Uluslararası Tarım Araştırmalarında Yenilikçi Yaklaşımlar Dergisi
International Journal of Innovative Approaches in Agricultural Research 2023, Vol. 7 (4), 403-412
https://doi.org/10.29329/ijiaar.2023.630.2

Original article

Black Carrot Extract Containing Polyvinyl Alcohol-Based Nanofibers: Structural Characterization and Determination of Total Oxidant-Antioxidant Capacity

Turgay Çetinkaya [a], Mehmet Aydın Dağdeviren [b] & Sibel Bayıl Oğuzkan [c, *]

[a] Department of Food Processing, Armutlu Vocational School, Yalova University, Yalova, Türkiye
[b] Deparment of Biology, Gaziantep University, Gaziantep, Türkiye
[c] Deparment of Medical Services and Techniques, Vocational School of Health, Gaziantep University, Gaziantep, Türkiye

Abstract

In this study three different electrospun nanofiber samples, named as polyvinly alcohol (PVA), polyvinly alcohol +black carrot extract (PVAB) and polyvinly alcohol+black carrot extract+tin dioxide (PVABT), were produced successfully using the electrospinning method. According to characterization analysis by scanning electron microscopy (SEM), it has been found that PVA nanofibers were continuous and they preserved their uniform structure and average diameters were measured as 215.76±75.47. With the addition of black carrot extract, fiber diameters increased to 637.97±91.85 nm. On the other hand, for PVABT accumulation of new structures observed and diamater thickness increased to 658.66±101.5 nm respectively. Energy Dispersive X-ray Spectroscopy elemental mapping results of PVABT sample proved the binding and integration of the tin dioxide to the surface of the electrospun nanofibers. Antioxidant properties are also compared to determine how nanofiber coating of plant extracts effect on Total Oxidant Level (TOL) – Total Antioxidant Level (TAL). While the antioxidant level of the black carrot extract covered with nanofiber was ~62% higher than the extract without nanofiber, it was determined that the extract-metal oxide combination showed higher antioxidant results. In addition, no oxidant was detected in electrospun nanofiber samples. Overall, it has been concluded that nanofibers can be fabricated in combination with plant extract and metal oxides and addition of these materials have ability to effect characteristic properties and antioxidant properties. Therefore, metal oxides and plant extracts have the potential to be used as an active food packaging ingredients for further applications in food industry. In the future, it will be important to determine other properties such as thermal stability, mechanical properties, or water vapor permeabilities of these nanomaterials.

Keywords: Black Carrot, Electrospinning, Nanofiber, TAL, TOL.

Received: 21 September 2023 * **Accepted:** 17 December 2023 * **DOI:** https://doi.org/10.29329/ijiaar.2023.630.2

* **Corresponding author:**

Sibel Bayıl Oğuzkan, Deparment of Medical Services and Techniques, Vocational School of Health, Gaziantep University, Gaziantep, Türkiye.
Email: bayil@gantep.edu.tr

INTRODUCTION

The enrichment of food matrices with carotenoids, antioxidants, bioactive and antimicrobial compounds have gained more interest in the lact decade. As known, anthocyanin compounds from fruit and vegetables has antioxidant properties (Espinosa-Acosta et al. 2019). Microencapsulation is one of the method of incorporating polyphenols, natural antioxidants in small capsules in order to stabilize and protect them against nutritional as well as health losses. Microencapsulation of black carrot extract using spray dryer at inlet and outlet temperature of 150°C and 76°C with 2 mL/min feed rate were reproted to be for efficient quality anthocyanin powder. However, high temperature may affect antioxidant properties negatively. Therefore alternative encapsulation methods such as electrohydrodynamic nanoencapsulation gaining interest (Kar et. al 2019, Cetinkaya, 2023).

Nanomaterials represent promising frontiers in the research for improving antioxidants properties. Nanofiber production by electrospinning process may influence antioxidant content of the materials (Valgimigli et al. 2018). In a recent study by Zhao et al. (2023) zein/gelatin based nanofibers had poor (7.67%) antixidant capacity, but the antioxidant value was increased to 72.3% after incorporation of 5% gallic acid, which shows that wrapping materials with polyphenols preserve cherries and can be used as active packaging materials. In antoher study Bharathi et al. (2020) zein nanofiber coating on chitosan films improved its antioxidant activity from 12.41% to 44.17%, which was used to prevent browning in apple slices.

On the other hand, several nanoparticles, including cerium oxide nanoparticles, iron oxide nanoparticles, titanium oxide nanoparticles, selenium oxide nanoparticles were developed for the purpose of antioxidant applications. Because metallic nanoparticles can cause oxidative stress in the cell and cell wall, which cause antioxidant activities. The oxidation mechanisms of nanoparticles are mostly ROS-mediated. The mechanism causing oxidative stress is slightly different amongst metal nanoparticles, and is dependent on size or shape of the nanoparticles. Some of these nanoparticles could exhibit antioxidant effects due to the decoration of the bioactivities or fabricated together with the antioxidant moieties (Ge et al. 2022, Samrot et al. 2022, Cetinkaya, 2023). It has been stated that the antioxidant properties of the metal nanoparticles are mostly rendered with the combination of plant extracts (Samrot et al. 2022). So, metal nanoparticles could be applied also with combination of plant extracts in the food industry. Therefore, aim of this study is to development, structural characterization, and evaluation antioxidant activity of plant extract containing electrospun polyvinilvy alcohol based nanofiber samples.

MATERIAL AND METHOD

Material

Black carrot extract 30W-05 (Ref:KN006298-1) is received from Döhler Chemical. Polyvinily alcohol (BP17) is provided from ZAG Industrial Chemicals. Distilled water was used as dispersant.

Method

Production of nanofibers

Distilled water was used as a solvent to dissolve 10% polyvinyl alcohol which represent control solution (PVA). 5% (w/w) black carrot extract and tin dioxide 5% (w/w) added to prepare PVAB and PVABT solutions, respectively. Solutions were blended approximately for 30 min at 600 rpm using a magnetic stirrer at 70 °C. Electrospun pure polyvinly alcohol nanofibers or with materials were fabricated by Inovenso NE100 equipment. Parameters arranged as 0.8 mL/h feed rate, voltage of 10 kV, and 17 G needle was used. The collector was covered with white silicon-based paper. Needle-collector distance was kept at 10 cm.

Scanning Electron Microscopy

SEM (FEI, Inspect S50, Hillsboro, OR USA) with an EDS (Energy Dispersive X-ray Spectroscopy) detector under 20-25 kV accelerating voltage and distance of 8.6-8.8 mm was used to examine the morphology of nanofibers. Team Prime (EDAX Inc V4.1) software was used for elemental analysis of tin dioxide containing sample. The minimum, maximum, and average diameter of nanofibers were obtained with selected 50 random nanofibers.

Antioxidant and Oxidant Level of nanofibers

Rel Assay Diagnostics kits (TAL, TOL) were used to detect the antioxidant activity of black carrot extract, black carrot containing elecrospun nanofibers (PVAB) and black carrot+tin dioxide containing elecrospun nanofibers (PVABT) samples.

Determination of Total Antioxidant Level (TAL) by ABTS Method

Oxidative stress index (OSI = total oxidation level-TOL/total antioxidant level-TAL), sensitive method, provides convenience in understanding the effectiveness of the substances used in the experiments. The antioxidant substances in the example convert the dark blue-green colored ABTS radical (2,2 AzinoBis (3-Ethyl Benzo Thiazoline-6- Sulfonic Acid) to the reduced ABTS form and the change in the absorbance at 660 nm indicates, for example, the total antioxidant level. Radical ABTS was used as a positive control and Trolox, a synthetic antioxidant and Vitamin E analogue, was used as a positive control (Halliwell et al. 2000). Rel Assay Diagnostics- TAL Assay Kit was used for this analysis.

Determination of Antioxidant Activity by Total Oxidant Level (TOL)

This test is calibrated with hydrogen peroxide. Ferrous ion chelator in the presence of oxidants it oxidizes its complex to ferric ion. The presence of oxidants in the reaction medium depending on the oxidation reaction intensity increases. Ferric ion acidic it creates a colorful complex in the environment. The color intensity is spectrophotometrically measured. The absorbance value indicates the oxidant value in the sample. The results obtained as µmol H_2O_2 Equiv. The TOL value is determined by comparing with /L (Tarpey et al. 2004). Rel Assay Diagnostics- TOL Assay Kit was used for this experiment.

RESULTS AND DISCUSSION

SEM Results

As shown in SEM image of pure PVA nanofiber sample (Figure 1), the average was measured as 215.76±75.47 nm without any beads. With the addition of black carrot extract, the diameter increased to 637.97±91.85 (Table 1) still with homogenious formation (Figure 2).

Table 1. Electrospun polvinly alcohol baed nanofiber sample diameters.

Sample	Diameter (nm)		
	Minimum	Maximum	Average
PVA	125.5	561.5	215.76±75.47
PVAB	485.44	822.42	637.97±91.85
PVABT	469.65	989.19	658.66±101.5

The addition of black carrot extract into the solution, increases the viscoelasticity of the polymer jet, making the stretching of fibers more difficult, thereby increasing the minimum, maximum and average fiber diameters (Sun et al. 2014; Prietto et al. 2018). Therefore, diamater differences may be attributed to the change of electrical conductivity, viscosity, and surface tension of the solution. Similarly, Shavisi and Shahbazi (2022) studied the mean diameters of chitosan-gum Arabic and chitosan-gum Arabic+Rosa damascene extract nanofibers and observed larger diameter of Rosa damascene extract added nanofibers (725.39±0.46 nm) compared to the neat ones (300.12±0.25 nm). As can be seen from Figure 3, the average fiber diameter increased to 658.66±101.5 and new structures have been observed on the nanofibers after the addition of tin dioxide (inset image) which are attributed to agglomorated metal oxides.

Furthermore, as can be seen from Figure 3, when these structures integrated on the surface of some parts, these nanofiber parts enlarged.

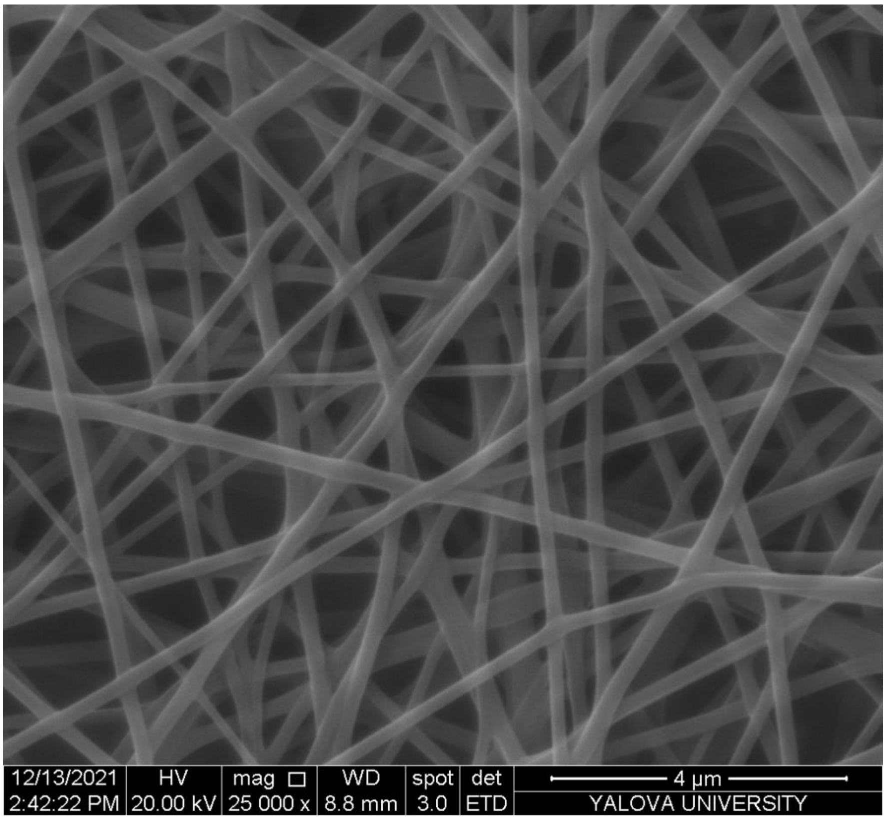

Figure 1. Nanostructure of pure polyvinly alcohol nanofibers.

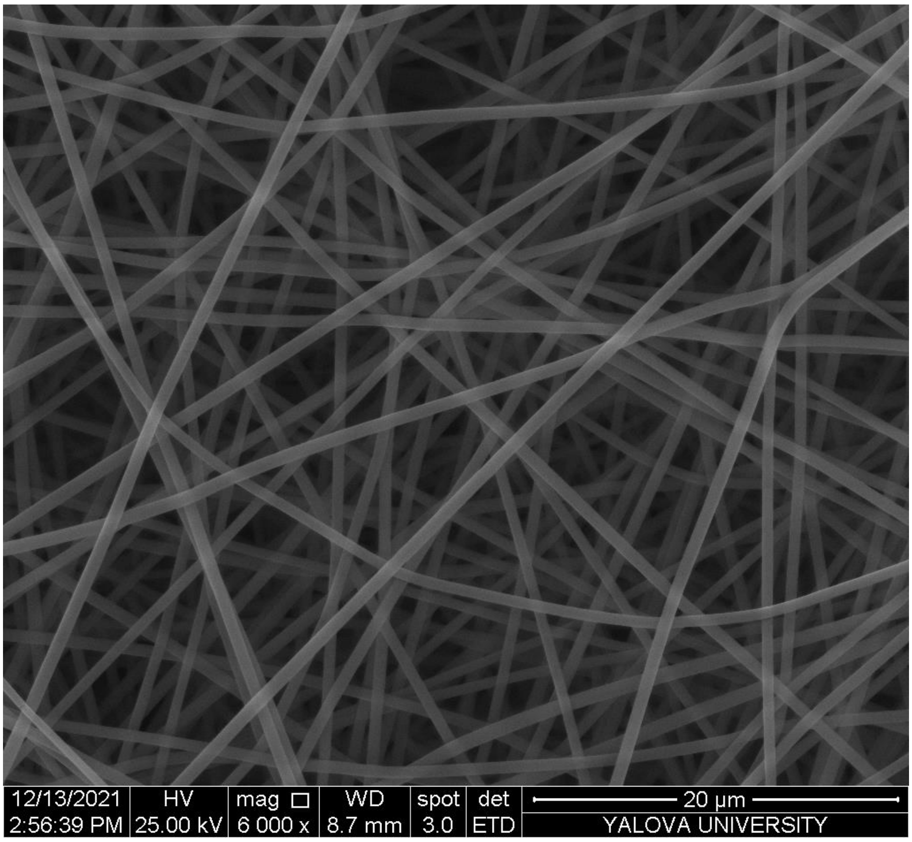

Figure 2. Nanostructure of black carrot extract containing polyvinly alcohol nanofibers.

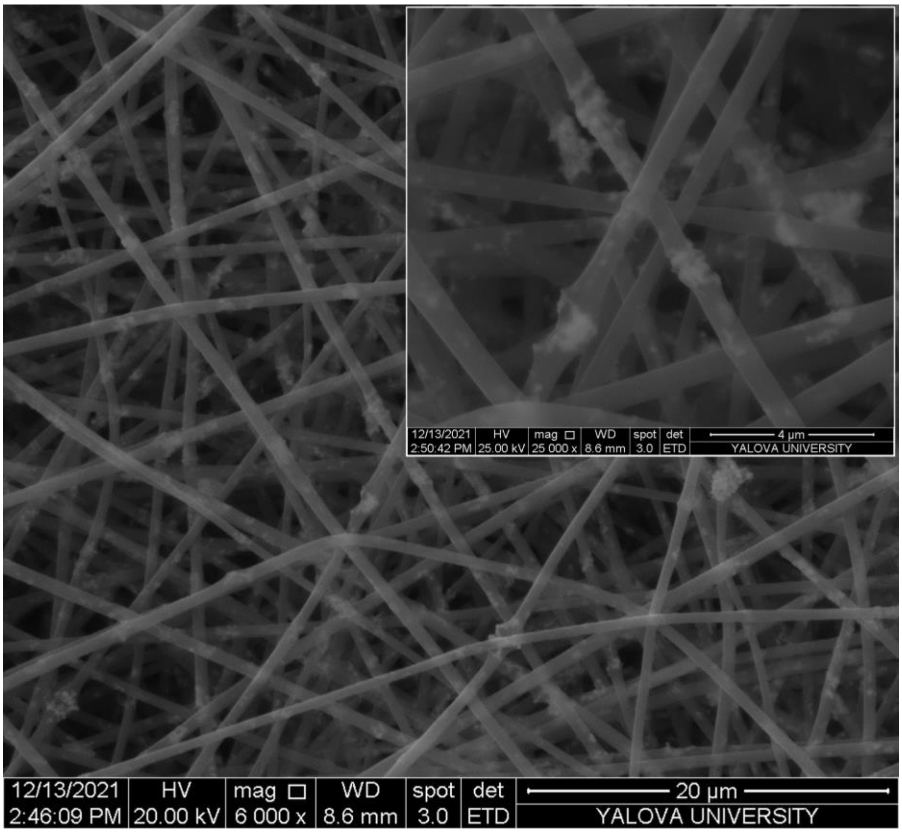

Figure 3: Nanostructure of black carrot extract and tin dioxide containing polyvinly alcohol nanofibers. Inset: enlarged image of showing tin dioxide$_2$ bumpbs.

The EDS analysis also confirmed the attachment of tin dioxide to the nanofibers (Figure 4). Carbon and oxygen are the main components of the nanofiber because they are present in proteins, polysaccharides, and anthocyanins (Alizadeh Sani et al, 2022). 3 EDS spots were chosen to exhibit metal dioxides. Sn peaks and one oxygen peak for proving that those peaks correspond to the structure of tin dioxide. EDS Spot 1 which have more white reflection (represent tin dioxide) had higher peak intensity compared to Spot 2 and Spot 3.

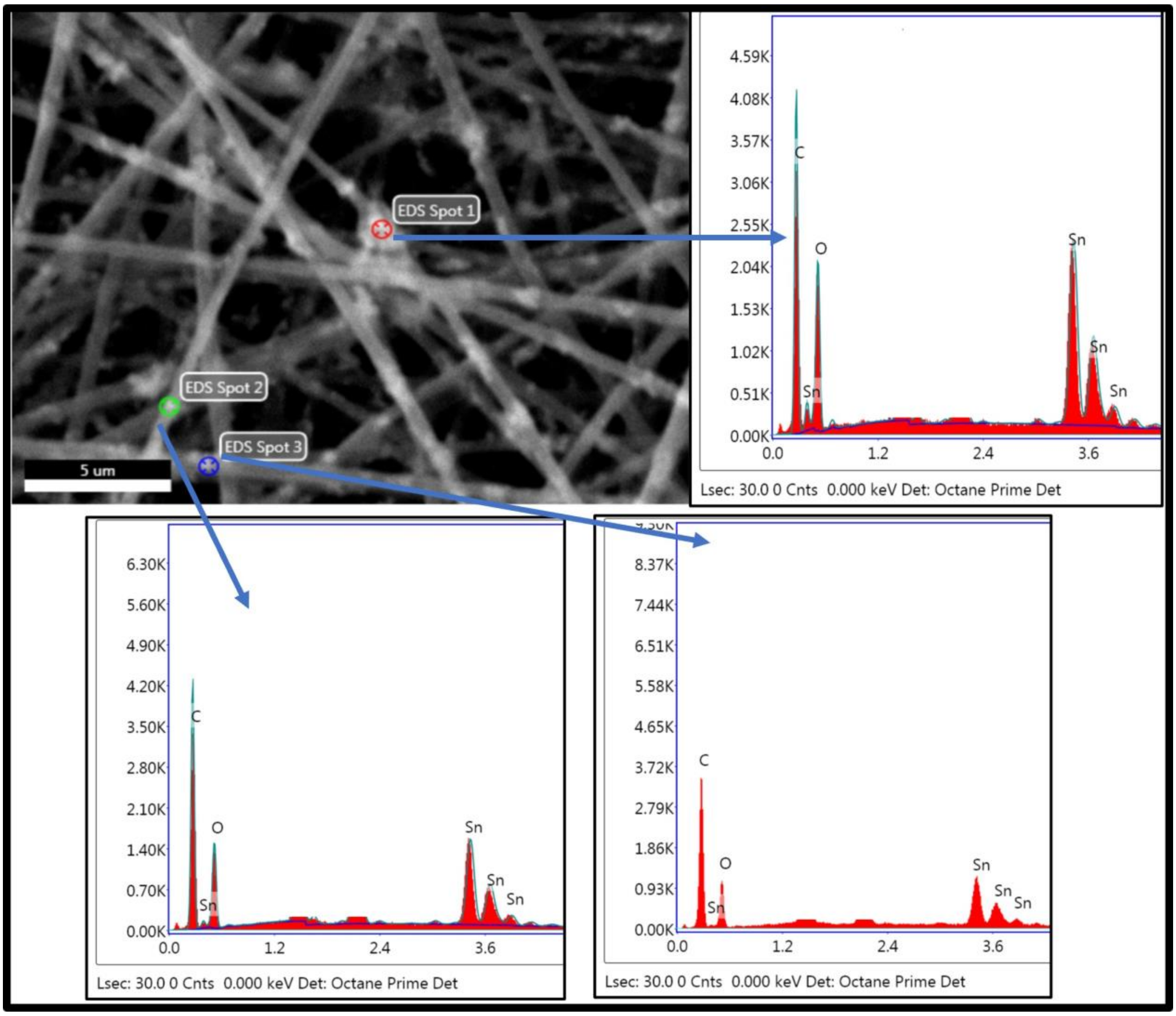

Figure 4. EDS elemental mapping three different spots of PVABT sample.

Results of TAL and TOL levels

Antioxidant and oxidant levels of black carrot extract (1), black carrot extract coated with PVA (2) and black carrot extract coated with PVA+metal (3) are as shown in table 2. In a study by Zambak et al. (2022) nanofibers without clove extract did not show any antioxidant activity. Similarly, Aslaner et al. (2021) stated that no antioxidant activity could be detected in extract free nanofibers. Therefore, in our study, we did not find necessary to measure pure PVA nanofiber's antioxidant activity.

Table 2. Total antioxidant and total oxidant levels of samples.

	TAL (mmol/L)	TOL (mmol/L)
1	1.2969	0.7068
2	2.1008	0.000
3	2.1628	0.000

References of TAL (mmol Trolox Equiv./L)

>2.0		Very good
1.45	2	Normal
<1.20		Very low antioxidant level

References of TOL (μmol H2O2 Equiv./L)

<5.00		Very good
8	5	Normal
>12.00		Very high oxidant level

When we compared the antioxidant and oxidant values with the reference ranges, it was found that the black carrot extract containing electrospun nanofiber samples had very good antioxidant properties, while the pure black carrot extract had a normal level of antioxidant value. When we look at the oxidant levels, black carrot extract is at a fairly low level, and the fact that the value is reset in nanocombined structures was evaluated as a very important data. Zambak et al. (2022) stated that encapsulation of clove extract with pullulan which is a water-soluble polymer increased its water solubility and as a result increased antioxidant activity. Since PVA is also water soluble polymer, results could be also attributed to higher water solubility of nanostructures compared to pure extract. According to the results of this study, electrospinning method increased the protection of the extracts by making them more stable both increased their antioxidant values and prevented all external exposures. In addition, the fact that the substance No. 2 coated with PVA nanofiber was detected lower than that coated with PVA+metal may also be due to the structure of tin itself. The fact that the oxidant level has never been detected in nanofiber structures shows that coating with PVA provides a serious protection and stability.

Conclusion

In this study, black carrot extract as an antioxidant compound was coated into polyvinly alcohol–based electrospun nanofibers. The smooth morphology of the all nanofibers samples as given by SEM images indicated succesfull production. Although morphologies were similar and smooth, addition of extract and extract with tin dioxide increased the averaga diameter sizes. Combination of black carrot and metal oxide improved the antioxidant properties (67% increase) of the nanofiber samples compared to black carrot containing nanofibers (62% increase) and only black carrot samples. In conclusion, plant extracts and metal oxides could be successfully coated with PVA polymer with improved antioxidant activity and to provide better stability. These nanomaterials with improved properties are promising for nanocoating or food packaging material applications.

REFERENCES

Aslaner, G., Sumnu, G., & Sahin, S. (2021). Encapsulation of grape seed extract in rye flour and whey protein–based electrospun nanofibers. *Food and Bioprocess Technology*, 14(6), 1118-1131.

Bharathi, S. K. V., Leena, M. M., Moses, J. A., & Anandharamakrishnan, C. (2020). Nanofibre-based bilayer biopolymer films: enhancement of antioxidant activity and potential for food packaging application. *International Journal of Food Science & Technology*, *55*(4), 1477-1484.

Bhushan Mishra, B., Singh Patel, A., & Kar, A. (2019). Storage stability of encapsulated anthocyanin-rich extract from black carrot (Daucus carota ssp. Sativus) using different coating materials. *Current Agriculture Research Journal*, *7*(1). https://doi.org/10.12944/carj.7.1.

Cetinkaya, T. (2023). Zein or gelatin nanofibers loaded with Au nanospheres, SnO_2 or black elderberry extract used as active and smart packaing layers for various fillets. PhD Thesis. Istanbul Technical University, Graduate School, Department of Food Engineering.

Espinosa-Acosta, G., Ramos-Jacques, A. L., Molina, G. A., Maya-Cornejo, J., Esparza, R., Hernandez-Martinez, A. R., Sánchez-González, I., & Estevez, M. (2018). Stability Analysis of Anthocyanins Using Alcoholic Extracts from Black Carrot (Daucus Carota ssp. Sativus Var. Atrorubens Alef.). *Molecules 2018, Vol. 23, Page 2744*, *23*(11), 2744. https://doi.org/10.3390/MOLECULES23112744

Ge, X., Cao, Z., & Chu, L. (2022). The antioxidant effect of the metal and metal-oxide nanoparticles. Antioxidants, 11(4), 791.https://doi.org/10.3390/antiox11040791.

Halliwell, B 1994. Free radicals and antioxidants:A personal view. Nutrition Reviews. 52(8) : 253-265.

Kar, A., Kumar Mahato, D., Singh Patel, A., & M. Bal, L. (2019). The Encapsulation Efficiency and Physicochemical Characteristics of Anthocyanin from Black Carrot (Daucus Carota Ssp. Sativus) as Affected by Encapsulating Materials. *Current Agriculture Research Journal*, *7*(1), 26–36. https://doi.org/10.12944/carj.7.1.04

Prietto, L., Pinto, V.Z., el Halal, S.L.M., de Morais, M.G., Costa, J.A.V., Lim, L.T., Dias, A.R.G. and Zavareze, E. da R. (2018) Ultrafine fibers of zein and anthocyanins as natural pH indicator. Journal of the Science of Food and Agriculture, 98(7), 2735-2741

Samrot, A. V., Ram Singh, S. P., Deenadhayalan, R., Rajesh, V. V., Padmanaban, S., & Radhakrishnan, K. (2022). Nanoparticles, a double-edged sword with oxidant as well as antioxidant properties—a review. *Oxygen*, *2*(4), 591-604.

Shavisi, N. and Shahbazi, Y. (2022) Chitosan-gum Arabic nanofiber mats encapsulated with pH-sensitive Rosa damascena anthocyanins for freshness monitoring of chicken fillets. Food Packaging Shelf Life, 32, 100827.

Sun, B., Long, Y.Z., Zhang, H.D., Li, M.M., Duvail, J.L., Jiang, X.Y. and Yin, H.L. (2014) Advances in three-dimensional nanofibrous macrostructures via electrospinning. Progress in Polymer Science, 39, 862–890.

Tarpey, M.M., Wink, D.A., Grisham, M.B 2004. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am JPhysiol, Regul Integr Comp Physiol. 286 (3): 431–44

Valgimigli, L., Baschieri, A., & Amorati, R. (2018). Antioxidant activity of nanomaterials. *Journal of Materials Chemistry B*, *6*(14), 2036-2051.

Zambak, Ö., Özkal, A., & Özkal, S. G. (2022). Production of clove extract loaded pullulan and whey protein nanofibers as antioxidant and antibacterial agent. *Journal of Applied Polymer Science*, *139*(46), e53141.

Zhao, Y., Guo, G., Xu, B., Liu, H., Tian, H., Li, J., ... & Kumar, R. (2023). Electrospun natural polypeptides based nanofabrics enriched with antioxidant polyphenols for active food preservation. *Food Chemistry*, *405*, 134991.