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Additional Information

Quality improvement of rainbow trout fillets by whey protein isolate coatings containing electrospun poly(ε-caprolactone) nanofibers with Urtica dioica L. extract during storage

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Abstract
Novel bioactive coatings were prepared to extend the quality and shelf life of fresh fish fillets of rainbow trout (Oncorhynchus mykiss). For this, whey protein isolate (WPI) with 96 g/100 g protein content was used as the coating-forming agent with glycerol in a ratio of 3.3 g/100 mL as plasticizer. Poly(ε-caprolactone) (PCL) nanofibers containing extract of Urtica dioica L., often called stinging nettle, at 60:40 (g:g) were produced by electrospinning. These were then incorporated into WPI at different concentrations, i.e., 50 and 70 g/100 g. Fish fillets protected by means of the functionalized coatings were stored for 15 days at 4 C. Protected fish fillets exhibited higher antimicrobial efficiency against mesophilic, psychrophilic, and lactic acid bacteria as well as Enterobacteriaceae than equivalent coatings containing Urtica dioica L. water extract. In particular, incorporation of 70 g/100 g PCL nanofibers exhibited the lowest counts in both bacterial growth and values in total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) during storage. Additionally, the coatings successfully provided antioxidant activity that could help to increase fish fillets' quality and favor their preservation.

1. Introduction

Large quantities of food are wasted because of poor logistics, inadequate storage, and lack of proper cold chain facilities (Ramos et al., 2012). Fish is globally demanded by many consumers not only content of polyunsaturated fatty acids (PUFA) (e.g, omega-3), amino acids, lipid soluble vitamins, and micronutrients (Medina, Gallardo, & Aubourg, 2009; Rodriguez-Turienzo et al., 2011). Nevertheless, marine food products including fish have a limited shelf life due to their high water holding capacity, low connective tissue content, neutral pH values, and tissular enzymes (Mahmoud, Yamazaki, Miyashita, Shin, & Suzuki, 2006; Bahram et al., 2016). As a result, fish is more perishable during refrigerated storage. This is mainly produced due to rapid microbial growth of microorganisms naturally present in fish or from external contamination, which usually result in either economic or health-related problems (RodriguezTurienzo et al., 2011; Gomez-Estaca, de Lacey, L opez-Caballero, Gomez-Guill en, & Montero, 2010). Fish fat, with high contents of lipid fraction of PUFA, can be rapidly oxidated by environmental oxygen, which causes quality deterioration during storage (Khan et al., 2015). This has received a great deal of attention due to the important role of PUFA in preventing human disaeas (Medina et al., 2009). Chilled temperature conditions, i.e., 4 C cannot fully preserve the quality and safety of fish because of still-continuing lipid oxidation and microbial and enzymatic spoilage during prolonged storage (Bahram et al., 2016; Goulas & Kontominas, 2005; Ojagh, Rezaei, Razavi, & Hosseini, 2010). Even freezing, i.e., the use of

temperatures below 0 C, which are widely used to preserve fish, suppresses the microbial growth but only slow down enzymatic activity (Rodriguez-Turienzo et al., 2011).

In order to avoid such rapid deterioration of fish-based foodstuffs, several preservation methods have been studied for years (Adeyeye, 2016; Cheng, Wang, & Weng, 2015; Sampels, 2015). One of these methods is based on developing coatings with biopolymers by incorporating synthetic or natural antimicrobial and/or antioxidant agents to prolong the shelf life of various food types including fish (Iturriaga, Olabarrieta, & de Maran~on, 2012; G omez-Estaca et al., 2010; Guilbert, Gontard, & Gorris, 1996; Campos, Gerschenson, & Flores, 2011; Song, Liu, Shen, You, & Luo, 2011). Many organic acids, enzymes, fungicides, and natural compounds such as spices, essential oils (e.g., phenolic compounds), and plant extracts have been incorporated into coatings in order to develop bioactive edible materials with antioxidant and antimicrobial properties. In this regard, the use of medicinal plants can represent an interesting strategy to develop novel antimicrobial materials. In this sense, Urtica dioica L. is known as a plant often used in the medical sector due to its antiviral properties. This also has a wide spread antimicrobial activity including Pseudomonas aeruginosa (P. aeruginosa), Proteus mirabilis (P. mirabilis), Citrobacter koseri (C. koseri), Staphylococcus aureus (S. aureus), Streptococcus pneumoniae (P. pneumoniae), Micrococcus luteus (M. luteus), Staphylococcus epidermidis (S. epidermis) (Gülçin, Küfrevioglu, Oktay, & Büyükokuroglu, 2004). Urtica dioica L. includes essential amino acids, ascorbic acid, minerals, and vitamin C and it is very rich in terms of b-carotene and w-3 fatty acid (Guil-Guerrero, FuentesRebolloso, & Isasa, 2003). Bioactive coatings can therefore extend shelf life of food products and/or provide microbial safety for consumers by inhibiting the pathogen growth inside, maintaining their quality longer, and enhancing the convenience of food processing, distribution, retailing, and consumption (Perez, Balagu e, Rubiolo, & Verdini, 2011). However, incorporation of components in large matrices often associate limitations such as burst release, inmiscibility, and heterogeneous distribution (Ni, Fan, Wang, Qi, & Li, 2014). In this sense, the application of nanotechnology to coatings may provide new approaches to remove some of these deficiencies (Sorrentino, Gorrasi, & Vittoria, 2007). Even if there are plenty of studies focused on edible coatings, a few of them describe the incorporation of nanoparticles into edible coatings in order to extend the shelf life of foodstuff (Mohanty, Nayak, Kaith, & Kalia, 2015; Siragusa & Dickson, 1992).

Electrospun nanofibers have been recently proposed as a novel platform to generate bioactive materials, including both the release of different biocides or the use of inherent antimicrobial polymers (Torres-Giner, Perez-Masi a, & Lagaron, 2016). In particular the electrospinning technology has been recently applied to preserve meat and meat products by means of polyurethane (PU), virgin olive oil encapsulated PU, and olive oil-zinc oxide (ZnO)/PU hybrid nanofibers (Amna, Yang, Ryu & Hwang, 2015). This includes the use of poly(ε-caprolactone) (PCL) nanofibers, which is a semicrystalline linear resorbable aliphatic polyester of petrochemical origin. PCL is biodegradable in nature, biocompatible, highly hydrophilic, and chemically and thermally stable due to the susceptibility of its aliphatic ester linkage to hydrolysis (Jin et al., 2013; Suganya, Senthil Ram, Lakshmi, & Giridev, 2011; Chellamani, Vignesh Balaji, Veerasubramanian, & Sudharsan, 2014; Sridhar et al., 2014). The by-products of PCL are either metabolised via the tricarboxylic acid (TCA) cycle or eliminated by direct renal secretion. The Food and Drug Administration (FDA) approves a number of medical and drug

delivery devices of PCL based on extensive in vitro and in vivo studies of biocompatibility (Filipczak et al., 2005; Kweon et al., 2003; Thomas et al., 2006).

In this study, fresh rainbow trout fillets will be coated with whey protein isolate (WPI) formulations including Urtica dioica L. lyophilized water extract and/or PCL nanofibers/Urtica dioica L. extract with the aim to prolong the shelf life of fresh fillets at refrigerator temperature, i.e., 4 C. To the best of our knowledge, this is the first study in which a WPI coating incorporating electrospun nanofibers functionalized with a plant extract is applied to improve food preservation.

2. Material and methods

2.1. Materials

BiPRO WPI with a protein content of 90 g/100 g was obtained from Davisco Foods International Inc. (La Sueur, MN., USA). Biomedical-grade PCL (440744), with an average molecular weight (MW) of 80,000 g/mol and density of 1.145 g/mL, chloroform, methanol, and glycerol were all purchased from Sigma Aldrich (Istanbul, Turkey). Leaves of Urtica dioica L. were collected from Trabzon province in Turkey.

2.2. Nanofibers formation by electrospinning

2.2.1. Preparation of powdery extract of Urtica dioica L

Urtica dioica L. leaves were dried for 3 days at 30e35 C to reach a maximum moisture level of 1e2%. These were then grounded and sieved through a 14 inch sieve (Alp & Aksu, 2010). After this 20 g of the material in the bottom of the sieve was added to 400 mL of boiling water and stirred for 15 min (Gülçin et al., 2004). After filtering (Whatman No.1) it was frozen at 38 C and lyophilized at 50 C. The Urtica dioica L. lyophilized water extract (UWE) was obtained.

2.2.2. Electrospinning process

PCL and the above-obtained powder extract of Urtica dioica L. were first dissolved in a chloroform/methanol 50/50 (mL:mL) solution at a ratio of 60:40 (g:g) to obtain a 12 g/100 mL solution and stirred at room temperature for 24 h. These conditions were selected based on the research reported previously by Jin et al. (2013). The prepared polymer solution was then fed into a 3-mL standard syringe attached to a 27G blunted stainless steel needle using a syringe pump at a flow rate of 1.0 mL/h. A high voltage of 15 kV (Gamma High Voltage Research, USA) was applied to the pumped polymer solution. Resultant electrospun PCL nanofibers containing the plant extract, i. e., the Urtica dioica L. nanofibers (UNFs), were collected as a continuous mat on a metallic plate kept at a distance of 12 cm from the needle tip. The solvent employed for the polymer solution was totally evaporated during the process.

2.3. Obtention of fish fillets

Eviscerated and ice-preserved rainbow trouts (Oncorhynchus mykiss) were purchased from a local fish market. The fish fillets were hand skinned and cut along the anteroposterior axis, removing the tail section in the laboratory. The average weight of the fish fillets was 108-200 g.

2.4. Preparation of coating solutions and application to fish fillets

Coating preparation was done according to the procedure described by Gounga, Xu, and Wang (2007). For this, five types of coating solutions were prepared: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g UWE with WPI; Test sample 2 (T2): 7 g/ 100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g UNF with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Coating solutions were prepared by slow stirring 96 g/100 g WPI in distilled water for 30 min at room temperature, i.e., 20 C. Then, glycerol in a ratio of 3.3 g/100 mL was added to plasticize the solution. Afterwards, dispersions were heated in a water bath at 90 ± 2 C for 30 min at pH 7 and cooled to room temperature to incorporate UWE and UNF at the above concentrations. Different concentrations of UWE (5 and 7 g per 100 g of WPI) were added to WPI and the solutions were manually stirred until the extracts were completely dispersed. Level of the additives were determined by preliminary studies. The concentration of Urtica dioica L. was limited to 7.0 g/L due to the generation of greenish color and high turbidity. Different concentrations of UNF (50 and 70 g per 100 g WPI) were added to the WPI and the dispersions were homogenized at 6500 rpm for 30 min, using a IKA T-25 digital homogenizer (IKA-Werke GmbH Co. KG, Staufen, Germany).

WPI coatings were then applied to the fish fillets by the dipping method for 1 min at room temperature. Materials were drained for 10 s, packed aerobically in polyethylene bags, and then stored at 4 ± 0.5 C for 15 days. All experiments were performed in triplicate. Quality assessment was determined on the initial day (0th), 3rd, 5th, 7th, 9th, 11th, and 15th days of storage. Fig. 1 shows the as-received fresh fillets and the protected fillets by means of the bioactive coatings of WPI containing the UNFs.

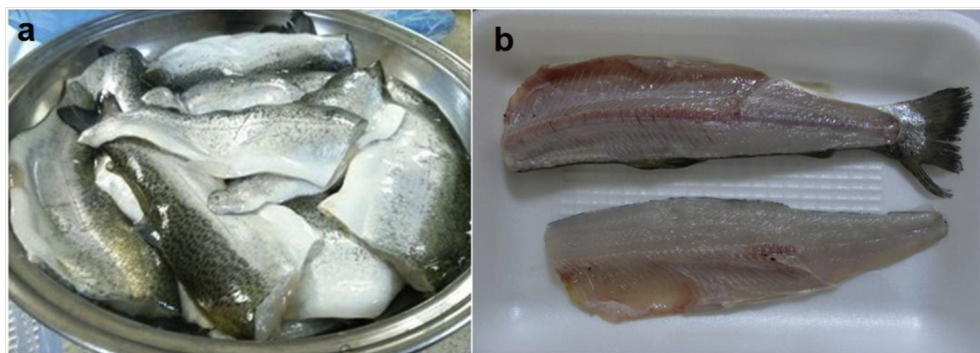

Fig. 1. Washed fresh fish fillets of rainbow trouts (Oncorhynchus mykiss) (a). Fish fillets obtained with the bioactive whey protein isolate (WPI) coating of electrospun poly(εcaprolactone) (PCL) nanofibers containing Urtica dioica L. (b).

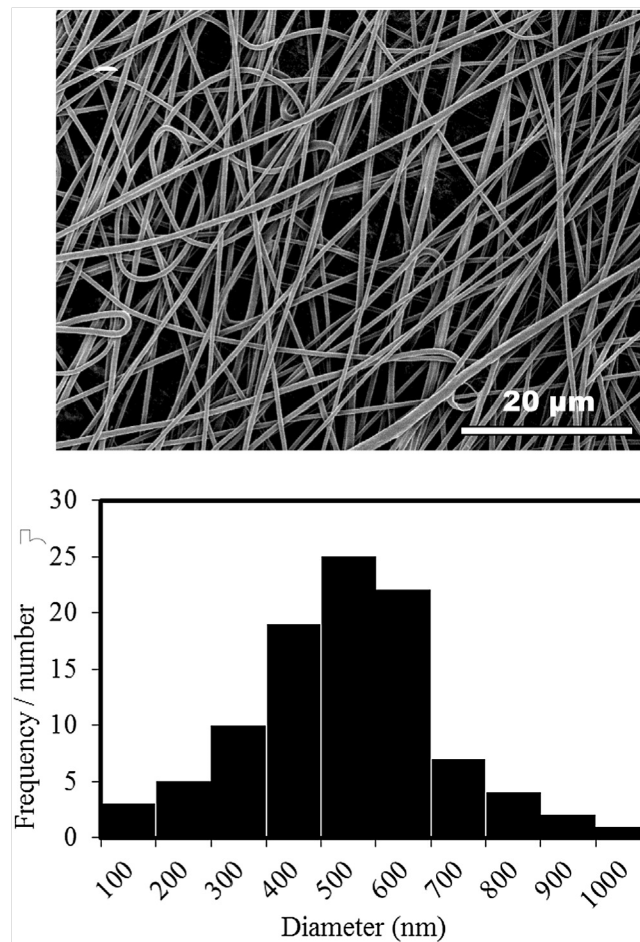

Fig. 2. Scanning electron microscopy (SEM) images of the electrospun poly(ε-caprolactone) (PCL) nanofibers containing Urtica dioica L. obtained with a magnification of 1000x and scale marker of 20 mm (a). Size distribution (b).

2.5. Morphological characterization

The morphology of the electrospun nanofibers was studied by Scanning Electron Microscopy (SEM) with an S-4100 model from Hitachi (Tokyo, Japan) on goldepalladium sputtered specimens. All SEM experiments were carried out at an accelerating voltage of
8.0 kV. Diameters of the electrospun materials were analyzed from the SEM micrographs in their original magnification using image analysis Adobe Photoshop 7.0 software by manual size determination.

2.6. Microbiological analysis

Fish fillet with a weight of 25 g of was aseptically placed in stomacher bags, with 225 mL of saline solution, i.e., 0.85 g/100 g NaCl, and homogenized for 1 min. Serial dilutions of the homogenates were prepared and the surface plate method was used for enumeration. Plate count agar (PCA) from Oxoid Thermo Scientific (Basingstoke, UK) was used to determine total aerobic mesophilic bacteria (TAMB) and plates were aerobically incubated at 30 C for 48 h. de Man Rogosa Sharpe (MRS) agar was used for lactic acid bacteria and incubation was done anaerobically at 30 C for 48 h. Violet red bile dextrose (VRBD) from Oxoid Thermo Scientific

(Basingstoke, UK) was used for Enterobacteriacea and plates were anaerobically incubated at 30 C for 48 h. Potato Dextrose Agar (PDA) was used for yeast and mold count and the plates were incubated aerobically at 25 C for 5 days. PCA was used to determine psychrophilic bacteria at an incubation temperature 7 C for 10 days.

2.7. Determination of TVB-N and TBA values

For the total volatile basic nitrogen (TVB-N) analysis,10 ± 0.1 g of fish fillet was weighed. The sample was placed into Kjeldahl tubes containing magnesium oxide (MgO) and 100 mL of distilled water was added. Then, 100 mL of distilled water was added into an erlenmayer flask containing 10 mL of 3 g/100 g boric acid and 8 drops of methylene red. Distillation process was maintained until 200 mL of distillate was collected in the flask. Distillate was titrated with 0.1 mol equi/L HCl until neutralization occured and following expression was employed:

TVB N mg=100g sample ¼ A 1:4 100=B

Where, A ¼ used 0.1 mol equi/L HCl amount (mL) and B ¼ Sample (g).

Similarly, 10 ± 0.1 g sample of fish fillet was weighed and placed into kjeldahl tubes for the determination of thiobarbituric acid (TBA). For this, 97.5 mL pure water and 2.5 mL of 1:2 HCl was added to the sample. Distillation process was maintained until 200 mL of distillate was collected in the flask. Then, 5 mL of distillate and 5 mL of TBA reactive were placed into the capped tubes and boiled in water bath for 35 min. After cooling the tubes to room temperature, absorbance of the solutions was measured at 532 nm. Measured values were multiplied by 7.8 and the amount of malondialdehyde (MDA), in mg, was determined in 1 kg sample.

2.8. Color measurements

Fig. 3. Total aerobic mesophilic bacteria (TAMB) expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

A Minolta Colorimeter model CR-410 (Minolta Co, Osaka, Japan) was used for the color measurement of the coated fillets. Measurements were done in three replicates from three differentregions of the fish fillets. The instrument was calibrated with standard plates before analysis. Color values (L*, a*, and b*) were measured according to following criteria: L* ¼ 0, darkness; L* ¼ 100, lightness; a*; þa* ¼ red, a* ¼ green; and b*; þb* ¼ yellow, b* ¼ blue.

2.9. Statistical analysis

A completely random-block design was used. All data were expressed as a mean ± standard deviation (SD). Data generated were analyzed using SPSS software, version 22 for Windows. The one-way ANOVA, Duncan post-hoc test ($p < 0.05$), and the mean SD were conducted.

3. Results and discussion

3.1. Electrospun morphology

SEM micrograph of the electrospun mat of PCL nanofibers containing Urtica dioica L., i.e., the here-called UNF mat, is shown in Fig. 2a. This revealed beadless ultrathin fibers with a mean diameter of 575 ± 130 nm (Fig. 2b), produced under the selected electrospinning conditions. The incorporation of the plant extract did not affect the uniform fibrilar shape and nanofibers presented similar morphological characteristics of that recently reported for neat PCL (Jin et al., 2013). UNFs were subsequently applied in the coatings for the fish fillets.

3.2. Microbiological changes

Both treatment and storage time had significant effect ($p < 0.05$) on TAMB counts. The highest average of TAMB counts was observed for C. i.e., neat WPI, while the lowest counts were for T4. Storage time had also significant effect ($p < 0.05$) on TAMB counts, which increased on a daily basis (Fig. 3). TAMB counts of the test samples based on 50 and 70 g/100 g UNF with WPI, i.e., T3 and T4 did not show a significant difference during the storage. However, TAMB counts of 5 and 7 g/100 g UWE with WPI, i.e., T1 and T2, and in the control sample (C), increased rapidly by the 7th day.

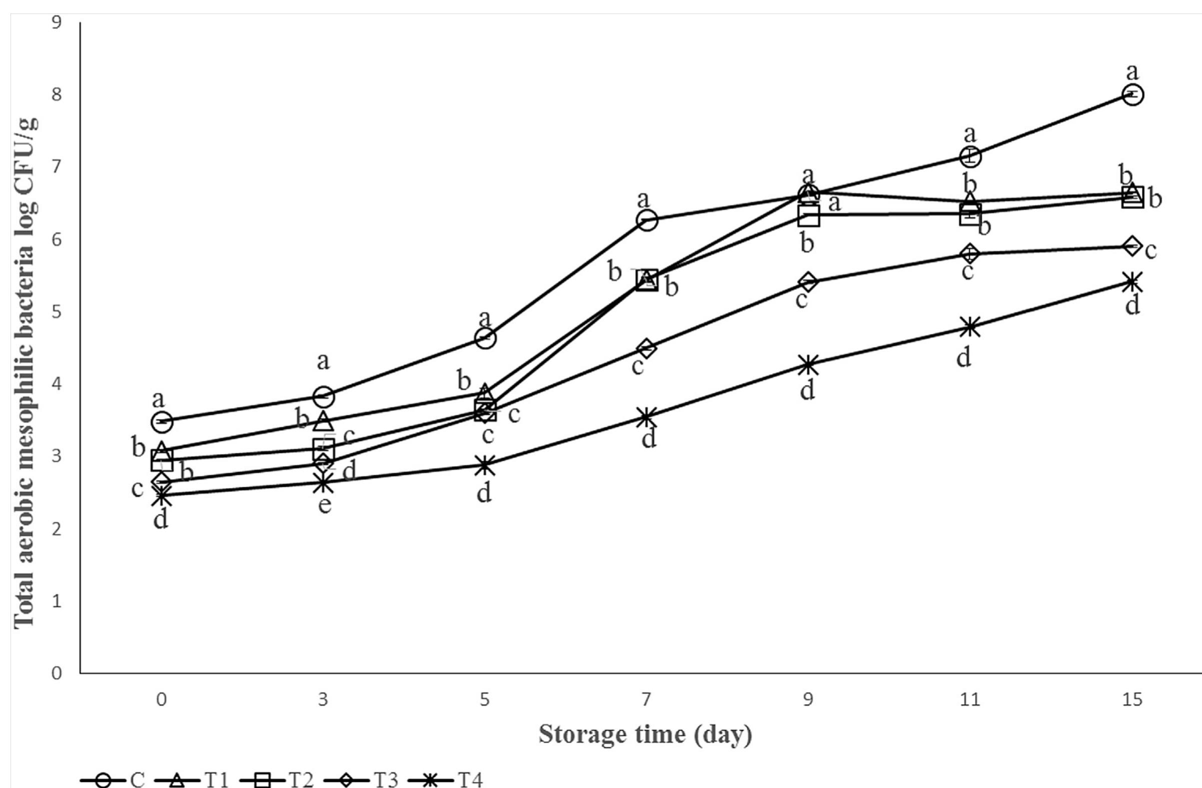

Fig. 3. Total aerobic mesophilic bacteria (TAMB) expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

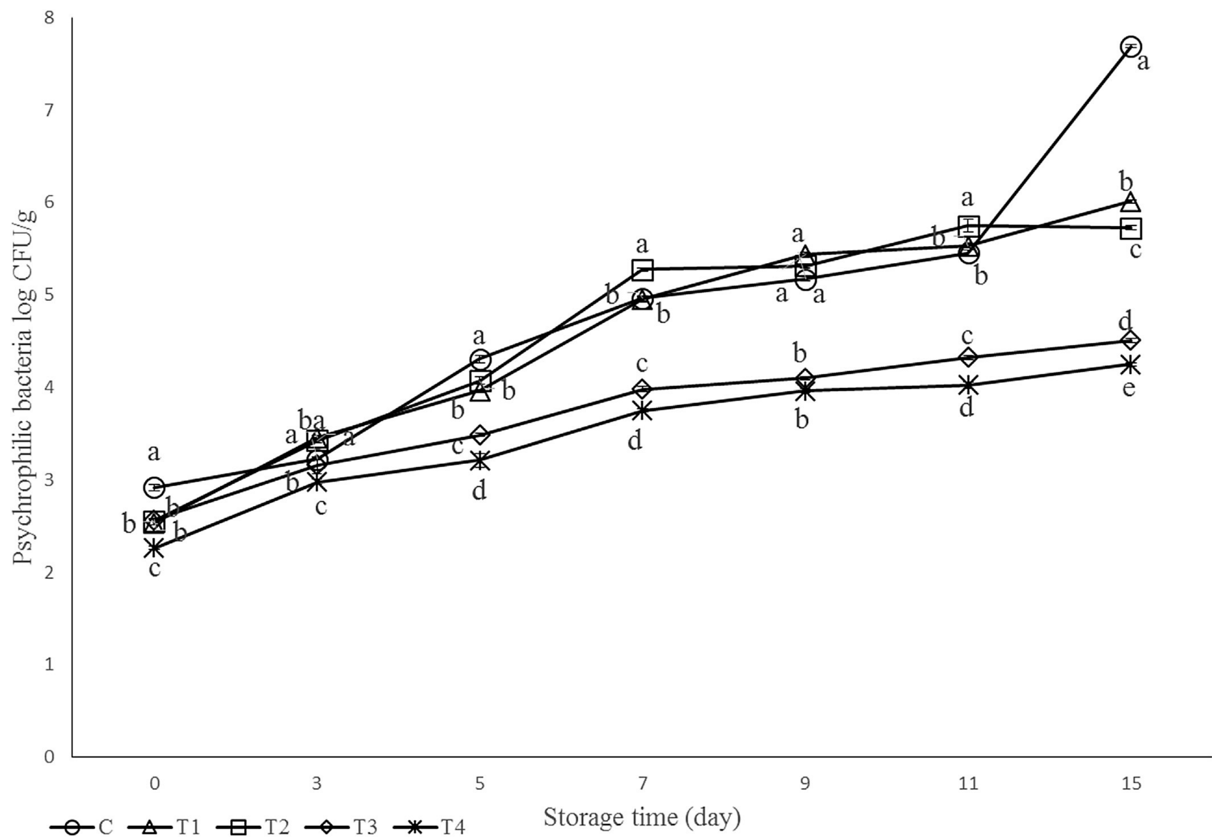

Fig. 4. Psychrophilic bacteria expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

Rainbow fresh trouts stored without any treatment in refrigerator conditions, i.e., 4 C, spoiled in six days according to TAMB counts. They particularly presented a 2 log CFU/g on the first two days, while reached an increasing level of 3.79 log CFU/g on the 3rd day. Finally, the samples spoiled on the 7th day of storage with the highest level, i.e., 6.27 log CFU/g, as previously reported by Mol, Erkan, Ücok, and Tosun (2007). It was evidenced that contribution of Urtica dioica L., either in the form of water extract or embedded in electrospun PCL nanofibers, prolonged the shelf life of the freshly stored fish fillets. As seen in Fig. 3, incorporation of UNFs into the WPI coating showed a significant difference from the 9th day of storage. It is considered that UNFs offered a large surface area-to-volume ratio for the WPI coatings. In addition, Urtica dioica L., as an antimicrobial agent, had enhanced capability to diffuse over an extended area of contact with the fish fillet.

Present results are in agreement with those recently obtained by Li and Peng (2015), who conferred antimicrobial and antioxidant effects of cellulose fibers by using layer-by-layer deposition technique with chitosan/lignosulfonates multi-layers. The previously reported antimicrobial results proved that modified fibers with chitosan in the outer layer exhibited highest antimicrobial activity against E. coli. This was mainly explained by the fact that

chitosan has inherent antimicrobial activity. Additionally, E. coli absorption and immobilization capacities of chitosan were increased because of its hydrophilicity and higher positive charge. Finally, the increase in surface uniformity of cellulose fibers led to a higher specific area that may provide an efficient contact and interaction between chitosan and E. coli.

The here-described results showed that the bioactive coating performed on the fish fillets had a significant effect ($p < 0.05$) on the psychrophilic bacteria counts. The highest average psychrophilic bacteria counts were determined for the control sample (C) with 7.69 ± 0.01 log CFU/g, while the lowest counts were for the test sample based on 70 g/100 g UNF with WPI (T4) with 4.25 ± 0.01 log CFU/g. As observed in Fig. 4, storage time had very significant effects ($p < 0.05$) on psychrophilic bacteria counts. Psychrophilic bacteria growth increased during the storage in all test samples (T1eT4) but in the control sample (C) increased rapidly after the 11th day of storage. Incorporation of Urtica dioica L. in WPI coatings showed an effective antimicrobial activity both in form of water extract (T1 and T2) and PCL nanofibers (T3 and T4). Interestingly, UNFs provided higher antimicrobial performance than that observed for UWE. This can be attributed to the larger surface area and better dispersion achieved for the phenolic compounds contained in Urtica dioica L., which could offer a more complete and sustained release profile by a diffusion mechanism from the PCL matrix. Alternatively, Urtica dioica L. in the water extract mainly provided a burst release. A similar approach in the biomedical field was carried out by means of PLA-based ultrathin fibers containing antibiotic (Torres-Giner, Martínez-Abad, Gimeno-Alcaniz, Ocio,~ & Lagaron, 2012). The previously reported electrospun membrane predominantly offered a dual-stage release profile: An initially faster diffusion of the antibiotic, followed by a somewhat arrested behavior until equilibrium was reached. The higher surface offered by the electrospun nanofibers could additionally improve the antimicrobial effect. The here-attained result was also in agreement with previous research findings in which the high porous characteristics of the electrospun fibers provided a larger surface area that increased exposure and release capacity of bioactive compounds to the surrounding bacteria (Torres-Giner, Martínez-Abad, & Lagaron, 2014; Song et al., 2015).

As shown in Fig. 5, lactic acid bacteria counts decreased significantly ($p < 0.05$) due to the presence and increasing level of UNFs. T4, i.e., 70 g/100 g UNF with WPI, had the lowest counts of 3.22 ± 0.04 log CFU/g, while the control sample (C) showed the highest counts, i.e., 5.76 ± 0.02 log CFU/g. Storage time had a very significant effect ($p < 0.05$). Lactic acid bacteria counts increased slowly in fish fillets during the storage time for the WPI coatings based on UNFs (T4 and T3) while the counts of lactic acid bacteria increased rapidly in control sample (C). Yeast and mold, originated from soil, are not natural flora of fish and their presence generally indicate a water contamination during fishing or from a latter process. There was not a very significant effect between the studied groups in terms of yeast and mold counts while there was a significant effect during storage time ($p < 0.05$). As it can seen in Fig. 6, yeast and mold counts showed a fluctuation among the studied samples during the storage time. Therefore, interestingly, Urtica dioica L. could inhibit a broad spectrum of bacteria but not yeast and mold. In this sense, it is known that some antimicrobial agents cannot inhibit yeast and mold growth effectively but inhibit a broad spectrum of bacteria. In this sense, Can, Arslan, and Ozdemir (2007)€ tested the antimicrobial activity of eugenol in fresh and raw fish fillets. It was reported that eugenol inhibited growth of mesophil aerobic bacteria, mesophilic anaerobic bacteria, total coliform, S. Typhimurium, S. aureus, and V. paraha-emolyticus.

However, it was not very effective against yeast and molds. Another example of existing antimicrobial materials is nitrite, which was used for bacterial inhibition, but it has no effect on yeast and mold growth (Horsch et al., 2014; Jackson, 2010). In contrast, some preservative agents like propionic acid and sorbic acid can inhibit yeast and mold growth, while these are not that effective for bacterial growth (Deak, 2007; Robinson, 2014).

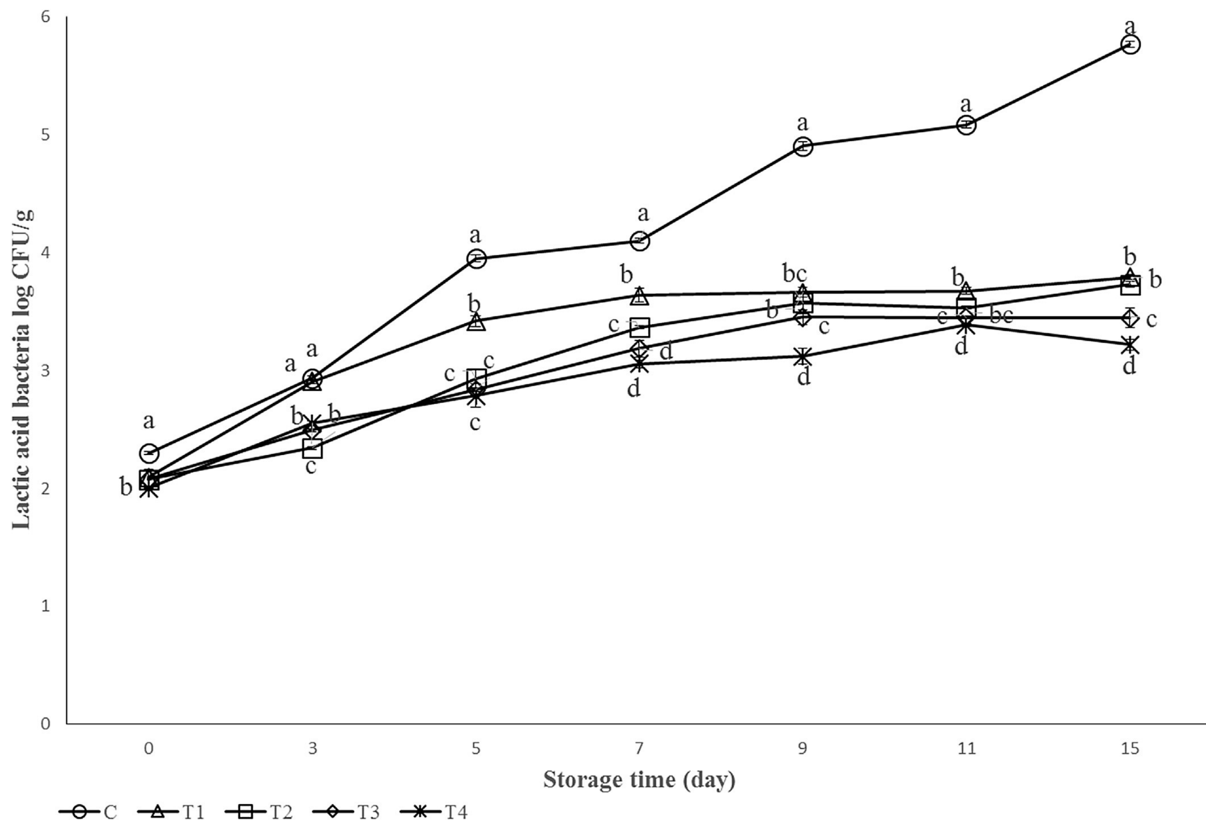

Fig. 5. Lactic acid bacteria expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

As shown in Table 1, Enterobacteriacea counts were lower than 2 log CFU/g in all tested samples. Similar to Gülçin et al. (2004), Urtica dioica L. also presented certain inhibition effect on Enterobacteriacea counts expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

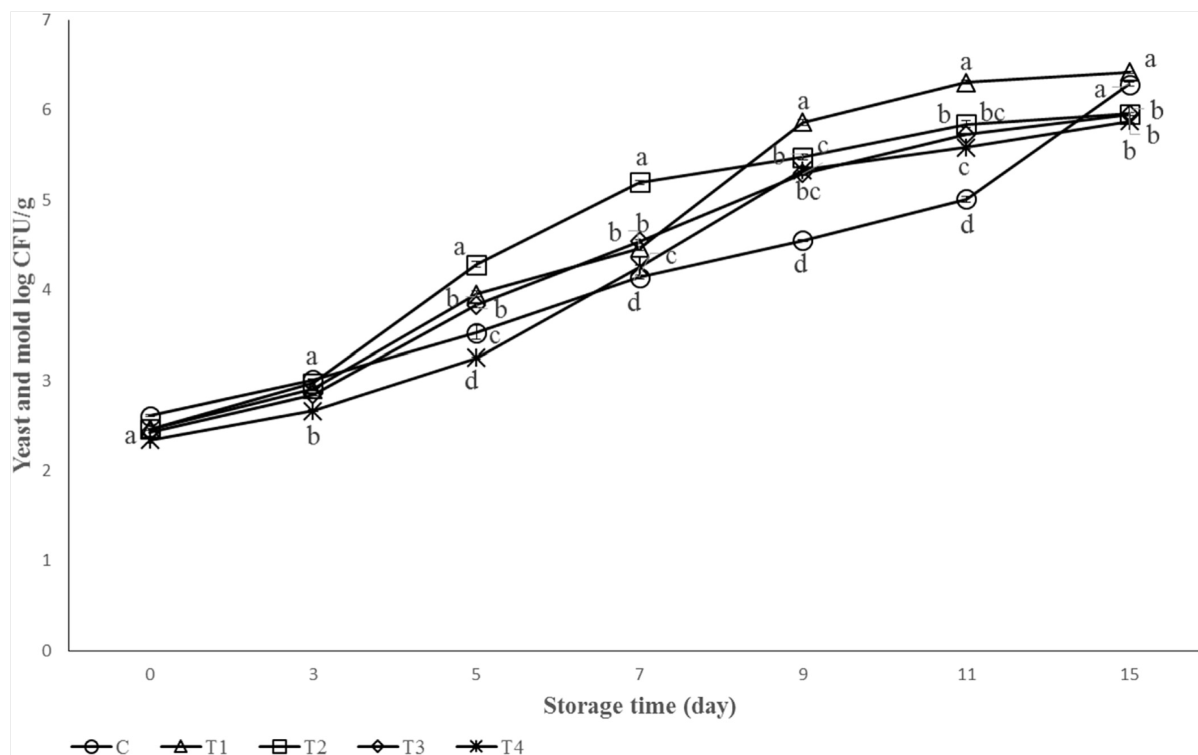

Fig. 6. Yeast and mold expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

Table 1. Enterobacteriacea counts expressed in colony forming units (CFU)/g of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

Samples	Storage Period (day)						
	0	3	5	7	9	11	15
C	1.14 ± 0.001a	1.14 ± 0.001a	1.16 ± 0.001a	1.24 ± 0.001a	1.24 ± 0.001a	1.34 ± 0.001a	1.35 ± 0.001a
T1	1.14 ± 0.001a	1.14 ± 0.001a	1.14 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.23 ± 0.001b
T2	1.12 ± 0.001b	1.14 ± 0.001a	1.14 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001a
T3	1.13 ± 0.001c	1.14 ± 0.001a	1.14 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001a
T4	1.13 ± 0.001c	1.14 ± 0.001a	1.14 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001b	1.22 ± 0.001a

3.3. TVB-N, TBA and pH values

The highest TVB-N value was observed for the control (C), i.e., 36.61 ± 0.33 mg/100 g at the end of the storage period while the lowest value was determined in the 70 g/100 g UNF with WPI (T4), i.e.,16.79 ± 0.34 mg/100 g. As it can be seen in Fig. 7 TVB-N values in each tested

sample, especially in the control (C), increased rapidly, while a slight increase occured for the here-called T4. This is in agreement with previous research done by Wang, Zhu, Li, and Tang (2007), in which electrospun polyaniline (PANI) nanofibers showed high antioxidant activity. The authors tested the antioxidant activity of polyaniline (PANI), which is an effective scavenger of free radicals in aqueous dispersions. This particularly showed similar antioxidant capability than ascorbic acid and catechin. That study additionally reported that nano-structured PANI had chemical and physical properties considerably higher than conventional PANI. The antioxidant activity of PANI nanofibers was also evaluated by Wang et al. (2007), who determined the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging ability with ultravioletevisible (UV-vis) spectroscopy. The authors indicated that PANI nanofibers showed excellent antioxidant activity as the average fiber diameter was decreased. According to that study, the antioxidant capability of the materials developed by electrospinning can be increased due to the larger surface area of the nanofibers.

Lipid oxidation occurs in fish since they are rich in PUFAs, which is one of the main issues related to early spoilage. This process can be retarded by using antioxidants such as plant extracts including phenolic materials. In this sense Urtica dioica L. is rich in phenolic substances and, as a result, it has excellent antioxidant activity (Alp & Aksu, 2010; Gülçin et al., 2004; Karabacak & Bozkurt, 2008). Phenolics in Urtica dioica L. include shikimic acid derivatives phenylpropanes, caffeic acid, and various esters of the latter acid such as chlorogenic acid and caffeoylmalic acid (up to 1.8%) (Upton, 2013). Pinelli et al. (2008) reported chlorogenic and caffeoylmalic acid concentrations of 71.5 and 76.5% of total phenolics per 1.5 g of cultivated and wild samples of Urtica dioica L., respectively. Leaf of this plant is also rich in carotenes such as b-carotene, i.e., 2.95e8 mg/100 g in fresh plants and 20.2 mg/100 g in dried young plants and 25e300 mg in dried plants, and vitamin C, i.e., 20e60 mg in dried samples (Upton, 2013). UNFs retarded lipid oxidation significantly during storage time as it can seen in Figs. 7 and 8 for TVB-N and TBA results, respectively. TVB-N values increased rapidly during the storage time in the control sample (C) while there was an acceleration after 9th day of storage in T4, i.e., in the WPI coating containing 70 g/100 g UNF. A similar trend was observed for TBA values in Fig. 8. In particular,

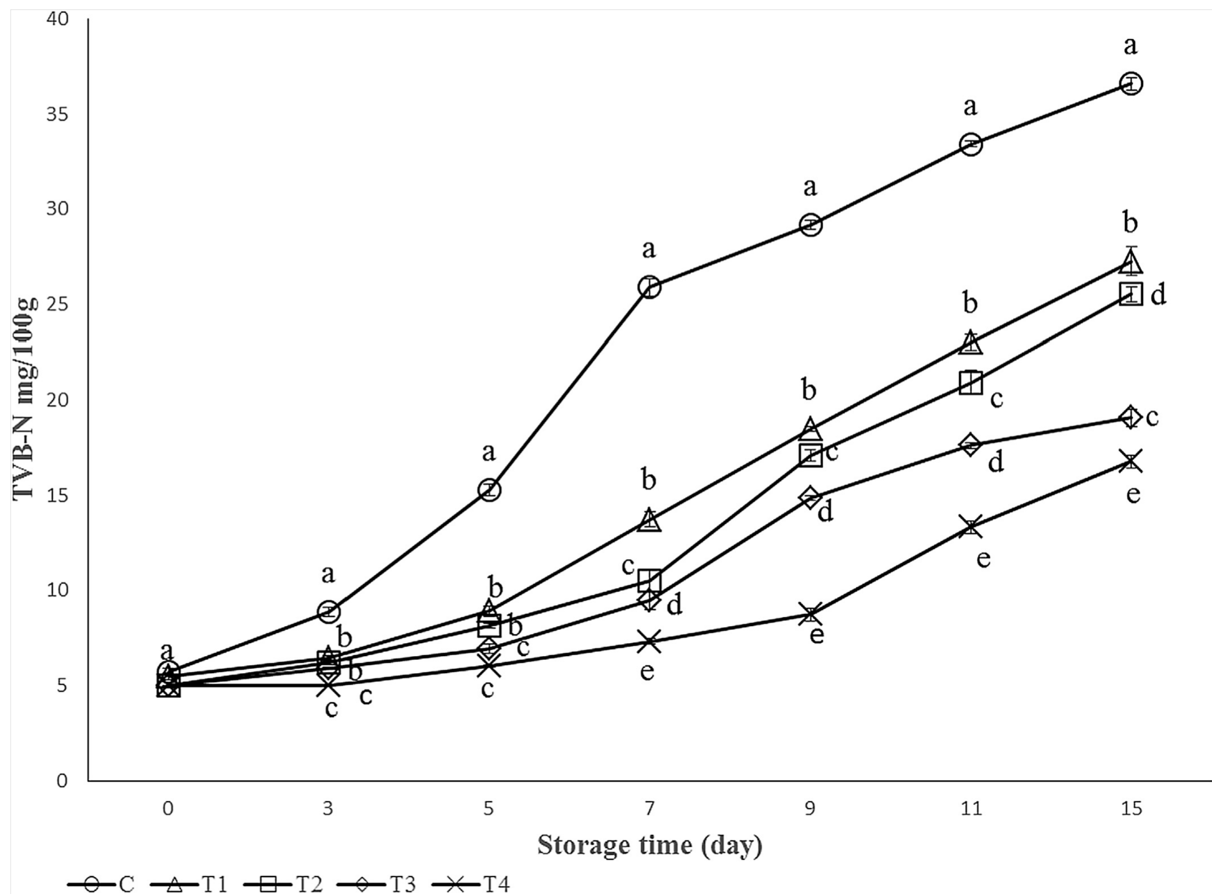

Fig. 7. Total volatile basic nitrogen (TVB-N) value of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

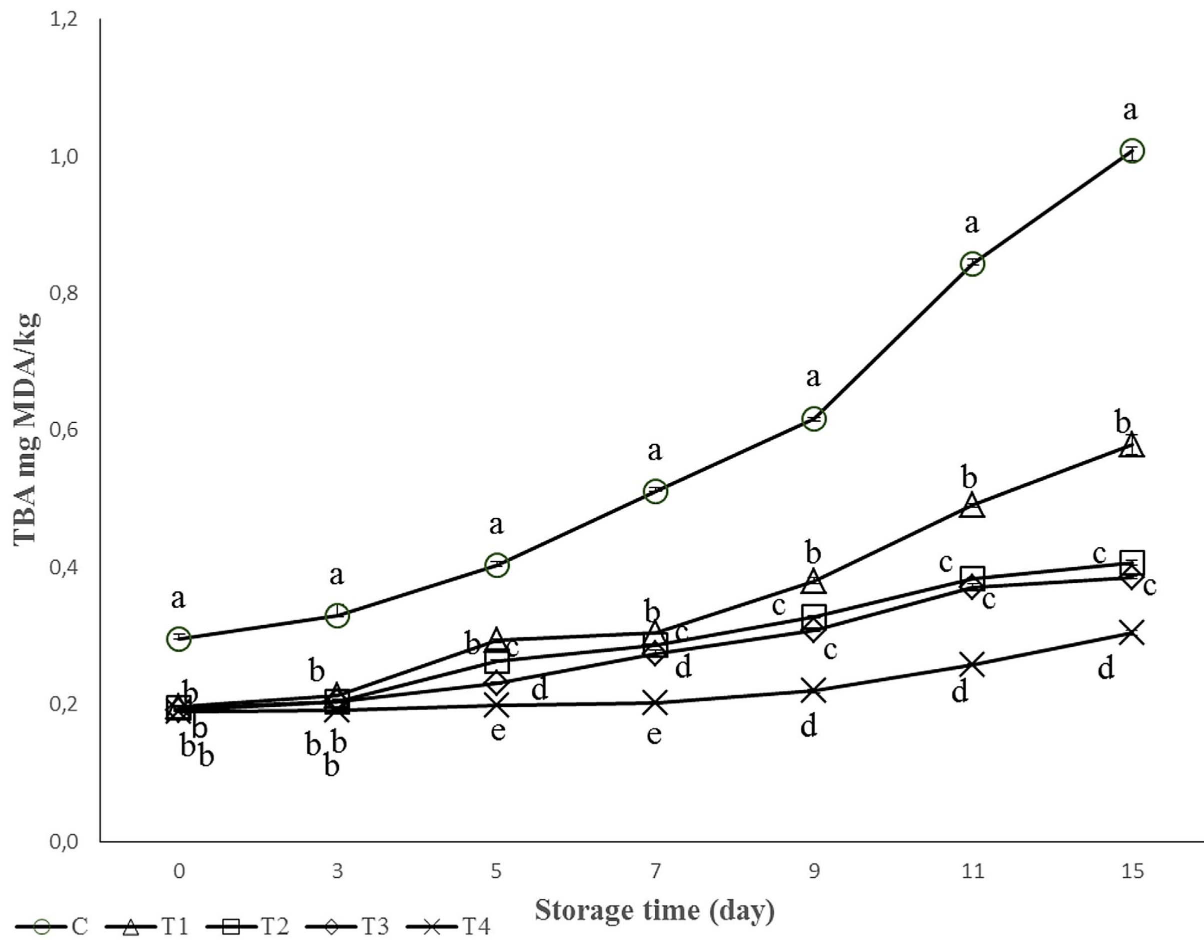

Fig. 8. Thiobarbituric acid value (TBA; mg malondialdehyde (MDA)/kg sample) of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/ 100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

TBA values of the control sample (C) increased constantly until 9th day and afterwards there was an acceleration that continued up to the end of the study. TBA values of the here-called T4 showed decreased values during storage in comparison to the other tested samples. As it can seen in Figs. 7 and 8, lipid oxidation was retarded considerably by using UNFs. This effect was higher than that observed for conventional UWE-based coatings. Although the antioxidant effect of electrospun functionalized nanofibers has been previously reported in different fields (Banerjee, Saikia, Kumar, & Konwar, 2010; Charernsriwilaiwat, Rojanarata, Ngawhirunpat, & Opanasopit, 2015; Wang et al., 2007), including the food industry (Fernandez, Torres-Giner, & Lagaron, 2009), this can be considered the first result in relation to the antioxidant activity achieved by Urtica dioica L.
Finally, pH values did not differ between both UNF-based coatings, T3 and T4, i.e., 6.46 ± 0.01 and 6.39 ± 0.01, respectively. However, it was shown a slight decrease in relation to the control and the test samples (Fig. 9). Despite the acceptable limit of pH, typically in the range 6.8e7.0 for fish, this is not habitually considered as a single quality criteria. At the end of storage, pH

of the control sample (C) reached 7.0 while in the here-called T3 and T4 it was approximately 6.4.

3.4. Color changes

Active coating treatment had very significant ($p < 0.05$) effect on L* values of the samples as it can be seen in Table 2. Storage time and interaction of treatment with storage time had very significant effects ($p < 0.05$) on L* values. During the storage time, L* values of the UNF-based coatings, i.e., of T3 and T4, remained almost constant. However, L* values of the control sample (C) and the WPI coatings produced with UWE, i.e., T1 and T2, presented non-statistically significant fluctuations. This is in agreement with the research work performed by Rodriguez-Turienzo, Cobos, and Diaz (2012). In that previous study it was detected L* values for fresh untreated Atlantic Salmon (Salmo salar) of 45.37, while whey coated samples increased to 49.85.

Both samples containing stinging nettle, i.e., UWE (T1 and T2) and UNFs (T3 and T4), had a significant ($p < 0.05$) effect on a* values while non-statistically significant for b*values (Table 2). In particular, a* values of the control sample (C), i.e., neat WPI, decreased during storage time more than the other samples while b* values increased. In particular, color values in all samples indicated that fish fillets changed from red to green and yellow to brown, according to a* and b*values, respectively.

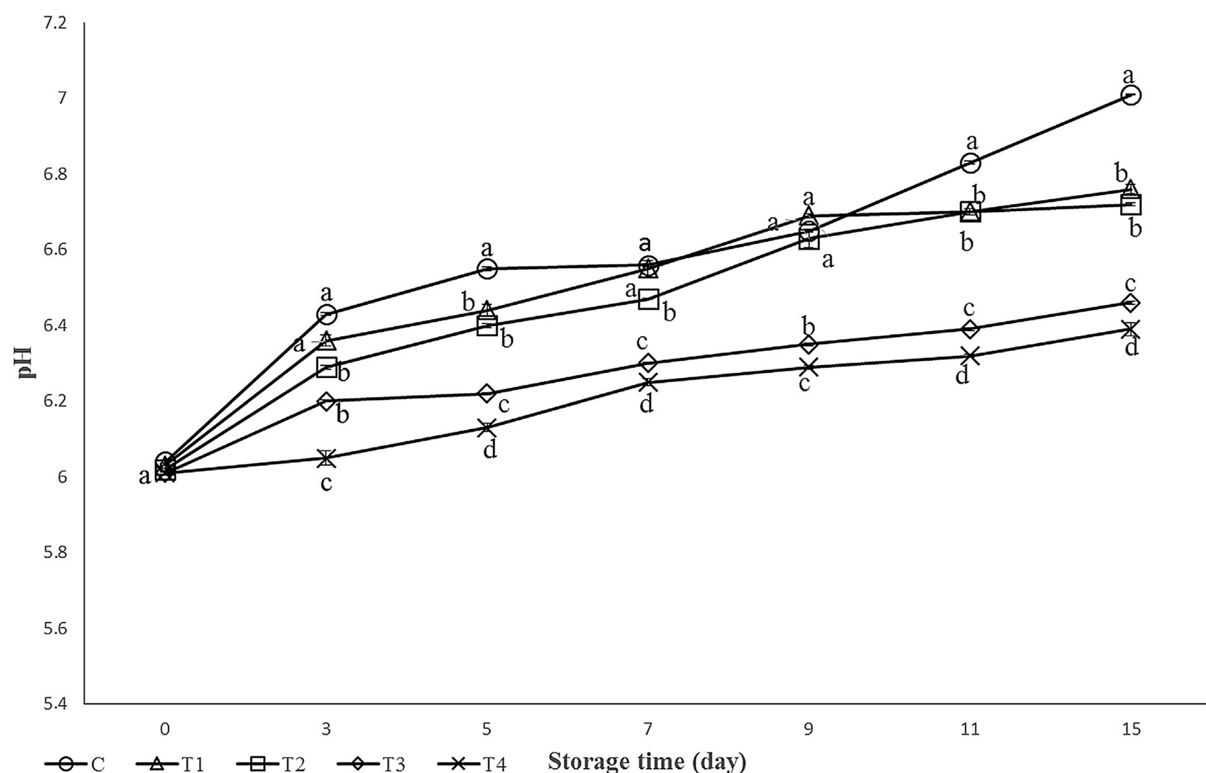

Fig. 9. pH value of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

Table 2

L*, a* and b* values of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/ 100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

Table 2. L*, a* and b* values of fish fillets coated with whey protein isolate (WPI) during storage at 4 C. Samples are identified as follow: Control (C): Neat WPI; Test sample 1 (T1): 5 g/ 100 g Urtica dioica L. water extract (UWE) with WPI; Test sample 2 (T2): 7 g/100 g UWE with WPI; Test sample 3 (T3): 50 g/100 g Urtica dioica L. nanofiber (UNF) with WPI; Test sample 4 (T4): 70 g/100 g UNF with WPI. Different letters indicate significant difference at $p < 0.05$.

Samples	L*,a*,b*	Storage period (day)						
		0	3	5	7	9	11	15
C	L*	36.41 ± 0.22a	41.94 ± 0.35a	42.61 ± 0.30a	43.93 ± 0.05a	45.35 ± 0.14a	46.00 ± 0.5a	48.77 ± 0.21a
	a*	2.74 ± 0.01a	2.04 ± 0.01d	1.97 ± 0.02d	1.77 ± 0.02e	1.05 ± 0.01e	0.97 ± 0.02e	0.94 ± 0.01e
	b*	9.77 ± 0.0a	9.88 ± 0.01a	9.91 ± 0.03a	9.89 ± 0.07a	10.07 ± 0.02a	10.27 ± 0.01a	10.36 ± 0.0a
T1	L*	36.41 ± 0.22a	39.86 ± 0.39b	41.54 ± 0.37b	43.15 ± 0.24a	44.5 ± 0.15b	45.07 ± 0.02b	46.23 ± 0.10b
	a*	2.55 ± 0.01b	2.42 ± 0.03c	2.23 ± 0.01c	2.04 ± 0.01d	1.94 ± 0.04d	1.78 ± 0.03d	1.30 ± 0.01d
	b*	9.75 ± 0.04a	9.84 ± 0.01a	9.88 ± 0.03a	9.84 ± 0.09a	9.97 ± 0.02b	10.12 ± 0.02a	10.24 ± 0.01a
T2	L*	36.34 ± 0.16a	38.32 ± 0.08bc	41.58 ± 0.09b	41.89 ± 0.04b	43.61 ± 0.10c	44.79 ± 0.15c	45.71 ± 0.07bc
	a*	2.47 ± 0.04c	2.49 ± 0.01b	2.32 ± 0.01b	2.28 ± 0.01c	2.11 ± 0.02c	2.08 ± 0.01c	2.00 ± 0.01c
	b*	9.70 ± 0.01a	9.74 ± 0.01a	9.79 ± 0.02a	9.89 ± 0.01a	9.92 ± 0.02b	10.10 ± 0.02a	10.23 ± 0.01a
T3	L*	36.05 ± 0.02a	37.38 ± 0.4 cb	37.31 ± 0.07c	38.36 ± 0.15c	39.51 ± 0.09d	40.2 ± 0.04d	41.32 ± 0.08c
	a*	2.50 ± 0.03d	2.50 ± 0.04a	2.46 ± 0.04a	2.42 ± 0.05b	2.39 ± 0.03b	2.29 ± 0.04b	2.11 ± 0.04b
	b*	9.67 ± 0.03a	9.73 ± 0.01a	9.78 ± 0.02a	9.86 ± 0.01a	9.91 ± 0.01b	9.95 ± 0.01b	9.92 ± 0.02b
T4	L*	35.97 ± 0.01b	36.22 ± 0.06c	36.72 ± 0.16d	37.01 ± 0.02d	37.14 ± 0.01e	37.8 ± 0.04e	37.89 ± 0.04d
	a*	2.50 ± 0.004d	2.48 ± 0.04ba	2.46 ± 0.02a	2.45 ± 0.02a	2.44 ± 0.02a	2.31 ± 0.02a	2.29 ± 0.03a
	b*	9.51 ± 0.00a	9.54 ± 0.01a	9.59 ± 0.01a	9.57 ± 0.01a	9.62 ± 0.00b	9.67 ± 0.02b	9.65 ± 0.01b

4. Conclusion

Novel PCL nanofibers containing Urtica dioica L. were fabricated by the electrospinning technique. Resultant nanofibers were embedded to WPI at different weigth contents and these were applied as a bioactive coatings to preserve rainbow trout fresh fish fillets. Obtained results demonstrated that the here-developed bioactive coatings present excellent antimicrobial and antioxidant activity that could extend the shelf life and quality of the fish fillets up to 15 days. In particular, the bioactive coatings obtained by PCL nanofibers containing Urtica dioica L. inhibited the bacterial growth and decreased the TVB-N and TBA values significantly. This was related to the large surface area and sustained release capacity offered by the electrospun morphology, in which the bioactive substances could diffuse in a controlled

manner to the food product and/or its media. This novel study opens up new opportunities for the application of Urtica dioica L. in food technology and the pharmaceutical industry for drug delivery applications. Additionally, the here-obtained results encourage the use of the electrospinning technology as a platform to encapsulate medicinal plant extracts that can preserve different types of foodstuff by bioactive coatings and packaging systems.

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