RESEARCH ARTICLE
Bull Biotechnol (2021) 2(2):32-37
https://doi.org/10.51539/biotech.1034044 Jurnal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Structural and thermal properties of electrospun whey protein/PEO nanofibers

Mustafa Gözler[1], Atike İnce Yardımcı[2], Özgür Tarhan[1,*]

*Corresponding author : ozguratarhan@gmail.com
Orcid No: https://orcid.org/0000-0001-7084-6253

Received : 08/12/2021
Accepted : 26/12/2021

Abstract: Nano-scale fibers or films obtained by adding natural active agents to natural food proteins are promising materials for packaging purposes. Whey proteins are one of the most popular matrices in application with their cheap and sustainable availability. They have many functional properties and their antimicrobial, antiviral and anticarcinogenic properties are highlighted as well. Enzymatic hydrolysis can increase their functionality such as inducing gelation and revealing bioactive peptides. These features can make whey proteins good candidates for the fabrication of talented fiber structures through electrospinning. Electrospinning technique based on the deposition of fine fibers on a collector surface under electrical forces can be used to form protein nanofibers. The purpose of this research is to produce whey-based protein nanofibers and determine their structural and thermal features by infra-red spectroscopy and thermogravimetric analysis. A high molecular weight polymer, polyethylene oxide (PEO) was used in combination with whey protein concentrate (WPC) to increase spinnability. Besides, enzymatic hydrolysis of WPC enhanced the viscosity of the protein/polymer solution and helped electrospinning ability. Both non-hydrolyzed and hydrolyzed WPC/PEO nanofibers exhibited promising morphological, structural and thermal properties for targeted use in food and biomedical applications. Further research will focus on the application of these protein nanofibers for the packaging of particular foods.

Keywords: electrospinning; nanofiber; whey; protein; PEO

1 Introduction

To meet the nutritional needs of the increasing world population, it is necessary to benefit from the innovations brought by the emerging technologies to food industry. Producing healthy and high quality products and proper storage ensuring the freshness and safety of these products for a long time without deterioration are critical in industrial manufacturing. Food decay occurs rapidly due to microbiological spoilage and chemical degradation. There are various packaging methods to retard spoilage and extend shelf life. In this context, food packaging and storage have great importance in the food industry. Methods such as modified atmosphere packaging (Erdinç and Acar 1996), vacuum packaging (Jin et al. 2003), and smart packaging (Kocaman and Sarımehmetoğlu 2010) have been developed to preserve the freshness of foods by providing longer shelf life. In recent years, nanofiber based packaging materials obtained from polymers/ biopolymers (eg. food proteins) have been investigated for better protection of food materials as well (Topuz and Uyar 2020). Nanofibers can be produced from both natural and synthetic polymers. Animal- and plant-based proteins are among the natural polymers successfully reported with their nanofiber forms for food applications (Ince Yardımcı and Tarhan 2020). Some animal-based proteins used for nanofiber formation are whey proteins, gelatin, albumin, casein and some plant proteins are soy, zein, gliadin (Zhong et al. 2018, Lin et al. 2019, Tomasula et al. 2016, Wang et al. 2013, Deng et al. 2019). In a general application of electrospinning, clasically, soluble protein solutions are loaded to the syringe needle. Then the positive pole of the power supply is connected to the tip of the needle, and the negative pole is connected to the collector surface. The injector pump starts to work and the pumped protein solution accumulates in the form of fibers on the collecting surface under the produced electric field. These nano-scale fibers gather to form nanofiber films to be used for coating/ packaging of foods. Antimicrobial active agents incorpotared in protein solutions to be electrospun resulted in the formation of nanofiber packaging materials possesing antimicrobial property promising for the extension of shelf life of the target foods.

Whey proteins are widely used sustainable sources for developing novel talented matrices including nanofibers. Protein hydrolysis reveals increased viscosity, thus might support spinnability of proteins. Here, the presented study proposed to compare spinnability of unhydrolyzed and hydrolyzed whey proteins, and investigate some properties. Determination of structural and thermal features of the

nanofiber materials are significant for designing specific coating/packaging applications.

This study aimed to investigate the formation of nanofibers from whey protein concentrate (WPC) and hydrolyzed whey protein concentrate (HWPC) blended with polyethylene oxide (PEO). Within the scope of the presented study, morphological, structural and thermal properties of the WPC/PEO and HWPC/PEO nanofibers were investigated through Scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FTIR), and Thermogravimetric analysis (TGA). Microstructure, typical bondings and interactions within the molecular structure, and thermal durability of the given protein nanofibers were helpful in providing insight regarding their applicability to food systems.

2 Materials and Method

2.1 Materials

To synthesize WPC/PEO nanofibers, PEO (MW of 100.000, Sigma-Aldrich, USA), WPC 80 %, meaning percent protein concentration in wt/wt, (Alfasol, Kimbiotek, Turkey) and ultra pure water were used. Alcalase enzyme (2.4 AU-A/g) was kindly supplied by Novozymes A/S (Bagsvaerd, Denmark).

2.2 Preparation of Protein Solutions

To prepare WPC/PEO and hydrolyzed WPC/PEO, three different solutions were prepared as follows. PEO solution was prepared by dissolving 15 wt % PEO in ultra pure water via mechanical stirring for 12 h at room temperature (RT). The 35 wt % WPC was dissolved in ultra pure water by mechanical stirring for 5 h at RT. The 10 wt % WPC dissolved in ultra pure water was hydrolyzed at 50 ^{o}C in a water bath for 12 h by adding 0.025 g alcalase enzyme (pH 6.5). To obtain a homogen WPC/PEO solution, 5 ml of 35 wt % WPC solution and 5 ml of 15 wt % PEO solution were stirred for 30 min at 1000 rpm (RT). To obtain a homogen HWPC/PEO solution, 2 ml of 10 wt % HWPC solution and 8 ml of 15 wt % PEO solution were stirred for 30 min at 1000 rpm (RT).

2.3 Electrospinning and characterization of WPC/PEO and HWPC/PEO nanofibers

The WPC/PEO and HWPC/PEO solutions were filled into a 20 ml syringe (0.80 mm in diameter) connected to a high voltage for electrospinning. For WPC/PEO sample, 21 kV voltage was applied and flow rate of solution was 0.7 µl/h; for HWPC/PEO sample, 15 kV voltage and 0.652 µl/h flow rate was carried out to obtain nanofibers. The kV values used were determined through preliminary studies. The distance between the syringe and collector was kept constant as 15 cm and the fibers were collected on an Aluminium foil for 3 h.

The morphology of WPC/PEO and HWPC/PEO samples were characterized by SEM (LEO 1430 VP) with an acceleration voltage of 20 kV and a secondary-electron detector. For this purpose, the samples were coated with gold and fixed onto metallic stubs with double-sided carbon tape.

FTIR characterizations of WPC/PEO and HWPC/PEO samples were carried out between 400 and 4000 cm^{-1} wavenumbers with Perkin Elmer UATR Spectrum Two FTIR. The resolution was 4 cm^{-1} and the number of scans collected was 128.

Thermal behavior of nanofibers was determined by TGA (Hitachi STA 7300) analysis in the temperature range of 25-600 ^{o}C under nitrogen atmosphere at a heating rate of 10 ^{o}C /min.

3 Results and Discussion

3.1 Microscopy

The morphological properties of the WPC/PEO fibers were examined with SEM images (Fig 1). While pure PEO is a polymer suitable for electrospinning, the addition of natural food grade protein WPC prevented PEO from being drawn effectively (Zhong et al. 2018). SEM images were carefully evaluated to understand how the whey proteins blended with PEO formed nanofiber.

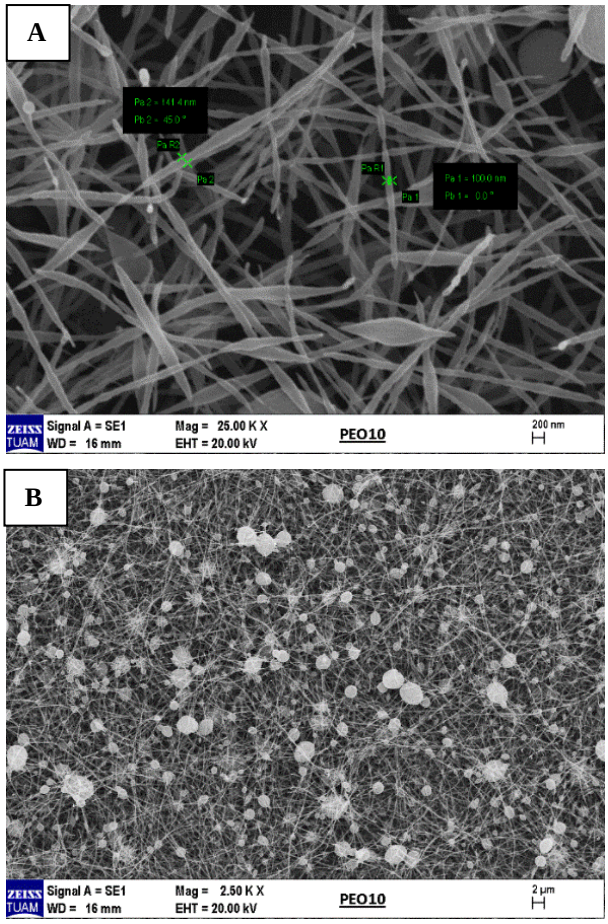

Figure 1. SEM images of WPC/PEO sample with A) high (25.00 KX), and B) low (2.50 KX) magnification

As clearly indicated in Fig 1, fiber formation was achieved in case of WPC/PEO blend. However, beads and short fibers/ breaks were also formed within the nanofiber network. One reason for that is probably the protein concentration in the polymer solution. Equal amount (1:1, v/v) of WPC (35 wt %) and PEO (15 wt %) were mixed and electrospun. This amount of WPC could possibly prevented the formation of regular long fibers to some extend. Besides, addition of higher ratio of WPC in WPC/PEO solution was found to increase bead formation (data not shown). In fact, trails on electrospinning of WPC alone did not result in fiber formation, but PEO alone formed nanofibers in this study (data not shown). Another

reason might be insufficient dissolution of WPC and PEO to be electrospun, thus unhomogenized polymer blend might interrupt the process and caused bead formation and breaks during electrospining. To facilitate electrospinning of WPC and PEO solutions, a better mixing of those should be achieved at higher speeds using an homogenizer. Future work will focus on the optimization of homogenization and electrospinning parameters for WPC/PEO blend. In fact, many factors affect fiber formation, such as electrospinning parameters including flow rate, voltage (Deitzel et al. 2001), distance to the collector (Thompson et al. 2007) and needle diameter (Tan et al. 2005); environmental parameters like temperature, pressure and humidity (De Vrieze et al. 2009; Theron et al. 2004); and solution parameters including polymer molecular weight (Koski et al. 2004), concentration (Deitzel et al. 2001), and electrical conductivity. Therefore, the properties of the solution to be electrospun should be well adjusted to achieve a desirable fiber formation.

SEM images of the HWPC/PEO fibers were given in Fig 2. Although the viscosity of the solution increased (data not shown), bead formation increased as well in case of using hydrolyzed protein instead of unhydrolyzed one. The protein content was lower in hydrolyzed WPC solution (10 wt %) than that of unhydrolyzed one (35 wt %). Thus, under the given conditions hydrolysis causing the formation of smaller protein fragments and aggregates negatively affected spinnability and fiber-forming ability of WPC.

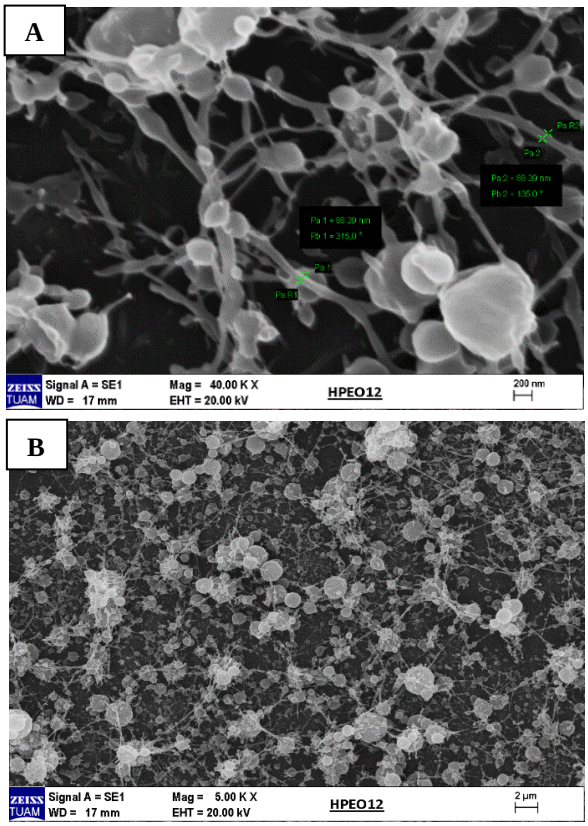

Figure 2. SEM images of the HWPC/PEO sample with A) high (40.00 KX), and B) low (5.00 KX) magnification

3.2. FTIR Analysis

FTIR analysis of protein/polymer nanofibers were given in Figure 3. It is clearly seen that, the peak signal at ~1631 cm^{-1} refers to Amid I stretching vibrations, at ~1541 cm^{-1} attributed to Amide II stretching vibrations (Tarhan et al. 2014). The signals at ~1399 cm^{-1}, 1453 cm^{-1}, ~1242 cm^{-1}, ~1061 and ~1104 cm^{-1} referred to oil, methyl, methylene groups and carbohydrate structures, respectively, in WPC sample (Andrade et al. 2019). Peaks detected at ~699 cm^{-1}, 1467 cm^{-1}, 1360 cm^{-1} and 2884 cm^{-1} indicated the presence of PEO (Ratna et al. 2006).

Similarly, remarkable signals corresponding to Amide I and II vibration modes of the WPC proteins and -CH_2 stretching modes of PEO were detected in HWPC/PEO sample, in agreement with the literature (Ratna et al. 2006, Tarhan and Şen 2022). The peak heights lowered since the amount of intact WPC was lower in the HWPC/PEO sample as a result of protein hydrolysis. The α-helical elements represented by the amide vibrational peaks at the given wavenumbers in unhydrolyzed protein degraded in case of hydrolysis. It was evident with the significantly lowered signals at ~1631 and 1541 cm^{-1} in the latter case. Alterations of the structural conformation of proteins revealed through hydrolysis. In general, helical structures are disturbed through enzyme digestion revealing exposed β-sheet and random coil structures leading to new conformational arrangements within protein structure (Tarhan and Şen 2022).

The presence of WPC in fiber prefixes was evident due to FT-IR findings (Fig3A). Besides, the peaks observed in the spectrum showed the presence of PEO in fiber samples obtained from WPC and PEO polymer blends.

3.3 TGA Analysis

Thermograms of WPC/PEO and HWPC/PEO were presented in Figure 4. The blue line in the graphs indicated the weight loss in WPC/PEO sample, the fracture at 100ºC indicated the evaporation of the water, after 300º C the weight loss of WPC itself were shown (Islam et al. 2014), and the weight loss after 400º C revealed the degredation of PEO (Balo et al. 2019).
The HWPC/PEO sample lost its weight as a result of a single stage decomposition after 400º C. The protein was hydrolyzed through 12 h before electrospinning and thus, the polymer solution contained hydrolysis products instead of intact protein. Nanofibers with beads were formed from peptide fragments of degraded WPC molecules. Different from WPC/PEO graph, there was no peak representing WPC loss in the HWPC/PEO graph and the transition was smooth. This clearly indicated that there was no intact WPC molecule in the nanofiber network.

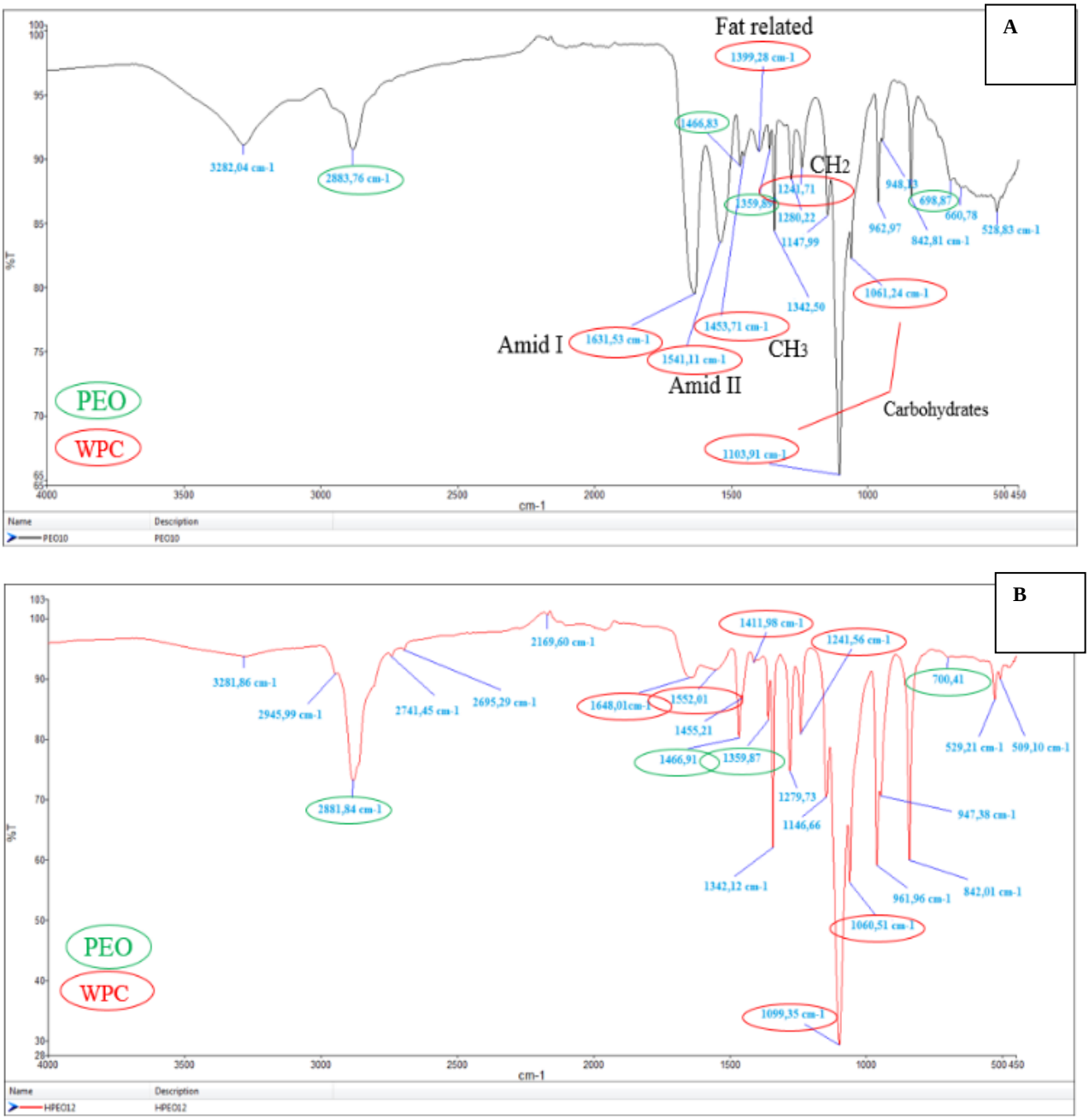

Figure 3. FTIR spectrum of the WPC/PEO (A), HWPC/PEO (B) samples

4. Conclusion

It was observed that WPC, a natural animal-based protein, could form continuous electrospun nanofibers in the presence of the synthetic polymer PEO with a ratio of 1:1 (v/v). Based on the SEM images, the WPC/PEO sample exhibited better spinnability than the HWPC/PEO sample. It was evident that HWPC increased bead formation due to some reasons such as the presence of low molecular weight peptide fragments discouraging spinnability and non-homogeneous mixture of protein/peptide and PEO solution. Thus, better mixing of the electrospinning solutions through the homogenizer will be beneficial for more effective fiber formation. FTIR plots confirmed the presence of WPC, HWPC and PEO in the resultant nanofiber structures. According to TGA findings, thermal degradation of WPC and PEO occurred at 300 ºC and 400 ºC, respectively, and almost all WPC was degraded during hydrolysis. Based on the structural and thermal properties of nanofibers derived from WPC could be considered as proper matrices for different applications. Further research will focus on investigating additional characteristics of WPC/PEO nanofiber materials and their efficiency in food packaging applications.

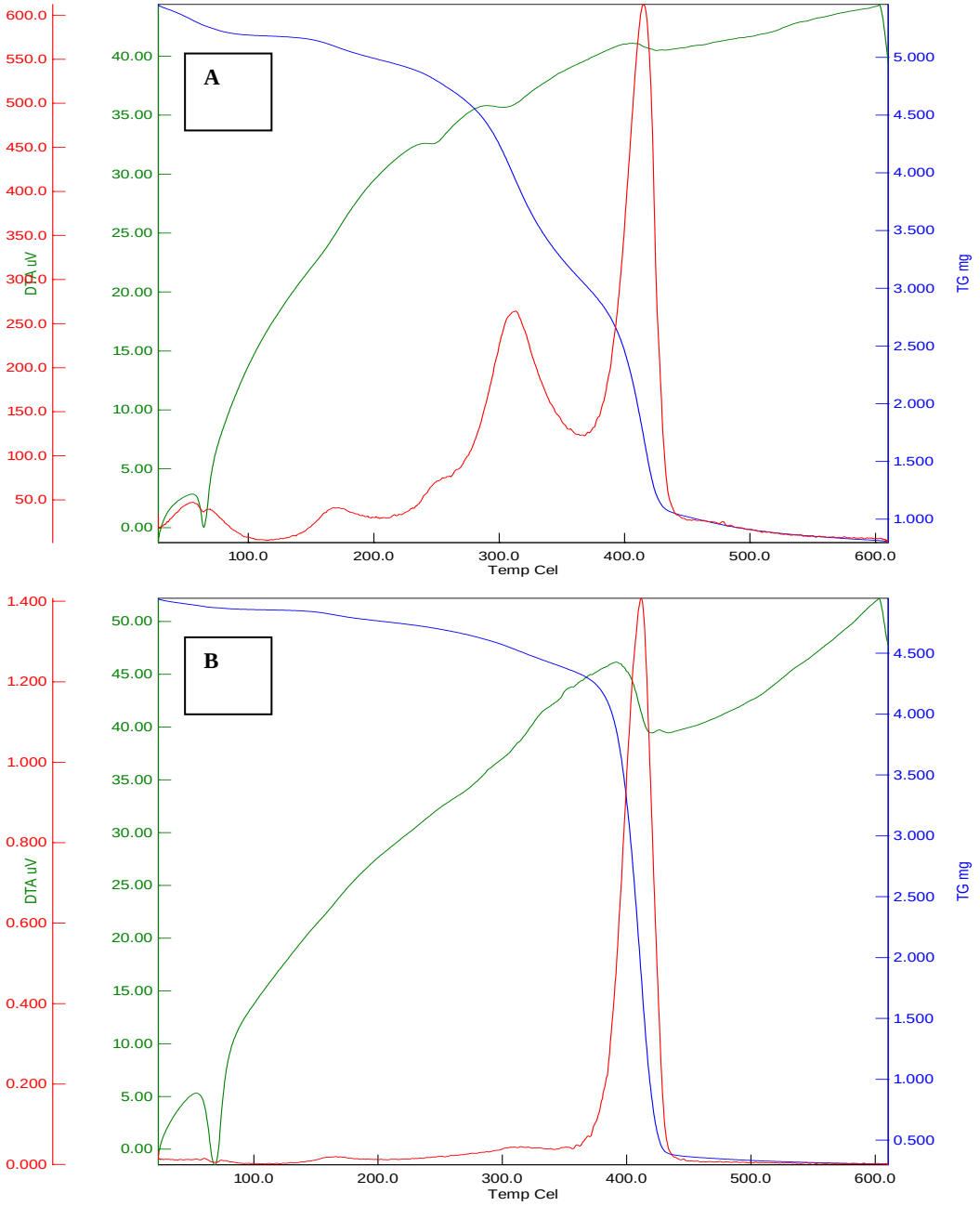

Figure 4. TGA scan of WPC/PEO (A), HWPC/PEO (B) samples

Acknowledgement

The authors are very thankful to Scientific Analysis, Technological Application, and Research Center of Usak University for FT-IR measurements.

References

Andrade J, Pereira CG, de Almeida Junior JC, Viana CCR, de Oliveira Neves LN, da Silva PHF, Bell MJV, dos Anjos VDC (2019) FTIR-ATR determination of protein content to evaluate whey protein concentrate adulteration. LWT-Food Sci and Tech 99: 166-172

Balo L, Gupta H, Singh SK, Singh VK, Tripathi AK, Srivastava N, Tiwari RK, Mishra R, Meghnani D, Singh RK (2019) Development of gel polymer electrolyte based on LiTFSI and EMIMFSI for application in rechargeable lithium metal battery with GO-LFP and NCA cathodes. J of Solid State Electrochemistry 23(8): 2507-2518

Deitzel JM, Kleinmeyer J, Harris DEA, Tan NB (2001) The effect of processing variables on the morphology of electrospun nanolifs and textiles. Polymer 42(1): 261-272

Deng L, Li Y, Feng F, Wu D, Zhang H (2019) Encapsulation of allopurinol by glucose cross-linked gelatin/zein nanofibers: Characterization and release behavior. Food Hydrocolloids 94: 574-584

De Vrieze S, Van Camp T, Nelvig A, Hagström B, Westbroek P, De Clerck K (2009) The effect of temperature and humidity on electrospinning. Journal of Mat Sci 44(5): 1357-1362

Erdinç B, Acar J (1996) Gıda muhafazasında modifiye atmosfer paketleme (MAP). Gıda 21(1): 17-21

Islam MS, Hamdan S, Ahmad MB, Hasan M, Hassan A, Haafiz MM, Jawaid M (2014) Effect of PVA-co-MMA copolymer on the physical, mechanical, and thermal properties of tropical wood materials. Advances in Mat Sci and Eng doi.org/10.1155/2014/626850

İnce Yardımcı A, Tarhan Ö (2020) Electrospun protein nanofibers and their food applications. Mugla J of Sci and Tech 6(2): 52-62

Jin Y, Wang ZF, Lim PC, Pan DY, Wei J, Wong CK (2003) MEMS vacuum packaging technology and applications. In: Proceedings of the 5th electronics packaging technology conference (EPTC 2003), IEEE, Singapore, 10-12 December 2003, pp 301-306

Kocaman N, Sarımehmetoğlu B (2010) Use of smart packaging in foods. Veteriner Hekimler Derneği Dergisi 81(2): 67-72

Koski A, Yim K, Shivkumar S (2004) Effect of molecular weight on fibrous PVA produced by electrospinning. Materials Letters 58(3): 493-497

Lin L, Gu Y, Cui H (2019) Moringa oil/chitosan nanoparticles embedded gelatin nanofibers for food packaging against Listeria monocytogenes and Staphylococcus aureus on cheese. Food Packaging and Shelf Life 19: 86-93

Ratna D, Divekar S, Samui AB, Chakraborty BC, Banthia AK (2006) Poly (ethylene oxide)/clay nanocomposite: thermomechanical properties and morphology. Polymer 47(11): 4068-4074

Tan SH, Inai R, Kotaki M, Ramakrishna S (2005) Systematic parameter study for ultra-fine lif fabrication via electrospinning process. Polymer 46(16): 6128-6134

Tarhan Ö, Tarhan E, Harsa Ş (2014) Investigation of the structure of alpha-lactalbumin protein nanotubes using optical spectroscopy. Journal of Dairy Research 81(1): 98-106

Tarhan Ö, Şen R (2022) Heat-denatured and alcalase-hydrolyzed protein films/coatings containing marjoram essential oil and thyme extract. Food Biosci 45: 101466 doi.org/10.1016/j.fbio.2021.101466

Theron SA, Zussman E, Yarin AL (2004) Experimental investigation of the governing parameters in the electrospinning of polymer solutions. Polymer 45(6): 2017-2030

Thompson CJ, Chase GG, Yarin AL, Reneker DH (2007) Effects of parameters on nanolif diameter determined from electrospinning model. Polymer 48(23): 6913-6922

Tomasula PM, Sousa AM, Liou SC, Li R, Bonnaillie LM, Liu L (2016). Electrospinning of casein/pullulan blends for food-grade applications. J of Dairy Science 99(3): 1837-1845

Topuz F, Uyar T (2019) Antioxidant, antibacterial and antifungal electrospun nanofibers for food packaging applications. International Int Food Res J 130: 108927 doi.org/10.1016/j.foodres.2019.108927

Wang S, Marcone MF, Barbut S, Lim LT (2013) Electrospun soy protein isolate-based fiber fortified with anthocyanin-rich red raspberry (Rubus strigosus) extracts. Food Research Int 52(2): 467-472

Zhong J, Mohan SD, Bell A, Terry A, Mitchell GR, Davis FJ (2018) Electrospinning of food-grade nanofibres from whey protein, Int J Biol Macromol 113: 764-773