www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 636.085:577.112
DOI: 10.5937/ffr0-51613

Original research paper

FOOD GRADE NANOSTRUCTURES OF PUMPKIN LEAVES PROTEIN/PULLULAN BLEND AS A POTENTIAL CARRIER FOR COBALAMIN

Ana Salević-Jelić[1], Zorica Knežević-Jugović[2], Branko Bugarski[2], Smilja Marković[3], Viktor Nedović[1], Verica Đorđević[2], Bojana Balanč[*4]

[1]University of Belgrade, Faculty of Agriculture, 11080 Belgrade, Nemanjina 6, Serbia
[2]University of Belgrade, Faculty of Technology and Metallurgy, 11000 Belgrade, Karnegijeva 4, Serbia
[3]University of Belgrade, Institute of Technical Sciences of SASA, 11000 Belgrade, Knez Mihailova 35/IV, Serbia
[4]University of Belgrade, Innovation Centre of Faculty of Technology and Metallurgy, 11000 Belgrade, Karnegijeva 4, Serbia

Abstract: Nowadays, nanostructures made of biopolymers, such as proteins and polysaccharides, have gathered the growing attention of food scientists. In this study, pumpkin leaves from field crop side streams were processed to produce the protein isolate. The leaf protein isolate was investigated given the ability to encapsulate cobalamin (vitamin B12) in a blend with pullulan by electrospinning method. The starting blend solutions were characterized regarding the key factors that influence the formation of the fibers: viscosity, charge density carried by the jet, and surface tension. The results showed that the addition of the protein isolate (1% w/v) increased the conductivity of the pullulan solution (5% w/v), from 0.163 mS/cm to 1.420 mS/cm and the viscosity from 1.74±0.07 to 8.34±0.09 mPas. Cobalamin (at a concentration of 0.3 mg/mL) decreased the conductivity (0.978 mS/cm) and slightly increased the surface tension and viscosity of the final solution. SEM micrographs showed the formation of beads-on-fiber structures after the electro-hydrodynamic processing of the solutions. The protein caused the reduction of the beads compared to the beads obtained from neat pullulan (176.68 nm *vs.* 357.52 nm), while the mean fiber diameter was not affected (~22.5 nm). The combination of biopolymer pullulan and protein-rich pumpkin leaf extract has shown the properties of a potential carrier for the model vitamin.

Keywords: *biopolymer, electro-hydrodynamic processing, pumpkin leaves, protein isolate, encapsulation, vitamin B12*

INTRODUCTION

Electro-hydrodynamic processing is a cost-effective, straightforward process for making ultrafine nanometer and submicrometer range fibers from polymer solutions (Xiao & Lim, 2018). During electro-hydrodynamic processsing, a very high electric field is applied to obtain continuous filaments from the starting solution of the polymer. For this purpose, various polymers and polymer blends can be used (Schiffman & Schauer, 2008), and the obtained nanofibers have numerous functional features. Namely, the nanofibers have a large

Corresponding author: Phone: +381 11 3370-472
E-mail address: bisailovic@tmf.bg.ac.rs

surface-to-volume ratio, usually a small pore size, and controllable physical and mechanical properties. These features support many application fields, such as drug delivery, nutraceuticals, controlled release, active packaging, tissue engineering, uses in textiles, etc. (Uyar & Besenbacher, 2008). The electro-hydrodynamic processing technique is applicable for encapsulating sensitive bioactive compounds due to ambient processing conditions and the ability to process aqueous instead of organic solutions. (Echegoyen, Fabra, Castro-Mayorga, Cherpinski, & Lagaron, 2017). Even though, electrospun nanofibers can have beads as nusproducts of the process. This phenomenon is associated with starting solution features (such as polymer concentration, viscosity, surface tension, and conductivity) and processing parameters (voltage, flowrate, distance between tip and collector), which affect the jet's stability during electro-hydrodynamic processing along with morphology and diameters of the resulting fibers. The generation of beads is commonly related to low solution viscosity and a small or large distance between the tip and collector (Bhardwaj & Kundu, 2010).

Regarding encapsulation application, another advantage of electro-hydrodynamic processing is the possibility of using a wide range of polymers and active compounds (Echegoyen et al., 2017). Some studies reported the application of pullulan, an edible linear extracellular polymer, for the production of food-grade electrospun nanostructures as carriers for active compounds (Drosou, Krokida, & Biliaderis, 2018, Ma et al., 2021). Pullulan is a microbial polysaccharide soluble in water, able to form hydrogen bonds with proteins (e.g., pea and amaranth protein isolate) (Aceituno-Medina, Mendoza, Lagaron & López-Rubio, 2013, Aguilar-Vázquez, Loarca-Piña, Figueroa-Cárdenas & Mendoza, 2018). This advantage can be used to impart bioactive functionality and optimize the final nanofibers' structural properties. In addition to pullulan, zein, collagen, gelatin, and whey proteins are also utilized in electro-hydrodynamic processing (Nieuwland et al., 2014, Balanč et al., 2024). Food-grade nanofibers were also fabricated using varyaty combinations: whey protein isolate with guar gum or maltodextrine (Kutzli, Gibis, Baier & Weiss, 2018., Ramazani, Rostami, Raeisi, Tabibiazar & Ghorbani, 2019), *Spirulina* protein concentrate with gelatin (Mosayebi, Fathi, Shahedi, Soltanizadeh, & Emam-Djomeh, 2022) or even three component combination with sage extract, gelatin and zein (Salević-Jelić et al., 2023). Recently, Wang and coautors (2022) reviewed electrospinning of natural biopolymers for food applications conclouding nanofibers have favorable future as food-grade materials. However, it is currently the focus of the science and food industry on the usage of proteins extracted from non-animal sources. Namely, there is a worldwide need for alternative low-cost protein sources to replace and compensate for the requirement for proteins of animal origin. In this paper, we used proteins isolated from pumpkin leaves as an unconventional, alternative, and innovative protein source. The main goal was to develop nanostructures composed of a blend of pullulan and pumpkin leaf-derived protein isolate, aimed at encapsulating active compounds like vitamins. Both the initial solutions and the resulting nanostructures were characterized to demonstrate their potential for further application as carriers of active compounds.

MATERIALS AND METHODS

The pumpkin leaves used for the protein Isolation were collected on fields owned by the company JS&O (Novo Miloševo, Serbia) and kept in a deep freezer at -80 °C until the isolation procedure. Pullulan was from J&K Scientific LLC (USA), and Cyanocobalamin (vitamin B12) was from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade, and the water used was double-distilled.

Pumpkin leaf protein isolation

In brief, around 300 g of pumpkin leaves were taken out of deep freezing and left at room temperature to melt away. The leaves were further mechanically processed by squeezing on a stainless steel cold press, so the protein-rich juice was obtained. The heat precipitation step was next applied, resulting in the precipitation of chloroplast and green proteins from the juice (30 minutes at 55°C). Then, the pH was adjusted using 2M HCl to 4.5 (Tenorio, Gieteling, De Jong, Boom & Van Der Goot, 2018). In this way, white protein isolate was precipitated, and centrifugation was used for the separation. The protein isolate was finally lyophilized to obtain a powder used in further experiments (Figure 1). This isolation protocol ensures 1g of protein isolate.

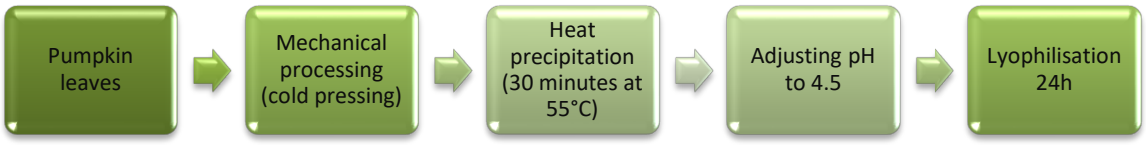

Figure 1. Scheme of the pumpkin leaves protein isolation procedure

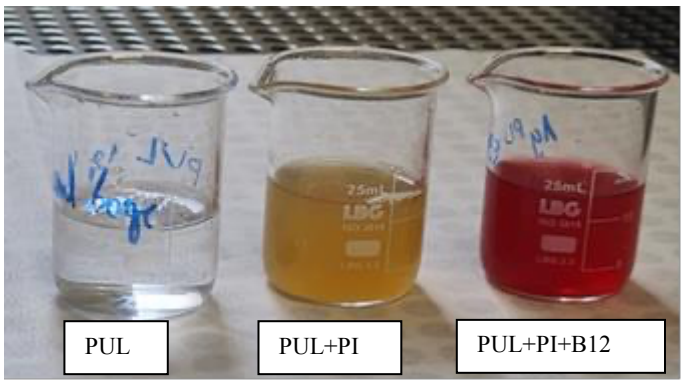

Figure 2. Solutions prepared as described in the section **Solutions**

Solutions

Pullulan (5% w/v) was dissolved in water at room temperature for 24 hours to ensure complete hydration (PUL). Then the pumpkin leaves protein isolate was added to the pullulan solution (1% w/v), and again, the mixture was stirred overnight at room temperature (PUL+PI). Cobalamin solution was added directly to the PUL+PI solution 30 minutes before the electro-hydrodynamic processing at a final concentration of 0.3 mg/mL (Fig. 2).

Characterization of the solutions

The electrical conductivity of formerly prepared solutions was determined using a digital Benchtop Conductivity Meter (HI 2315, HANNA Instruments, Ltd, USA). Further, the surface tension by the Wilhelmy plate method was measured on a Krüss K20 tensiometer (Krüss GmbH, Hamburg, Germany). The same instrument was used for density measurements, equipped with a DE01 set. The IKA ROTAVISC LO-VI viscometer (KA, Staufen, Germany) was used to measure the viscosity of the solutions. All measurements were done in triplicate at room temperature (25 ± 1°C).

Electro-hydrodynamic processing

Previously prepared pullulan-based solutions were subjected to an electro-hydrodynamic process. For this purpose, a blunt stainless steel needle (18G) was attached to the syringe, and the syringe pump (Razel Scientific Instruments, Stamford, Conn., U.S.A.) was adjusted to a steady flow rate of 0.5 mL/h. The needle was connected to the positive electrode, while the collector plate was connected to the ground electrode. The applied voltage was 17 kV, while the distance between the needle tip and the collector was 8 cm (Figure 3). The experiments were done at room temperature (~25°C).

Scanning electron microscopy (SEM) of the obtained nanostructures

SEM micrographs were obtained using a voltage of 10 kV and a magnification of 10000 and 30000 (TESCAN MIRA3XMU, Czech Republic). The micrographs were further analyzed using Image J software (National Institutes of Health, Bethesda, MD, U.S.A.) to get insight into the size distribution of the obtained nanostructures.

Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy was used to estimate the structural properties of the nanostructures as well as the chemical interactions between components. The samples were analyzed in attenuated total reflection mode (ATR) in the wavenumber range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1} and 100 accumulations per scan using an IRAffinitty-1S (Shimadzu, Japan).

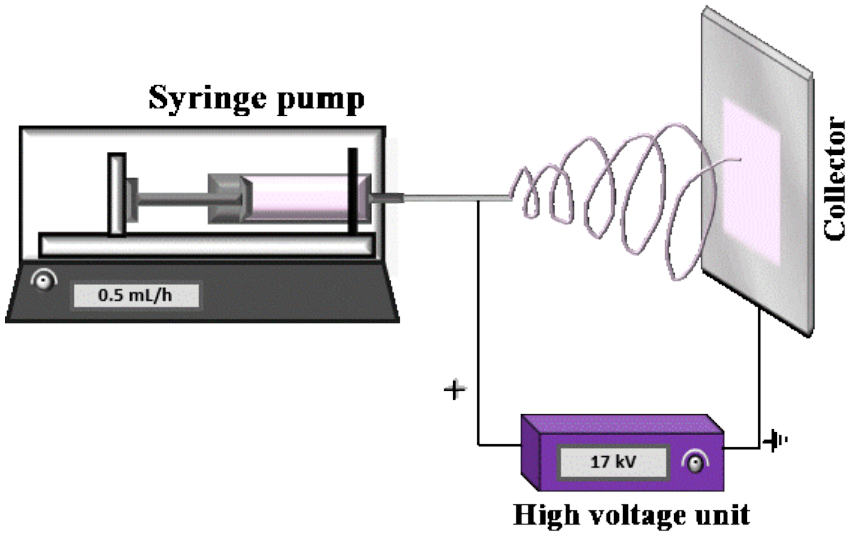

Figure 3. Schematic illustration of electro-hydrodynamic process applied to obtain nanostructures

RESULTS AND DISCUSSION

The focus of the present work is on the production of electrospun fibers by blending the leaf protein extract with pullulan as a spinnable carbohydrate polymer. Pullulan is a linear polysaccharide able to produce ultrathin and smooth electrospun fibers but only at a high enough polymer concentration.

According to Drosou et al. (2018), this concentration is 20% w/w, otherwise, fibers with beads (beaded fibers) would be formed. The idea is to establish how new protein extracted from the green leaf and vitamin B12 would affect electrospun fiber diameter and morphology related to the solution parameters and properties.

Generally, fiber morphology is greatly affected by the features of the starting solution. The pH values of all prepared solutions were around 5, and the pH value of the neat pullulan solution was not significantly affected by the addition of protein isolate or vitamin, even though the solution of protein isolate itself had a higher pH (Table 1).

On the other hand, the addition of protein isolate changed the viscosity of the starting solution. Compared to the neat pullulan solution, the viscosity increased with the addition of protein isolate and the vitamin.

This was reflected in the resulting nanostructure's morphology, and the number of beads in the structure decreased due to the viscosity increase. Wang et al. (2019) reported that more viscous solutions provide fewer beads and beaded fibers during the electrospinning of polymers. The same trend is evident in the presented results since PUL+PI+B12 had the highest viscosity. In addition, the distance between the beads on the fiber is longer as the viscosity increases (Fong, Chun & Reneker, 1999).

This is due to the influence of the solutions' viscosity on the entanglement of polymer chains and consequently, stabilization and thinning of the jet during the electro-hydrodynamic processsing, determining the morphology of the resulting structures (Busolo, Castro & Lagaron, 2017).

The protein isolate addition reduced the surface tension compared to the sole pullulan solution (26.2 to 25.2 mN/m), providing smoother fibers. A similar finding was reported by Drosou et al. (2018) who prepared whey protein-pullulan nanofibers, but they did not find any change in surface tension depending on protein proportion.

On the other hand, the reduction in surface tension after the addition of pumpkin leafe protein isolate was recently reported when gelatin was used for the production of nanofibers (Balanč et al., 2024).

Evendou the samples are aqueous solutions, a slight difference in density values was detected when protein was added to pullulan solution.

The addition of vitamin B12 did not affect density values significantly (Table 1). Generally, higher density creates smoother fibers because of the more forceful whipping instability of the liquid jet. The results are similar to those shown in our previous paper where gelatin and leaf protein isolate were used to produce electrospun fibers (Balanč et al., 2024).

More regular fibers' morphology, prompted by the addition of protein isolate and vitamins could also be attributed to the increased conductivity. Among the samples, the neat pullulan solution exhibited the lowest electrical conductivity, around 0.163 mS/cm. The addition of protein isolate resulted in a significant increase in conductivity (1.42 mS/cm).

The increased solution conductivity is beneficial to charge density on the jet surface during the electro-hydrodynamic processing and thus increases the elongation forces and production rate (Busolo et al., 2017). Namely, in the literature, it can be found that the increase in the conductivity of starting solutions results in the formation of bead-free fibers since a higher electrostatic force is established along the spin jet, extending the polymer into a thinner nanofiber (Uyar & Besenbacher, 2008, Xiao & Lim, 2018).

The values given in Table 1 and the discussion in the previous section are confirmed through the SEM micrographs given in Figure 4. SEM micrographs were also used to calculate the average diameter of the fibers and beads. For that purpose, 100 diameters were measured, and the results are presented in Fig. 5. The highest average bead diameter was detected in nanostructures made from pure pullulan solution (~357 nm). A slightly smaller average diameter was found in the other two samples (~176 and 202 nm for PUL+PI and PUL+PI+B12, respectively).

This change can also be due to the abovediscussed changes in the viscosity and conductivity of the solutions. High values of the standard deviation were found, indicating instability of the jet during the electrohydrodynamic processing. On the other hand, the average fiber diameter was similar for all samples, and it was around 22.5 nm. As discussed previously, the distance between the beads on the fiber is longer with the addition of proteins, and their appearance is less frequent.

FT-IR spectroscopy analysis was employed to evaluate potential interactions between the constituents of the nanostructures along with structural properties. Figure 6. presents the spectra of electro-hydrodynamic processing of PUL, PUL+PI and PUL+PI+B12 nanostructures in the range 500–4000 cm^{-1}. A broad peak located at around 3320 cm^{-1} was present in all spectra and assigned to O-H stretching.

The absorption band at around 2930 cm^{-1} corresponds to stretching vibrations of CH_2 groups present in the structure of pullulan.

There were bands at ~1647 and 1362 cm^{-1}, corresponding to the O–C–O bond and C–O–H bond, respectively (Kamali, Yavarmanesh, Najafi & Koocheki, 2022). The highly coupled modes of the C-C, C-O, and C-H stretching and COH bending of pullulan were in the region between 950 and 1245 cm^{-1} (Aceituno-Medina, Mendoza, Lagaron & López-Rubio, 2013,).

The bands at around 755, 848, and 931 cm^{-1} can be ascribed to α-(1-4)-D-glycosidic bonds, α-D-glucopyranoside units, and α-(1-6)-D-glycosidic bonds, respectively (Prasad, Guru, Shivakumar & Sheshappa Rai, 2012). Neither new bands nor band splits appeared after blending pullulan with pumpkin leaf protein isolate and incorporating vitamin B12 within pullulan-pumpkin leaf protein isolate blend-based nanostructures, indicating a homogenous dispersion of the constituents and efficient vitamin B12 incorporation.

However, there was a shift of the band from 995 cm^{-1} for the plain pullulan structure to 1015 cm^{-1} for the neat pullulan-pumpkin leaf protein isolate blend-based structure and further to 1022 cm^{-1} for the pullulan-pumpkin leaf protein isolate blend-based structure incorporating vitamin B12. This observation suggests intermolecular interactions between the constituents of the pullulan-pumpkin leaf protein isolate-vitamin B12 nanostructure constituents. A similar spectral change was reported after blending pullulan with other proteins, such as lactoferrin (Zhao, Xiong, Zhou & Xiao, 2019).

Table 1.
Characteristics of the starting solutions

Sample	***PI***	***PUL***	***PUL+PI***	***PUL+PI+B12***
pH	7.5 ± 0.2^{a}	5.1 ± 0.1^{b}	4.9 ± 0.2^{b}	5.0 ± 0.2^{b}
Conductivity(mS/cm)	2.67 ± 0.02^{a}	0.163 ± 0.009^{d}	1.42 ± 0.01^{b}	0.978 ± 0.007^{c}
Density (g/ml)	0.999 ± 0.0001^{c}	1.002 ± 0.0003^{b}	1.015 ± 0.0000^{a}	1.016 ± 0.0001^{a}
Viscosity (mPa·s)	1.15 ± 0.08^{d}	1.74 ± 0.07^{c}	8.34 ± 0.09^{b}	10.1 ± 0.09^{a}
Surface tension (mN/m)	33.3 ± 0.04^{a}	26.2 ± 0.00^{c}	25.2 ± 0.07^{d}	28.6 ± 0.10^{b}

**The results are expressed as mean ± standard deviation (n = 3). Means with different letters in the same row are significantly different (p < 0.05)*

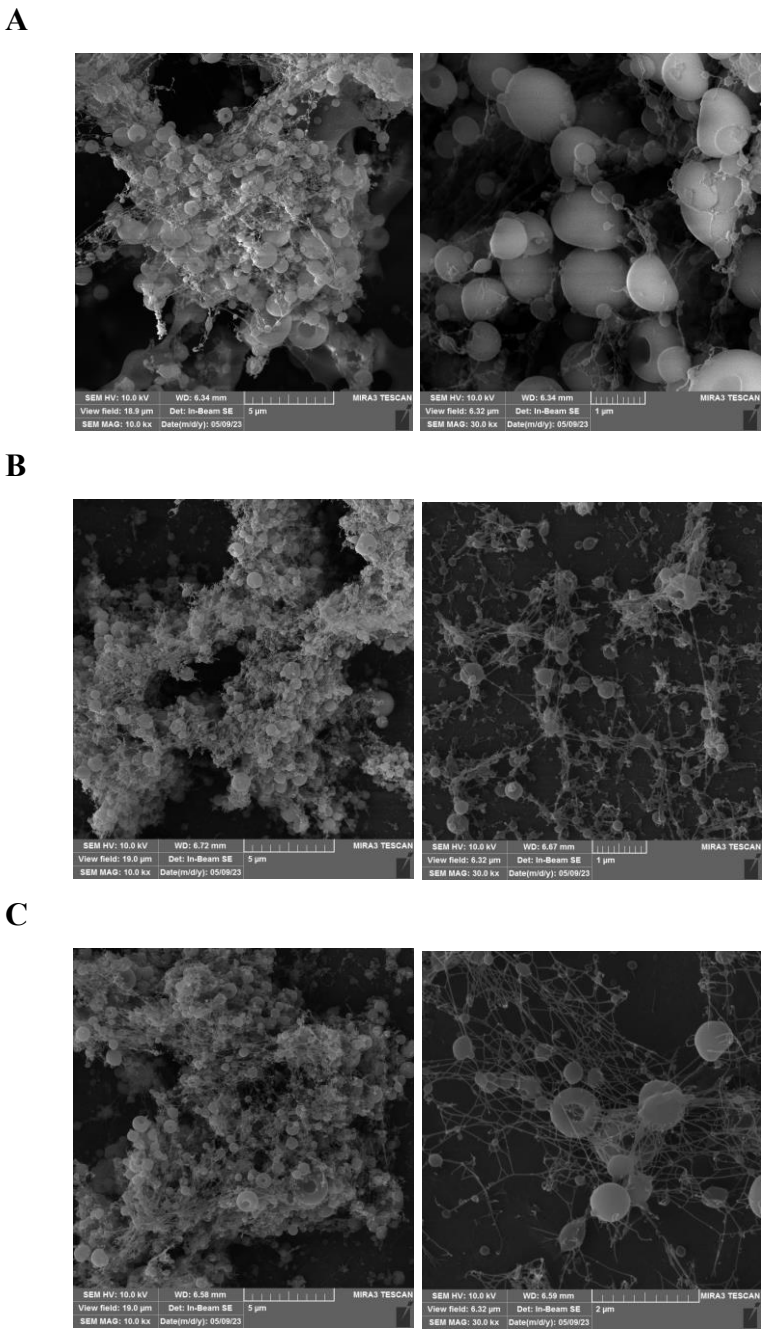

Figure 4. SEM micrographics in two magnifications 10000x (left) and 30000x (right) of A) pullulan nanostructures (PUL), B) pullulan nanostructures with the pumpkin leaf protein isolate (PUL+PI), C) pullulan nanostructures with the pumpkin leaf protein isolate and vitamin B12 (PUL+PI+B12)

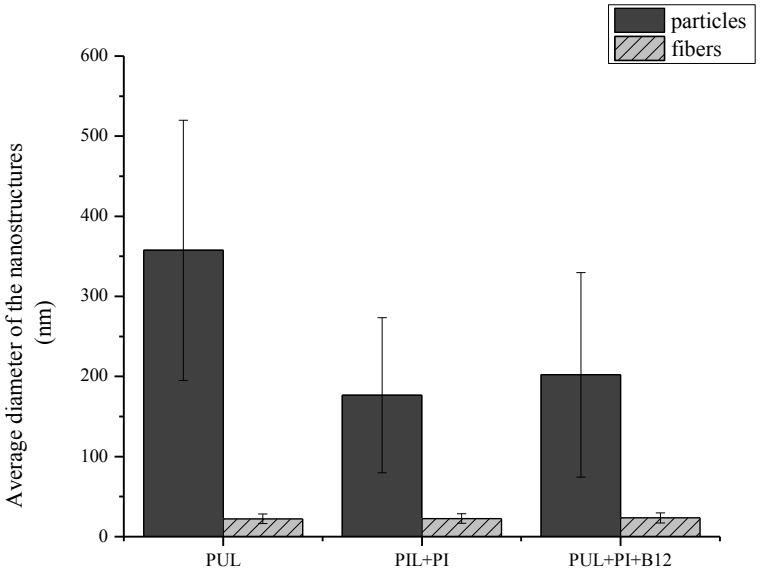

Figure 5. The average diameter of the nanostructures. Data are expressed as mean ± SD (n = 100)

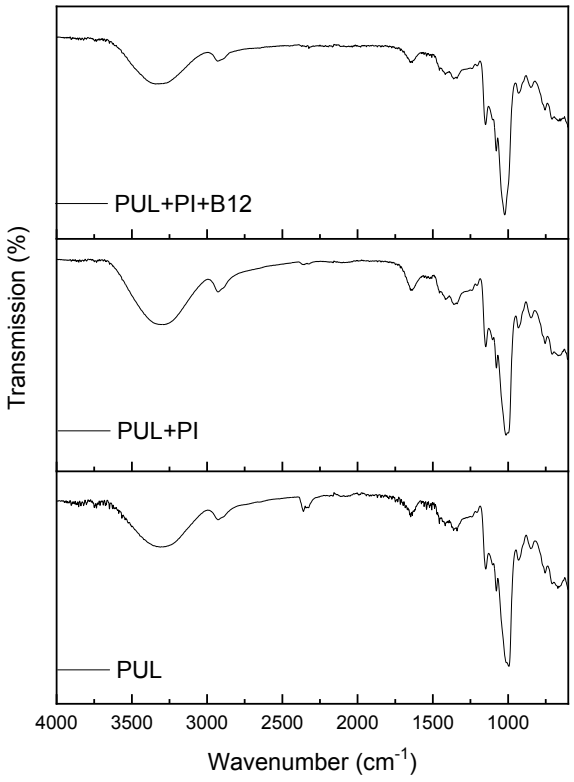

Figure 6. FTIR spectra of the obtained nanostructures

CONCLUSIONS

In summary, nanostructures based on proteins isolated from pumpkin leaves (PI) and natural polymer pullulan were obtained by the electro-hydrodynamic process. The physicochemical properties of the starting biopolymer solutions (viscosity and conductivity) were significantly

changed with the addition of PI. The solution of PI itself was not spinable, but in a blend with pullulan, the nanostructures were formed, as confirmed by SEM. The average size of the particle formed decreases from 357.52 nm for native pullulan nanostructures to 176.68 nm for pullulan with the isolate. Still, an increase in the average particle size to 202.01 nm was noted after cobalamin addition. The mean fiber diameter was not affected by the protein isolate addition. As confirmed by FTIR analysis, slight intermolecular interactions between the constituents of the pullulan-pumpkin leaf protein isolate-vitamin B12 nanostructure constituents were detected. According to the results, the combination of biopolymer pullulan and protein-rich pumpkin leaf isolate has shown the properties of a potential carrier for the model vitamin. Further research should be focused on the formation of more uniform nanostructures with a higher protein ratio. This will be challenging due to the low solubility of the protein isolated from alternative and innovative protein source*s*-pumpkin leaves.

AUTHOR CONTRIBUTIONS

Conceptualization, A.S., B.Ba. and Z.KJ.; Methodology, A.S., B.Ba., V.Đ., and S.M.; Investigation A.S., B.Ba. and V.Đ., formal analysis A.S., B.Ba., V.Đ., and S.M.; writing-original draft, A.S., B.Ba., V.Đ., Z.KJ.; Writing-review and editing, Z.KJ, B.B., V.N.; Supervision, Z.KJ, B.B., V.N

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

ACKNOWLEDGEMENTS

The authors acknowledge the generous funding support of the Science Fund of the Republic of Serbia, grant no. 7751519, Multifunctional leaf protein and assembled nanocarrier structures delivered by enzyme technology – MultiProMis and also Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract no. 451-03-65/2024-03/200135, contract no. 451-03-66/2024-03/200287 and contract no. 451-03-65/2024-03/200116).

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Aceituno-Medina, M., Mendoza, S., Lagaron, J. M., & López-Rubio, A. (2013). Development and characterization of food-grade electrospun fibers from amaranth protein and pullulan blends. *Food Research International, 54*(1), 667-674. https://doi.org/10.1016/j.foodres.2013.07.055

Aguilar-Vázquez, G., Loarca-Piña, G., Figueroa-Cárdenas, J. D., & Mendoza, S. (2018). Electrospun fibers from blends of pea (Pisum sativum) protein and pullulan. *Food Hydrocolloids, 83,* 173-181. https://doi.org/10.1016/j.foodhyd.2018.04.051.

Balanč, B., Salević-Jelić, A., Đorđević, V., Bugarski, B., Nedović, V., Petrović, P., & Knežević-Jugović, Z. (2024). The application of protein concentrate obtained from green leaf biomass in structuring nanofibers for delivery of vitamin B12. *Foods*, *13*(10), 1576. https://doi.org/10.3390/foods13101576.

Bhardwaj, N., & Kundu, S. C. (2010). Electrospinning: A fascinating fiber fabrication technique. *Biotechnology Advances*, *28*(3), 325-347. https://doi.org/10.1016/j.biotechadv.2010.01.004.

Busolo, M.A., Castro, S., & Lagaron, J.M. (2017). Electro-hydrodynamic processes (electrospinning and electrospraying): nonthermal processes for micro- and nanoencapsulation. In Krokida, M. (Ed.), *Thermal and nonthermal encapsulation methods* (pp. 115-136). Boca Raton, Florida, USA: CRC Press, Taylor & Francis Group.

Drosou, C., Krokida, M., & Biliaderis, C. G. (2018). Composite pullulan-whey protein nanofibers made by electrospinning: Impact of process parameters on fiber morphology and physical properties. *Food Hydrocolloids*, *77,* 726-735. https://doi.org/10.1016/j.foodhyd.2017.11.014.

Echegoyen, Y., Fabra, M. J., Castro-Mayorga, J. L., Cherpinski, A., & Lagaron, J. M. (2017). High throughput electro-hydrodynamic processing in food encapsulation and food packaging applications. *Trends in Food Science & Technology*, *60,* 71-79. https://doi.org/10.1016/j.tifs.2016.10.019.

Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, *40*(16), 4585-4592. https://doi.org/10.1016/S0032-3861(99)00068-3.

Kamali, S., Yavarmanesh, M., Najafi, M. B. H., & Koocheki, A. (2022). Development of whey protein concentrate/pullulan composite films containing bac-teriophage A511: Functional properties and anti-Listerial effects during storage. *Food Packaging and Shelf Life, 33,* 100902. https://doi.org/10.1016/j.fpsl.2022.100902.

Kutzli, I., Gibis, M., Baier, S. K., & Weiss, J. (2018). Fabrication and characterization of food-grade fibers from mixtures of maltodextrin and whey

protein isolate using needleless electrospinning. *Journal of Applied Polymer Science*, *135*, 46328. https://doi.org/10.1002/app.46328

Ma, J., Xu, C., Yu, H., Feng, Z., Yu, W., Gu, L. & Hou, J. (2021). Electro-encapsulation of probiotics in gum Arabic-pullulan blend nanofibres using electrospinning technology. *Food Hydrocolloids*, *111,* 106381. https://doi.org/10.1016/j.foodhyd.2020.106381.

Mosayebi, V., Fathi, M., Shahedi, M., Soltanizadeh, N., & Emam-Djomeh, Z. (2022). Fast-dissolving antioxidant nanofibers based on Spirulina protein concentrate and gelatin developed using needleless electrospinning. *Food Bioscience*, *47*, 101759. https://doi.org/10.1016/j.fbio.2022.101759

Nieuwland, M., Geerdink, P., Brier, P., Van Den Eijnden, P., Henket, J. T., Langelaan, M. L.& Martin, A. H. (2014). Reprint of "Food-grade electrospinning of proteins". *Innovative Food Science & Emerging Technologies, 24,* 138-144. https://doi.org/10.1016/j.ifset.2014.07.006.

Prasad, P., Guru, G. S., Shivakumar, H. R., & Sheshappa Rai, K. (2012). Investigation on miscibility of sodium alginate/pullulan blends. *Journal of Polymers and the* Environment, 20, 887-893. https://doi.org/10.1007/s10924-012-0427-4

Ramazani, S., Rostami, M., Raeisi, M., Tabibiazar, M., & Ghorbani, M. (2019). Fabrication of food-grade nanofibers of whey protein Isolate–Guar gum using the electrospinning method. *Food Hydrocolloids*, *90*, 99-104. https://doi.org/10.1016/j.foodhyd.2018.12.010

Salević-Jelić, A., Lević, S., Stojanović, D., Jeremić, S., Miletić, D., Pantić, M. & Nedović, V. (2023). Biodegradable and active zein-gelatin-based electrospun mats and solvent-cast films incorporating sage extract: Formulation and comparative characterization. *Food Packaging and Shelf Life*, *35*, 101027. https://doi.org/10.1016/j.fpsl.2023.101027

Schiffman, J. D., & Schauer, C. L. (2088) A review: Electrospinning of biopolymer nanofibers and their applications. *Polymer Reviews*, *48,* 317–352. https://doi.org/10.1080/15583720802022182.

Tenorio, A. T., Gieteling, J., De Jong, G. A., Boom, R. M., & Van Der Goot, A. J. (2016). Recovery of protein from green leaves: Overview of crucial steps for utilisation. *Food Chemistry, 203,* 402-408. https://doi.org/10.1016/j.foodchem.2016.02.092.

Uyar, T. & Besenbacher, F. (2008) Electrospinning of uniform polystyrene fibers: The effect of solvent conductivity. *Polymer, 49,* 5336–5343. https://doi.org/10.1016/j.polymer.2008.09.025.

Wang, Y., Guo, Z., Qian, Y., Zhang, Z., Lyu, L., Wang, Y., & Ye, F. (2019). Study on the electrospinning of gelatin/pullulan composite nanofibers. *Polymers*, *11*(9), 1424. https://doi.org/10.3390/polym11091424.

Wang, Y., Khan, M. A., Chen, K., Zhang, L., & Chen, X. (2023). Electrospinning of natural biopolymers for innovative food applications: A review. *Food and Bioprocess Technology*, *16*(4), 704-725. https://doi.org/10.1007/s11947-022-02896-z

Xiao, Q., & Lim, L.T. (2018) Pullulan-alginate fibers produced using free surface electrospinning. *International Journal of Biological Macro-molecules*, *112,* 809–817. https://doi.org/10.1016/j.ijbiomac.2018.02.005.

Zhao, Z., Xiong, X., Zhou, H., & Xiao, Q. (2019). Effect of lactoferrin on physicochemical properties and microstructure of pullulan-based edible films. *Journal of the Science of Food and Agriculture*, *99*(8), 4150-4157. https://doi.org/10.1002/jsfa.9645.

PREHRAMBENE NANOSTRUKTURE NA BAZI MEŠAVINE PROTEINA LISTA BUNDEVE I PULULANA KAO POTENCIJALNI NOSAČI ZA KOBALAMIN

Ana Salević-Jelić[1], Zorica Knežević-Jugović[2], Branko Bugarski[2], Smilja Marković[3], Viktor Nedović[1], Verica Đorđević[2] , Bojana Balanč[*4]

[1]Univerzitet u Beogradu, Poljoprivredni fakultet, 11080 Beograd, Nemanjina 6, Srbija
[2]Univerzitet u Beogradu, Tehnološko-metalurški fakultet, 11000 Beograd, Karnegijeva 4, Srbija
[3]Univerzitet u Beogradu, Institut tehničkih nauka SASA, 11000 Beograd, Knez Mihailova 35/IV, Srbija
[4]Univerzitet u Beogradu, Tehnološko-metalurški fakultet, Inovacioni centar, 11000 Beograd, Karnegijeva 4, Srbija

Sažetak: U današnje vreme nanostrukture pripremljene upotrebom prirodnih (zelenih) proteina privlače sve veću pažnju naučnika koji se bave hranom. U ovoj studiji, listovi bundeve koji predstavljaju otpadnu biomasu su iskorišćeni u cilju dobijanja proteinskog izolata. Mešavina ovog izolata i biopolimera pululana ispitana je sa aspekta mogućnosti inkapsulacije vitamina (vitamina B12) elektro-hidrodinamičkom metodom. Polazni rastvori ispitani su u pogledu ključnih faktora koji utiču na formiranje vlakana: viskozitet, gustina naelektrisanja koju nosi mlaz i površinski napon. Rezultati su pokazali da je dodavanje proteinskog izolata (1% w/v) povećalo provodljivost rastvora pululana (5% w/v), sa 0,163 mS/cm na 1,420 mS/cm i viskozitet sa 1,74±0,07 do 8,34±0,09 mPa·s. Kobalamin (0,3 mg mL^{-1}) je smanjio provodljivost (0,978 mS/cm) i blago povećao površinski napon i viskozitet konačnog rastvora. Mikrografije elektronske mikroskopije su pokazale formiranje struktura čestica na vlaknima nakon primene elektro-hidrodinamičke metode. Protein je izazvao smanjenje čestica u poređenju sa česticama dobijenim od čistog pululana (176,68 nm naspram 357,52 nm), dok srednji prečnik vlakna nije bio promenjen (~22,5 nm). Kombinacija prirodnog biopolimera pululana i izolata listova bundeve bogatog proteinima pokazala je svojstva pogodnog nosača za vitamin kobalamin.

Ključne reči: *biopolimer, elektro-hidrodinamički proces, protein lišća bundeve, izolat proteina, enkapsulacija, vitamin B12*

Received: *14 June 2024/* ***Received in revised form:*** *21 August 2024/* ***Accepted:*** *22 August 2024*

Available online: *October 2024*