and mold growth in the samples can be observed during the cold storage period (Akarca & Tomar, 2019). In this respect, nanoencapsulation of material rich in phenolic compounds, fatty acids, and vitamins, with economic importance food industry is evaluated as novel approach (Ceylan, Unal Sengor, & Yilmaz, 2018; Garavaglia et al., 2016). Grape seed oil may be evaluated as a potential nanoencapsulated material having higher encapsulation efficiency.

The main aim of the present work was to successfully fabricate grape seed oil-loaded PVA-based nanofibers using the electrospinning technique. The next step aim of the study was to characterize the nanomaterials with morphological, molecular properties, encapsulation efficiency, and then treat the fish meat and kashar samples with obtained and characterized nanomaterials. The final goal of the study was to reveal the potential effect of the nano-processing on oxidative properties, total mesophilic bacteria growth, total yeast, and mold growth of kashar cheese and fish meat samples stored at the same cold storage conditions.

2. Material and method

2.1. Material

Raw fish (rainbow trout) and kashar cheese samples were obtained from a national market. All samples were transferred in isolated boxes to the food processing laboratory. The skin and bones of whole fish samples were removed for further treatment. Whole kashar cheese samples were sliced using a knife, which was sterilized in an autoclave for this application. Food materials after pre-treatments were stored at cold storage conditions for the nano-coating process. In addition to food materials, grape seed (GS) oil was obtained from Arpaş Arifoğlu Marketing Distribution and Trade Inc. (İstanbul-Turkey), Poly(vinyl alcohol) (PVA: Mw: 89.000–98.000,99+%hydrolyzed) was purchased from Sigma-Aldrich (Germany).

2.2. Methods

2.2.1. Electrospinning process

For fabrication of nanofibers, Fytronix ESP-900 Electrospinning system (Elazığ-Turkey) consisting of a high voltage unit, syringe pump unit, the flat collector was used. Before the electrospinning process, the dope solution which was used directly for the fabrication of nanofibers was prepared. For this purpose, PVA was solubilized (10% w/v, in pure water) at 80 °C for 180 min. The dope solution was prepared by mixing 10 mL of PVA solution with 0.5 g of grape seed oil and 0.1 g of Tween 20. The dope solution was transferred within a plastic syringe. The flow rate of the dope solution was adjusted to 0.04 mL/min. The other electrospinning parameters were as follows: the voltage was 26 kV. The tip-to-collector distance was kept at 11.5 cm.

2.2.2. Morphological characterization of nanofibers

Morphological characterization of electrospun nanofibers was defined under low vacuum in a field emission scanning electron microscope: SEM (FEI, Quanta Feg 250, and the USA) at more than one magnification with a working distance adjusted to 8 mm. An accelerating voltage of 5 kV was defined in order to obtain secondary electron images. The mean value of GS electrospun nanofibers with standard deviation was revealed by using different measurements.

2.2.3. Molecular characterization of nanofibers

The molecular structures of grape seed oil-loaded nanofibers were revealed using ATR-FTIR (Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy). A Bruker Tensor 27 spectrometer (Bremen-Germany) attached with a DLATGS detector and a KBr beam splitter was used to obtain the spectrum. Diamond ATR cell was used in an ATR accessory. The analysis was controlled and saved using an OPUS software version 7.2 for Windows. The ATR-FTIR spectra of grape seed oil-loaded nanofibers were saved with a resolution of 2 cm⁻¹, accumulating 16 scans per spectra. The spectra, subtracted against the background air spectrum in grape seed oil-loaded nanofibers were saved between 4000 and 600 wavenumber/cm.

2.2.4. Encapsulation efficiency

Encapsulated grape seed oil amount was calculated by UV–vis absorbance value of supernatant that was obtained following centrifugation of the GS-loaded nanofibers. UV–vis absorbance value of supernatant given formula below, which was obtained from the calibration curve, was determined.

$$\text{Encapsulation Efficiency} = \frac{\text{Total GS Amount} - \text{Free GS Amount}}{\text{Total GS Amount}} \times 100$$

2.2.5. Total mesophilic aerobic bacteria (TMAB)

TMAB count of the samples coated with grape seed oil-loaded nanofibers and control samples were determined as stated by Maturin and Peeler (1998). 10-g fish meat and kashar cheese nano and control samples were separately homogenized during 150 s in a stomacher (Spain, IU Instruments) with 90 mL peptone water prepared 0.1%. Serial dilutions (10¹ to 10⁶) were prepared for all samples during the experimental day. Plate count agar (Merck, Darmstadt-Germany) was placed on diluted samples. All diluted samples prepared from nano and control samples were incubated at 35 °C for 48 h in order to determine TMAB growth during nine days (n = 12).

2.2.6. Total yeast and mold

Dicloran rose bangal agar (DRBC, Merck) was used in order to observe TYMc by incubating the samples at 23 °C during 5 days (Halkman, 2005). All microbiological measurement was repeated twelve times for each group (n = 12).

2.2.7. Thiobarbituric acid

Homogenized (10 g) fish samples treated with grape seed oil-loaded nanofibers and control group samples, distilled water (97.5 mL) and HCl (4 N, 2.5 mL) were added into round bottom flask with joint and then heated. 5 mL distillate was added to tubes. All test tubes were heated in hot water at 70 °C for 30 min. Once color changes in the tubes were occurred, absorbance values were measured at 538 nm using spectrophotometer. Obtained results were defined to be mg malondialdehyde per kg (mg MDA/kg) in all analyzed samples (Tarladgis et al., 1960).

2.2.8. Statistical evaluation

Measurements in the present study were repeated twice with three replications. All obtained data were subjected to analysis of variance in order to reveal the TBA, TMAB, and TYM in all samples. Graphpad Prism software Version 5.00 (California Corporation, CA) was performed to

determine significant differences between the two groups and also comparisons of all differences were evaluated using Tukey's Multiple Range Test ($p < 0.05$).

3. Result and discussion

3.1. Morphological properties of grape seed oil-loaded nanofibers

SEM images of electrospun grape seed oil-loaded nanofibers (gsN) are given in Fig. 1. These images reflected that the beadless grape seed oil-loaded nanofibers were obtained. The average diameter of gsN was measured to be 414.8 ± 58.7 nm. On the other hand, smooth surface nanofibers without encapsulation had 250 ± 40.3 nm diameter. As were seen from the diameters, nanoencapsulation of grape seed oil into nanofibers increased the average diameter of nanofibers. This increase in the diameter could successfully confirm the encapsulation of grape seed oil into nanofibers. Already, the average diameters of nanostructures could change depending on the type of material, the parameters of electrospinning, etc (Ceylan, Meral, Özogul, et al., 2020). The average diameters of kafirin-based fish oil nanocapsules were determined in the range of 552 and 861 nm (Cetinkaya et al., 2021) while nisin and curcumin-loaded poly(vinyl alcohol) (PVA)-based nanofibers had a lower (172 nm) average diameters (Ceylan, Meral, Alav, et al.,). There is no doubt that the type of used materials such as oil or protein-based materials etc is able to take a role in the definition of the average diameter of the nanofibers. Being revealed with the present study that loading of grape seed oil into PVA-based nanofibers clearly increased the mean value of the nanofibers.

3.2. Molecular characterization of grape seed oil-loaded nanofibers

The spectra obtained from the grape seed oil-loaded nanofibers are shown in Fig. 2. FTIR spectrum reflects molecular structures of the analyzed sample. As could be seen from the literature comments, the band at around 3270 cm^{-1} could associate with the OH stretching modes. The broad band peaking at around 3009 cm^{-1} is correlated with the C-H stretching of the *cis*-double bond ($=\text{CH}$). As well, asymmetric and symmetric stretching vibrations at 2923 and 2853 cm^{-1} define CH₂

groups, which are classified within the hydrocarbon chains of the lipid or lignin. C=O bonds of the ester groups (at 1744 cm^{-1} and 1716 cm^{-1}) are associated with the fatty acids and their glycerides, pectins, and lignins. The bands at around 1600 cm^{-1} are related to the stretching of C=O- and aromatic C=C groups, for example in pectins and phenolic compounds. The spectra region between 1500 and 800 cm^{-1} includes significant knowledge based on organic compounds, like organic acids, sugars, and alcohols. When they are more clearly examined, the bands found at round 782, 1035 and between 1520, and 1443 define phenolic compounds and polysaccharide structures (Kennedy et al., 2000; Wilson et al., 2000; Heredia-Guerrero et al., 2014; Lupoi et al., 2015; Gao et al., 2015; (Torres-Climent et al., 2015); (Fasoli et al., 2016); (Ceylan, Unal Sengor et al., 2017). In the present nano-study, especially, the characteristic bands were obtained at 3871.13, 3327.21, 2926.01, around 2775, 2312.65, 1735.93, 1535.34, 1379.10, 1249.87, 1168.86, 1089.78, 1053.13, 1028.06, 948.98, 840.96, 669.30, 638.44. The bands obtained from grape seed oil-loaded nanofibers were found to associate with literature studies as given above.

3.3. Encapsulation efficiency

The encapsulation efficiency of the grape seed oil was found to be $92.4 \pm 0.1\%$. This ratio revealed that the grape seed oil was effectively encapsulated into nanofibers, which were used as a nanocoating material for fish meat samples. Snehalatha et al. (2008) noted that encapsulation efficiency of etoposide (into PCL) was found as 80.15%. On the other hand, the encapsulation of fish oil into carbohydrate particles having 0.1 and $1.5 \mu\text{m}$ size, was defined around 70% as described by García-Moreno et al. (2017). According to Prieto & Lagaron (2020), selected encapsulation process and composition of the shell material could play significant role in the determination of encapsulation efficiency. In this respect, being clearly seen that the obtained encapsulation efficiency may affect the oxidative stability and the growth in bacterial count in fish meat and kashar samples stored at refrigerated conditions.

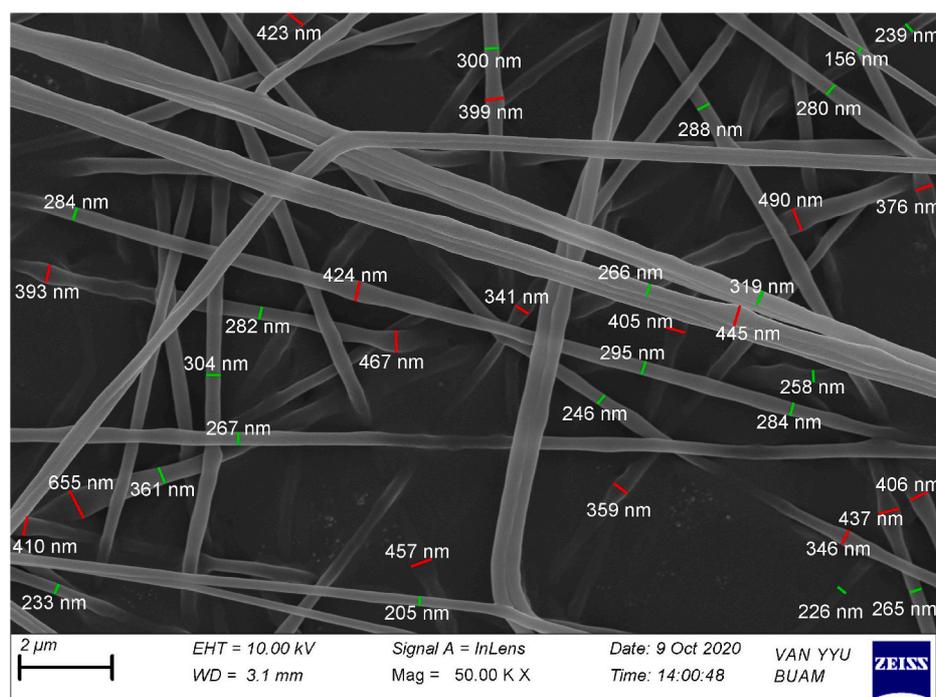


Fig. 1. Morphological characterization of grape seed oil-loaded nanofibers.

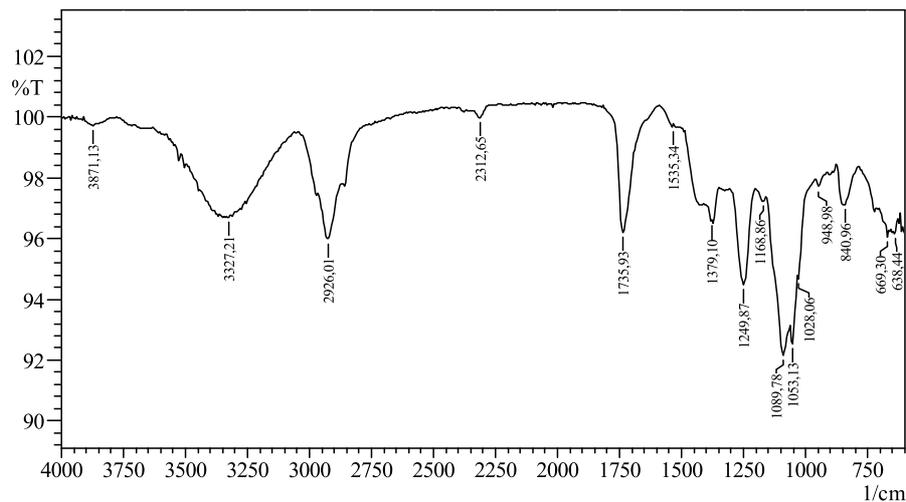


Fig. 2. Molecular characterization for grape seed oil-loaded nanofibers.

3.4. Total mesophilic aerobic bacteria count

Mesophilic aerobic bacteria counts of nano-coated and control group samples for fish meat and kashar are given in Tables 1 and 2. The initial TMAB loads of fish meat and kashar samples were defined to be 3.00 (± 0.07) and 4.22 (± 0.03) log CFU/g, respectively. The initial TMAB value of fish samples was in the scope of consumption value described by ICMSF (1986). For kashar cheese, the initial TMAB count could be determined as higher. In this respect, this value was found to be 2.6×10^6 CFU/g as stated by Yıldız Akgül et al., (2019). As known, the initial load of the food materials depends on different factors such as processing and initial contamination or environmental conditions (Ceylan, Meral, et al., 2018). Fish fillets coated with grape seed oil nanofibers had a lower TMAB count than control group fish meat samples during the 9-day storage period ($p < 0.05$). At the same cold storage conditions ($4 \pm 1^\circ\text{C}$) with fish samples, the initial TMAB count of kashar samples was higher during-six the day's cold storage period. In some food matrix, nanotechnology application can decrease the initial bacterial count following nanoprocess (Meral et al., 2019; Ceylan, Meral, Özogul, et al., 2020). Moreover, the changes in TMAB count of fish meat samples and kashar samples as compared with the control group samples were

Table 1

Microbiological quality parameters and TBA values of control and coated rainbow trout fillets.

Storage Period		Microbial Growth	Initial	1st day	3rd day	7th day	9th day
Control	TMABc		3.00 \pm 0.07	3.45 \pm 0.07 ^{aD}	4.63 \pm 0.15 ^{aC}	6.23 \pm 0.28 ^{aB}	7.70 \pm 0.11 ^{aA}
	TYM		2.03 \pm 0.05	2.83 \pm 0.01 ^{aD}	4.07 \pm 0.02 ^{aC}	5.12 \pm 0.01 ^{aB}	6.23 \pm 0.18 ^{aA}
	TBA		1.38 \pm 0.02	1.44 \pm 0.01 ^{aB}	0.93 \pm 0.06 ^{aC}	2.00 \pm 0.14 ^{aA}	2.06 \pm 0.07 ^{aA}
gsN	TMABc		3.24 \pm 0.04 ^{aD}	3.24 \pm 0.02 ^{bC}	3.69 \pm 0.14 ^{bB}	5.08 \pm 0.16 ^{bB}	6.17 \pm 0.06 ^{bA}
	TYM		2.73 \pm 0.11 ^{aD}	3.64 \pm 0.05 ^{bC}	4.12 \pm 0.16 ^{bB}	4.96 \pm 0.01 ^{bA}	
	TBA		1.39 \pm 0.02 ^{aB}	0.68 \pm 0.00 ^{bD}	1.14 \pm 0.01 ^{bC}	1.65 \pm 0.04 ^{bA}	

^{a-b} Within each column, different superscript lowercase letters show differences between treatment groups for same storage day ($p < 0.05$). ^{A-D} Within each row, different superscript uppercase letters show differences between the storage days within same analysis group ($p < 0.05$). C: Fillets uncoat ed, gsN: fish fillets treated with grape seed oil loaded nanofiber, TMABc: Total mesophilic aerobic bacteria counts; TYM: Total yeast and mold; TBA: thiobarbituric acid value.

calculated as 33.4% and 8.53%, respectively. A study related to fresh-cut apple treated with probiotic bacteria revealed that during the first 4 days at 5°C , no TMAB growth was determined as compared with the initial value as described by Alegre et al. (2011). With the present study, the differences between control and the kashar samples coated with grape seed oil nanofibers reached 1.40 log on the 3rd day of the cold storage ($p < 0.05$). In addition to kashar samples, during the experimental period for fish meat samples, up to 1.53 log CFU/g limitation was observed as compared to control group samples ($p < 0.05$). Encapsulation of grape seed oil into nanofibers by using electrospinning technique provided better preservation for kashar and fish meat samples. There is so limited study related to nanofiber on different foods in the literature. But, it could be observed that the presence of lactic acid bacteria in foods could have an antimicrobial activity for foods (Angiolillo, Danza, Conte, & Del Nobile, 2017; Angiolillo et al., 2014). The rapid increase in TMAB growth could not have been observed, because of the fact that kashar consists of bacteriocin or lactic acid bacteria. However, it was clearly seen that grape seed oil-loaded nanofibers could effectively delay the TMAB growth in fish meat and kashar samples stored at 4°C .

3.5. Total yeast & molds

The results for TYMc values in kashar and fish meat samples are presented in Tables 1 and 2. The TYMc of the raw material of kashar and fish meat samples were defined as ND and 2.03 log CFU/g, respectively. For kashar samples as compared with control group samples, TYMc of the kashar samples coated with grape-seed oil were significantly lower ($p < 0.05$) after the 13th day. On the other hand, the same nanomaterial obtained from grape seed oil provided significant limitations in the TYM growth of fish meat after the first day of cold storage ($p < 0.05$). Furthermore, while grape seed oil-loaded nanofibers provided a limitation up to 29% in kashar samples, this nanoapplication delayed the rapid TYM growth in the fish meat samples around 20%. The inhibitory effect of grape seed extract on the growth of TYM in Bacon samples was defined as stated by Wang et al. (2015). Also, grape seed extract with 0.2% effectively reduced the growth rate of TYM in buffalo veal slices (Singh et al., 2018). According to (Ceylan, Unal Sengor et al., 2017), the particle size could play a key role in order to limit the rapid increase in TYM count in fish meat. In this respect, with the present study results, grape seed oil in nanoformulation with 414.8 nm diameter showed a good antimicrobial effect on the surface of kashar and fish meat samples.

Table 2
Microbiological quality parameters and TBA values of control and coated kashar.

Storage Period		Initial	1st day	3rd day	6th day	13th day	20th day
Group	Microbial Growth						
Control	TMABc	4.22 ± 0.03	4.37 ± 0.1 ^{8aB}	5.05 ± 0.09 ^{aA}	5.18 ± 0.04 ^{aA}	5.14 ± 0.18 ^{aA}	5.63 ± 0.25 ^{aA}
	TYM	0.00 ± 0.00	0.00 ± 0.00 ^{aE}	1.99 ± 0.01 ^{aD}	2.22 ± 0.04 ^{aC}	3.57 ± 0.1 ^{aB}	5.36 ± 0.06 ^{aA}
	TBA	1.32 ± 0.02	1.32 ± 0.01 ^{aBC}	1.22 ± 0.01 ^{aC}	1.29 ± 0.0 ^{aBC}	1.81 ± 0.09 ^{aB}	2.45 ± 0.32 ^{aA}
gsN	TMABc		3.35 ± 0.07 ^{bC}	3.65 ± 0.07 ^{bBC}	4.21 ± 0.13 ^{bAB}	4.71 ± 0.21 ^{aA}	4.58 ± 0.2 ^{bA}
	TYM		0.00 ± 0.00 ^{aD}	1.92 ± 0.07 ^{aC}	2.17 ± 0.06 ^{aC}	3.19 ± 0.01 ^{bB}	3.76 ± 0.25 ^{bA}
	TBA		0.61 ± 0.07 ^{bB}	0.81 ± 0.01 ^{bB}	0.97 ± 0.01 ^{bB}	1.10 ± 0.11 ^{bB}	2.18 ± 0.18 ^{bA}

^{a-b} Within each column, different superscript lowercase letters show differences between treatment groups for same storage day ($p < 0.05$). ^{A-E} Within each row, different superscript uppercase letters show differences between the storage days within same analysis group ($p < 0.05$). TMABc: Total mesophilic aerobic bacteria counts; TYM: Total yeast and mold; TBA: thiobarbituric acid value gsN: kashar cheese treated with grape seed oil loaded nanofiber

3.6. Oxidation

TBA values of control and coated samples of fish meat and kashar are presented with Tables 1 and 2, respectively. Initial TBA values in fish meat and kashar were 1.38 and 1.32 mg MDA/kg. Depending on the increase in the experimental period, the TBA value of fish meat control samples reached 2.06 mg MDA/kg while that of kashar samples was found to be 2.45 MDA/kg. TBA value lower than 3 mg MDA/kg like could be evaluated as good quality for fish samples as stated by Ceylan, Sengor, and Yilmaz (2017). In this respect, more rapid oxidation in control group kashar samples was observed as compared to the fish control group samples. The reason could be associated with the nutritional composition of the samples especially fat content could have been played an important role in this oxidation. As stated by Ünal et al. (2013), the uncoated cheese (control: 1.36 mg MDA/kg) samples possessed higher TBA values than samples packed with films. Ceylan, Sengor, and Yilmaz (2017) reported that TBA value of fish fillets (sea bass) could reach 2.5 mg MDA/kg at 4 ± 1 °C. As were seen from TBA results and compared with control group results, grape seed oil nanofiber coating was highly effective to protect the rapid oxidation in fish and kashar samples stored at 4 ± 1 °C ($p < 0.05$). Furthermore, release and amounts of phenolic compounds, shown by FTIR in the present study, from grape seed oil within nanofibers were sufficiently effective in order to suppress the oxidative changes in kashar and fish meat samples at 4 ± 1 °C.

4. Conclusion

Electrospun grape seed oil-loaded nanofibers (gsN) possessing 414.8 nm average diameters was successfully obtained. Characterization analyses revealed that encapsulation was effectively provided within nanofibers, which were used as coating materials in fish meat and kashar samples. TMAB and TYM growth in two different foods coated with electrospun gsN was effectively delayed (~ 1.50 log CFU/g) at the same storage conditions. Furthermore, rapid oxidation in both food samples was limited (up to 0.85 mg MDA/kg) by using grape seed oil-loaded nanofibers. These nanostudy results related to fish meat and kashar samples have presented an alternative approach for the use of the food industry.

Author statement

Zafer Ceylan: Data Curation, Investigation, Visualization, Conceptualization, Writing-Original Draft Preparation, Review & Editing. **Nazan Kutlu:** Formal Analysis, Validation, Investigation. **Raciye Meral:** Resources, Formal Analysis, Data Curation, Methodology, Review & Editing. **Mehmet Mustafa Ekin:** Formal analysis, Validation, Investigation. **Yagmur Erim Kose:** Resources, Validation, Data Curation.

Declaration of competing interest

The authors have declared that they have no conflict of interest for this publication.

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