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Biomaterials for Food Packaging: Innovations from Natural Sources

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Today, innovation in the food packaging field can be summed up in the concepts of active and intelligent packaging. These two concepts refer to systems capable of interacting and monitoring the storage conditions of packaged food products, allowing them to control their shelf-life and quality at any time to ensure safer products for consumers. Among the active packaging, antimicrobial and antioxidant films seem to be the most promising as they allow to extend the shelf-life by reducing the proliferation of unwanted microorganisms and allow to maintain the organoleptic and nutritional qualities of food. Furthermore, considering the serious environmental impact caused by the volumes of plastic waste, the scientific world has turned towards the use of natural and biodegradable materials. Hence, this work is aimed at developing bio-films using several green techniques including electrospinning and solvent casting of biodegradable polymers such as zein, a prolamin extracted from corn, and polycaprolactone (PCL), a biodegradable synthetic polymer. The polymeric matrices obtained were functionalized by adding natural active compounds such as vanillin, present in vanilla pods, characterized by antimicrobial activity and α-tocopherol, contained in olive oil with high antioxidant properties. The results obtained are reported in terms of morphological characterization, migration tests, which have shown how it is possible to obtain a total release of the active compounds in 24 h under accelerated release conditions, showing the potential of these materials to be used as active food packaging.

1. Introduction

Active and intelligent packaging represent two innovative ways of conceiving food packaging. The innovation contained in these two concepts lies in the fact that it is no longer desirable to attribute to packaging the only passive roles of protection, containment, convenience and communication, typical of traditional packaging; indeed, the innovation starts from the concept of packaging as a system capable of playing an active role in the conservation of products, in order to maintain and monitor the food quality. The European Regulation 450/2009/EC defines active packaging as systems designed to deliberately incorporate components capable of releasing or absorbing substances into or from the packaged product or from the environment surrounding the food, thus extending its shelf life, while intelligent packaging is defined as a system capable of monitoring the conditions of food products and of the surrounding environment (EC, 2009). Both types have been extensively discussed in Drago et al. 2020. Among active packaging, antimicrobial and antioxidant films seem to be the most promising as they allow to extend the shelf-life by reducing the proliferation of unwanted microorganisms and allow to maintain the organoleptic and nutritional qualities of foods (Motelica et al., 2020; Baldino et al., 2017). Furthermore, the research and development of active food packaging films are also directed towards the use of natural materials and biodegradable polymers, with the aim of obtaining packaging that is not only performing in terms of food preservation but also with a lower impact than that caused, globally, by traditional disposable packaging. Regarding the production of these packages, conventional techniques include, for example, extrusion, injection molding, melt pressing and foaming, but nowadays the interest is aimed at the use of green techniques such as electrospinning and solvent casting, suitable for the treatment of both natural polymers and thermolabile substances. In particular, electrospinning is a process that allows the production of continuous fibers with diameters in the range from nano to micrometers. It is a versatile technique that uses only electrical force to guide the spinning process and to produce polymer fibers

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with a high surface/volume ratio due to high porosity and nanostructure (Topuz, 2020). Solvent casting, on the other hand, allows to obtain a thin, transparent and almost perfectly isotropic film; the technique consists of dissolving the polymer and the various additives in a solvent, depositing the solution onto a support and evaporating the solvent (Karki et al., 2016). For these reasons, in this work electrospinning and solvent casting techniques were selected for the production of antimicrobial films using zein, a prolamin extracted from corn characterized by low solubility in aqueous solutions with excellent film-forming properties (Niu et al., 2020), as polymeric material, and vanillin as active compound, an aromatic aldehyde extracted from vanilla pods, with excellent antimicrobial properties against, for example, *Escherichia coli* and *Saccharomyces cerevisiae* (Sangsuwan et al., 2015). Electrospinning was also used for the production of an antioxidant packaging using polycaprolactone (PCL), a biodegradable aliphatic polyester, widely used in various fields, as polymeric matrix and α-tocopherol, naturally contained in olive oil with high antioxidant properties as active substance (Campardelli, 2015).

2. Materials, Equipment and Methods

2.1 Materials

Zein purified, polycaprolactone (PCL, average MW 80,000), vanillin (purity ~ 99%), α-tocopherol (purity ≥ 97%) and glycerol were purchased by Sigma Aldrich, Milan, Italy. Ethanol and acetone were provided by Carlo Erba Reagents, Milan, Italy. Acetic acid glacial was provided by Scharlau, Barcelona, Spain. Distilled water was produced in the laboratory of the Department of Civil, Chemical and Environmental Engineering, Genoa, Italy.

2.2 Equipment

The electrospinning technique was used to produce both antimicrobial and antioxidant films. The electrospinning equipment (Spinbow, Bologna, Italy) reported in Figure 1, is composed of a syringe pump (KDS-100, KD Scientific, Holliston, MA, USA), a high voltage power supply (PCM series, Spellman, NY, USA), a needle (18 gauge) and a collector (metal plate). For the antimicrobial film, the zein solutions were prepared by dissolving the polymer in an aqueous solution of 80% v/v ethanol at different concentrations of zein (20%, 25% and 35% w/w) and adding a fixed amount of vanillin to obtain a theoretical vanillin load from 5 to 15% w/w with respect to the zein content. The solutions were stirred at room temperature for 30 min, after which 10 mL were electrospun with a flow rate of 1.20 mL/h, a voltage of 17.0 kV and a distance of 16.5 cm between the tip of the needle and collector. Similarly, for the production of the antioxidant film, the solutions were prepared by dissolving PCL at different concentrations (5%, 10%, 20% and 25% w/w) in a mixture of acetone and acetic acid 3:7 v/v and by adding an amount of α-tocopherol to reach a theoretical load of 5 to 15% w/w with respect to the PCL content. In this case, the solutions were stirred at 60 °C for 1 h to obtain a complete solubilization of the components, then 10 mL of the prepared solutions were electrospun by varying the flow rate from 0.5 to 2.2 mL/h, the voltage from 15 to 19 kV with needle-collector at a fixed distance of 16.5 cm. In both cases, the films produced were placed in a desiccator overnight before being analyzed.

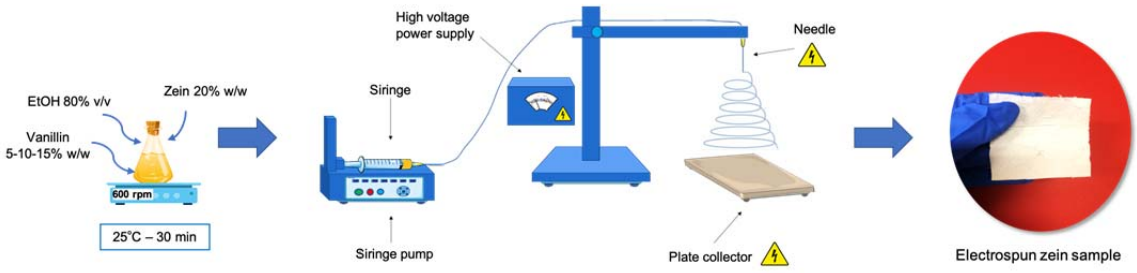

Figure 1: Scheme of electrospinning technique for the production of zein film loaded with vanillin. Some components of the figure were obtained and modified from Medical Servier Art based on a Creative Commons Attribution 3.0 Unported License available at www.servier.com

The solvent casting technique was used to produce the antimicrobial film. The solution was prepared similarly to that described for electrospinning, but in this case, the quantity of zein was fixed at 20% w/w in the 80% v/v ethanolic solution, stirred at 70 °C for a complete dissolution of the polymer, then cooled to 40 °C. At this point, vanillin was added, from 5 to 15% w/w with respect to the zein, and 0.24 mL of glycerol was also added as plasticizer, left under stirring for 8 minutes. Finally, the prepared solutions were poured onto Petri dishes

(2.5 mL for each plate) which were then placed in an oven at 50 °C for 2 h before being peeled and analyzed. The scheme of the solvent casting steps is shown in Figure 2.

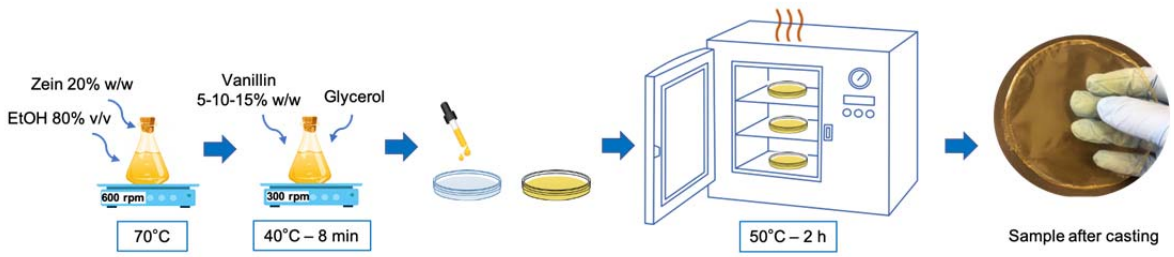

Figure 2: Scheme of solvent casting technique for the production of zein film loaded with vanillin

2.3 Methods

2.3.1 Antimicrobial film

All the samples obtained were analyzed by Field Emission Scanning Electron Microscope (FE-SEM) in order to study the morphology of the fibers obtained through electrospinning and the thickness of the continuous film obtained by solvent casting. The mean diameter (MD) of the electrospun fibers was also calculated using the Sigma Scan Pro 5.0 software. The study of the morphology of the fibers was initially performed on films produced only with polymer, without active ingredient, in order to evaluate the processability of the zein solution. Once the optimal parameters were found, the tests for the incorporation of the antimicrobial compound were repeated and analyzed at the FE-SEM to investigate the influence of the vanillin load on the morphology of the polymeric fibers. The presence of vanillin in the electrospun fibers was investigated by determining the encapsulation efficiency (EE); it was obtained by dissolving a known quantity of sample (5 mg) in 5 mL of the 80% v/v ethanolic solution until complete dissolution and then measuring the concentration of vanillin by means of UV-vis spectrophotometer (Lambda 25, Perkin Elmer, Wellesley, MA, USA) at 280 nm. Knowing the theoretical load of the active compound, it was possible to calculate the EE through Eq(1), where the real load was calculated from the ratio between the mass of the measured compound and the weight of the polymer sample analyzed.

$$EE = \frac{Real\ loading}{Theoretical\ loading}\% \quad (1)$$

Migration tests of vanillin from zein films produced both by electrospinning and by solvent casting were also conducted, using the total immersion method proposed in Xavier et al. (2015). Since migration depends on the food matrix, the tests were performed using one of the simulant fluids recommended by the European Regulation No 10/2011 (EC, 2011): ethanol 10% v/v to simulate lipidic foods. The samples were immersed in bottles containing the simulant fluid in order to maintain a ratio between the surface exposed to the fluid and the volume of the fluid always equal to 0.8. The samples thus prepared were placed in an oven at 37 °C to accelerate the mass transfer and analyzed after 24 h by means of a UV-vis spectrophotometer. All tests were performed in triplicate.

2.3.2 Antioxidant film

As for the case of the antimicrobial film, the PCL samples obtained by electrospinning were analyzed by FE-SEM. First, the morphology of the PCL fibers was studied by varying operating conditions of the process and the PCL concentration, as reported in the section 2.2 above, to find the optimal parameters for which regular fibers were obtained. Therefore, the matrices were reproduced by loading the antioxidant compound and again analyzed at FE-SEM to investigate the influence of the presence of α-tocopherol on the morphology of the PCL fibers. Also, the α-tocopherol release tests from PCL films were conducted at 40 °C using ethanol 50% v/v as simulant fluid (EC, 2011), following the protocol reported by Franco et al., 2019, and analyzing the samples for 24 h by UV-vis spectroscopy at 295 nm. By plotting the release curve relating to the amount of α-tocopherol detected in the simulant fluid as a function of time, it was possible to calculate the EE, expressed as the percentage ratio between the maximum quantity released, corresponding to the value, in milligrams, for which a constant value has been reached, and the theoretically loaded milligrams. All tests were conducted in triplicate.

3. Results

3.1 Antimicrobial film results

The preliminary experiments carried out to test the processability of the hydroalcoholic solution of zein by electrospinning were conducted by varying the concentration of zein between 20%, 25% and 35% w/w with respect to the solvent and testing, as operating conditions, a flow rate of 1.2 mL/h, a voltage of 17 kV, a distance between the needle (18 gauge) and the collector (metal plate) of 16.5 cm. The samples obtained were analyzed by FE-SEM which showed that, for all three concentrations of zein tested, it was possible to obtain a film with an average thickness of 60 μm and a mean weight of 20 g/m^2, with a homogeneous fibrous structure, without obvious imperfections, consisting of fibers with a ribbon structure, distributed in a random way. Furthermore, for the case with 20% and 25% zein concentrations, the distribution of the diameters was found to be comparable and made it possible to obtain sub-micrometric fibers with a mean diameter of about 0.70 μm. Conversely, the 35% zein concentration resulted in the production of micrometric fibers with a mean diameter of about 1.62 μm. Having obtained films with a similar morphology, it was decided to process by electrospinning the 25% zein solution prepared by adding vanillin loaded at 5%, 10% and 15% w/w. Through the FE-SEM analysis of the films obtained, the mean diameter does not change significantly as the vanillin load increases, in fact, the average diameter obtained was approximately 0.80 μm. Figure 3 shows the SEM images of the fibrous structures obtained with zein at 25% w/w and with vanillin loaded zein fibers.

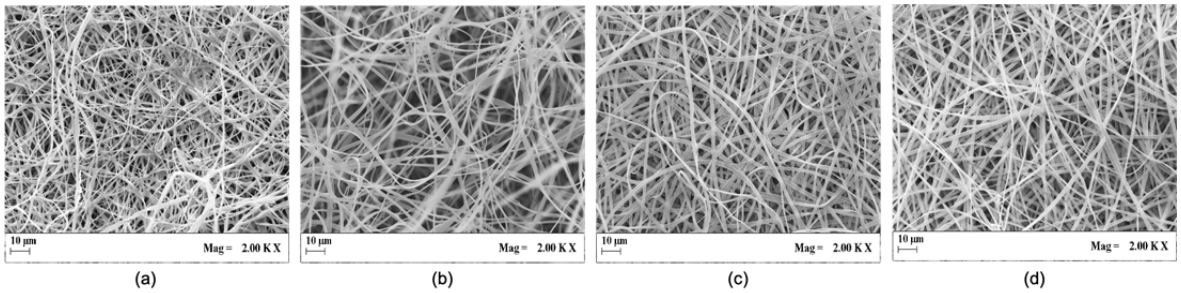

Figure 3: FE-SEM images of electrospun fibers produced with (a) unloaded zein 25% w/w, (b) zein loaded with 5% vanillin, (c) 10% vanillin, (d) 15% vanillin

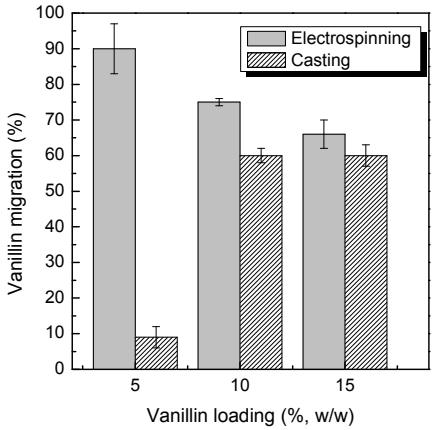

Figure 4: Vanillin migration (%) from zein film obtained by electrospinning and solvent casting using ethanol 10% (v/v) as simulant fluid

As for the antimicrobial films produced with the solvent casting technique, 20% of zein (w/w) was found to be the optimal concentration to obtained transparent films without surface defects, over 20% the solution is too viscous to be poured uniformly. The presence of glycerol facilitated the removal of the film from the Petri dishes. The FE-SEM was used to measure the thickness of the films produced, which was found to be between 10 and 20 μm, independent of the vanillin load and with an average weight of 170 g/m^2. In the case of solvent casting, the EE was 100%, as the process is not subjected to material losses, while for the case of electrospinning the EE has always been around the value of 75%, due to loss of vanillin during processing.

Finally, migration tests in 10% v/v ethanol were conducted to study the dissolution kinetics of vanillin from zein films produced both by electrospinning and by solvent casting. The results obtained by analyzing the electrospun samples after 24 h are reported in Figure 4 and show that the release percentage is maximum in the case of the lower vanillin load and that it decreases with increasing vanillin load. For comparison, Figure 4 also shows the results of the vanillin migration tests from the zein films produced by solvent casting, again in the same simulant fluid. From these last data it has been observed that in the case of the 5% vanillin load, the migration is very reduced compared to the other two cases, 10% and 15% and much lower than the corresponding electrospun samples, in fact the exposed surface is much smaller compared to the electrospun zein.

3.2 Antioxidant film results

Preliminary experiments carried out to test the processability of the 3:7 acetone/acetic acid solution of PCL by electrospinning were conducted by varying the PCL concentration between 5%, 10%, 20% and 25% w/w with respect to the solvent and testing, as process operating conditions, a flow rate between 0.5 and 2.2 mL/h, a voltage between 15 and 19 kV, and a fixed distance between the needle (18 gauge) and the collector (metal plate) of 16.5 cm. The tests carried out with 5% and 25% PCL concentration led to a negative result; however, it was allowed to identify the lower concentration limit (10% w/w) below which only particles and not fibers are obtained, and an upper limit (20% w/w) beyond which the solution is too viscose to be electrospun. Through the analysis of the morphology of the fibers by FE-SEM, the best fibers structure, reported in Figure 5 at two different magnifications, was obtained in the case of 20% PCL concentration, voltage 19 kV and flow rate 1.7 mL/h, while in the other cases, a mixed fibers-beads structure was obtained. For this reason, it was decided to use these optimal conditions for the realization of the antioxidant film by varying the load of α-tocopherol between 5%, 10% and 15% w/w with respect to the polymer.

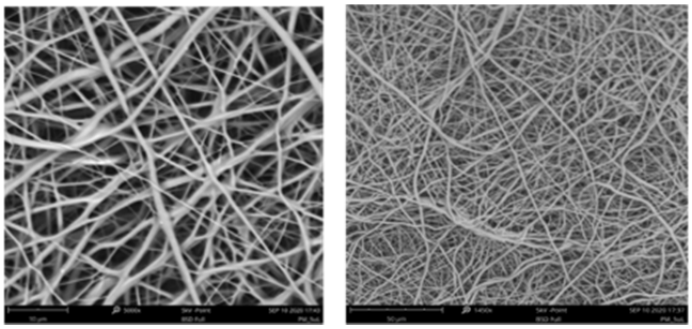

Figure 5: FE-SEM images of electrospun PCL 20% w/w fibers produced at 19 kV, 1.7 mL/h

The α-tocopherol release test from PCL films was conducted, as reported in section 2.3.2, at 40 °C using ethanol 50% v/v as simulant fluid and analyzing samples after 1 h of immersion, after 2 h, 4 h, 6 h, 15 h, 24 h. Figure 6 shows the graph of the release curve obtained expressed as mg of α-tocopherol released into the simulant over time.

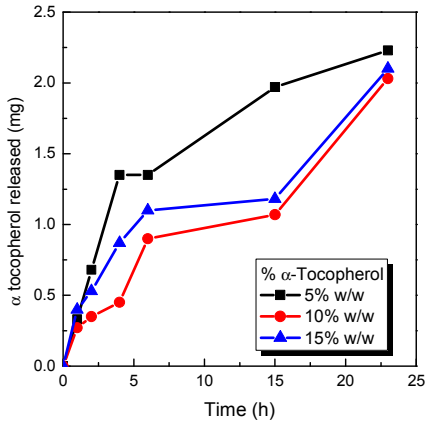

Figure 6: Release curve of mg of α-tocopherol released in 24 h from PCL matrix in ethanol 50% v/v

From the graph shown in Figure 6 it can be seen that, in the first 20 hours, the 5% case has a higher release kinetics than the other two loads. All three curves reach the maximum release value in 24 h. The EE was found to be practically equal to 100%.

4. Conclusions and perspectives

Electrospinning allowed the production of opaque polymeric matrices with fibers of mostly nanometric dimensions and independent of the presence of the loaded active compounds, with an EE of 75% in the case of vanillin and 100% in the case of α-tocopherol. These fibrous structures showed a better ability to release the active compound, especially if present at low concentrations, compared to the solvent casting techniques, which instead allowed the production of a thin and transparent film, a feature certainly useful for food packaging, with an EE of 100%. The good results obtained by both techniques show a potential application of the zein-vanillin film as antimicrobial active packaging and of the PCL-α-tocopherol film as antioxidant active packaging, since it was possible to obtain a total release of the active compounds in 24 h under accelerated release conditions. Future development of this work will include gas and water vapor permeability testing, antimicrobial activity and antioxidant activity testing.

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