Food Packaging and Shelf Life 18 (2018) 42–50

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: www.elsevier.com/locate/fpsl

Release kinetics of rosemary (*Rosmarinus officinalis*) polyphenols from polyvinyl alcohol (PVA) electrospun nanofibers in several food simulants

Santiago Estevez-Areco[a], Lucas Guz[a], Roberto Candal[b,*], Silvia Goyanes[a,*]

[a] Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Física, Laboratorio de Polímeros y Materiales Compuestos (LP&MC), Instituto de Física de Buenos Aires (IFIBA-CONICET), Ciudad Universitaria (1428), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina

[b] Instituto de Investigación e Ingeniería Ambiental, CONICET, Universidad Nacional de San Martín, Campus Miguelete, 25 de mayo y Francia, San Martín (1650), Pcia, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Active packaging
PVA
Rosemary
Electrospinning
Release kinetics
Food simulants

ABSTRACT

Polyvinyl alcohol (PVA) electrospun mats with antioxidant activity (PVA/Ros) were obtained by incorporating rosemary extract (RE) into the electrospinning precursor solution. RE compounds interact with PVA, favoring the retention of polyphenols (88 ± 2%), and improving its thermal stability. The components extracted from PVA/Ros mat were the same as those of the RE. PVA/Ros mats showed a polyphenol content of (15.4 ± 0.5) mg gallic acid equivalent/g of mat (mg GAE/g) and achieved an antioxidant activity of (120 ± 8) μmoles Trolox equivalent/g of mat (μmol TE/g), as measured by Folin-Ciocalteu method and DPPH· assay. The release rate of rosemary polyphenols to several food simulants was measured and kinetic data was adjusted by Fick's diffusion law, Power Law, and Weibull model. The resulting parameters suggested that polymer chain relaxation is the leading mechanism in hydrophilic simulant, while an anomalous release occurred in acid medium. A burst release was observed in lipophilic food simulant, limiting its effectiveness over time. This work shows the potential application of PVA/Ros mats as active food packaging, particularly for hydrophilic and acid food products.

1. Introduction

A new trend in the food packaging industry is the incorporation of bioactive natural components in the packaging material, in order to preserve food quality and extend its shelf life. In particular, the addition of antioxidant natural plant extracts to food packaging avoids oxidative damage during storage. The availability of bioactive compounds can be increased if the packaging material surface is designed as a nanostructure, due to its higher surface to volume ratio with respect to a bulk material. Additionally, the nanostructure could influence the release rate of these compounds (Wen, Zong, Linhardt, Feng, & Wu, 2017). A very promising technology to produce nanostructured materials for food packaging containing different active components is the electrospinning technique (Jacobsen, Garcia-Moreno, Mendes, Mateiu, & Chronakis, 2018).

Recently, it was demonstrated that rosemary extract is a very effective "green" antioxidant for food (Bonilla, Sobral, & do, 2017; López-Córdoba, Medina-Jaramillo, Piñeros-Hernandez, & Goyanes, 2017). Rosemary (*Rosmarinus officinalis*) leaves are an important source of antioxidant molecules, including phenolic compounds and diterpenes. They are commonly used to prepare different rosemary extracts or rosemary essential oils for various applications (Ho et al., 1998; Tu, Moss-Pierce, Ford, & Jiang, 2013; Urbančič, Kolar, Dimitrijević, Demšar, & Vidrih, 2014; Yang et al., 2016). Some authors have reported that rosemary extract composition depends on which solvent and extraction techniques are employed (Carvalho, Moura, Rosa, & Meireles, 2005; Herrero, Plaza, Cifuentes, & Ibáñez, 2010; Ibanez et al., 2003; Moreno, Scheyer, Romano, & Vojnov, 2006). Among the numerous compounds extracted from rosemary leaves, antioxidant activity is mainly attributed to rosemarinic acid, carnosic acid and carnosol content.

Rosemary extracts have previously been incorporated into different polymeric films to produce a potential active packaging. For example, Gómez-Estaca, Bravo, Gómez-Guillén, Alemán, and Montero (2009) studied antioxidant activity of gelatin-based films with the addition of aqueous rosemary extract, showing that the source of gelatin considerably affected the release of polyphenols due to different molecular interactions; Farghal, Karabagias, El Sayed, and Kontominas (2017) have coated polyethylene terephthalate commercial films with rosemary extract in order to protect fish from oxidation; Piñeros-Hernandez, Medina-Jaramillo, López-Córdoba, and Goyanes (2017)

* Corresponding authors.
E-mail addresses: rcandal@unsam.edu.ar (R. Candal), goyanes@df.uba.ar (S. Goyanes).

https://doi.org/10.1016/j.fpsl.2018.08.006
Received 14 March 2018; Received in revised form 12 June 2018; Accepted 13 August 2018
2214-2894/ Published by Elsevier Ltd.

incorporated rosemary aqueous extract in casting cassava starch films; and López-Córdoba et al. (2017) reported that the incorporation of rosemary ethanolic extract in starch filmogenic solution led to the formation of dispersed nanoparticles containing the extract active principles. The formation of nanoparticles was a consequence of the solvent displacement phenomenon. Solvent displacement occurs when the solute solution is incorporated into another solvent (normally referred as anti-solvent) in which the solute is poorly soluble. This incorporation leads to a supersaturation state, which is the driving force for nanoparticle formation (Joye & McClements, 2013).

Nevertheless, there are few studies regarding the incorporation of rosemary extract to electrospun mats. Colín-Orozco, Zapata-Torres, Rodríguez-Gattorno, and Pedroza-Islas (2015) have electrospun whey protein isolate/poly (ethylene oxide) with the addition of rosemary extract, and have studied the dependence of the rosemary release rate on the pH of the medium. Electrospinning is a well-established and scalable technique that allows the production of nanofibers with the capability to entrap and deliver different molecules (Celebioglu & Uyar, 2017; Celebioglu, Yildiz, & Uyar, 2018; Echegoyen, Fabra, Castro-Mayorga, Cherpinski, & Lagaron, 2017; Mujica-Garcia et al., 2016; Ribba, Parisi, D'accorso, & Goyanes, 2014). The use of electrospinning mats could change the release kinetics of active components compared to continuous films, due to the high surface to volume ratio of the mat nanofibers.

Poly(vinyl alcohol) (PVA) is an electrospinable, biodegradable (DeMerlis & Schoneker, 2003), water soluble, and non-toxic polymer permitted for use in food contact materials by the U.S. Food and Drug Administration (US Food & Drug Administration, 2003a, 2017), with a recommended maximum edible dose of 3 mg/kg body weight/day (US Food & Drug Administration, 2003b). PVA electrospun mats have been used by many authors to encapsulate different natural active molecules and extracts, including geraniol, vanillin, cinnamaldehyde, eugenol, d-limonene, and aloe vera, among others (Camerlo, Vebert-Nardin, Rossi, & Popa, 2013; Echegoyen et al., 2017; Kayaci & Uyar, 2012; Kayaci, Ertas, & Uyar, 2013; Kayaci, Sen, Durgun, & Uyar, 2014; Lemma et al., 2015; Torres-Giner, Wilkanowicz, Melendez-Rodriguez, & Lagaron, 2017; Wen et al., 2016). Reticulating PVA with an edible agent reduces PVA water solubility -one of the main limitations for its use as a controlled release system in aqueous mediums- without losing its potential application in food industry. Recently López-Córdoba, Castro, and Goyanes (2016) showed that citric acid (CA) crosslinking improves water stability of PVA mats, and consequently they obtained a controlled release system for tetracycline hydrochloride.

To the best of our knowledge, there are no reports about the release kinetics of rosemary polyphenols in different food simulant mediums from PVA electrospun mats containing rosemary extract. In this work the incorporation of rosemary extract into a crosslinked PVA electrospun mat in order to obtain an active material with controlled release capability was reported. First, a rosemary extract was obtained by solvent extraction in an ethanol:water solution (70:30 v/v) and was characterized by measuring polyphenol content and antioxidant activity. The resulting rosemary extract was incorporated into a PVA aqueous solution and electrospun. The incorporation efficiency of rosemary polyphenols and the antioxidant activity of electrospun PVA active mats were measured. Bioactive components of the original rosemary extract were compared to those present in the PVA mats containing rosemary extract and to those released from the mat in hydrophilic, lipophilic and acid food simulants. Morphological, thermal and chemical characterizations were performed in order to understand the obtained results. Finally, rosemary polyphenols release kinetics was studied in several food simulants and modeled according to Fick's diffusion law, Power Law model, and Weibull model in order to elucidate mechanisms involved in its release.

2. Experimental

2.1. Materials

Polyvinyl alcohol Mowiol 10–98 (Mw = 61000, Sigma-Aldrich, Germany), citric acid (Stantom, Argentina), Folin-Ciocalteu reagent (Anedra, Argentina), DPPH· reagent (Sigma-Aldrich, Germany), gallic acid (Anhydrous p.a., Biopack, Argentina), and Trolox (Sigma-Aldrich, Germany) were used as received. Rosemary dried leaves were purchased in a local trade; distilled water and food grade ethanol (96% v/v) were used.

2.2. Rosemary extract

Extraction was performed according to the procedure recently optimized by Oliveira, Oliveira, Conceição, and Leles (2016). Briefly, 10 g of dried rosemary leaves were milled and dispersed in 50 mL of ethanol:water solution (70:30 v/v). The mixture was placed in a thermostatic bath at 50 °C for 55 min. The obtained rosemary extract was cooled at room temperature and filtered (pore size 0.8 μm). The resulting solution was named RE; it was clear and without any precipitates. The extraction yield was determined gravimetrically, by drying 5 mL of RE at 50 °C until constant weight. The yield was (59 ± 1) g of dried solids extract/L of RE. The obtained rosemary powder was characterized by FTIR and TGA measurements. These experiments were performed on the powder instead of RE in order to avoid interferences from the solvent and interactions between solvent and rosemary compounds.

The total polyphenols content of RE was determined by Folin Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Briefly, 400 μL of each sample were mixed with 2 mL of Folin-Ciocalteu reagent (1:10 diluted). Then, 1.6 mL of sodium carbonate (7 g/100 mL) were added to each sample. Absorbance was measured at 760 nm after 30 min reaction using a spectrophotometer (SHIMADZU UV-1800, Japan). Calibration curve was obtained using gallic acid (Biopack, Argentine) as a standard, in a range between 10 mg/L and 150 mg/L. Results were expressed as gallic acid equivalents (GAE/mL of RE).

Antioxidant activity of RE was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH·) reagent as a free radical (Brand-Williams, Cuvelier, & Berset, 1995). RE was diluted in order to achieve different antioxidant concentrations. Then, 100 μL of each dilution were mixed with 3.9 mL of DPPH· in methanol (25 mg/L). Absorbance was measured at 516 nm after 30 min reaction. The DPPH· scavenging activity is defined by:

$$DPPH \quad inhibition\,(\%) = 100.\frac{A_b - A_s}{A_b}, \tag{1}$$

where A_b is the absorbance of the blank and A_s is the absorbance of the sample. Antiradical activity was first expressed as the amount of antioxidant necessary to decrease the initial DPPH· concentration by 50%, called efficient concentration (EC_{50}) (Brand-Williams et al., 1995). Inhibition of DPPH free radicals was compared with a Trolox calibration curve (5–40 mM), and antioxidant activity was also expressed as μmol Trolox equivalent (TE)/mL of extract (Delgado, Galleano, Añón, & Tironi, 2015).

2.3. Electrospinning process

PVA electrospun solution was obtained by dissolving 12 g of powdered PVA with 0.6 g of citric acid (5% w/w respect to polymer) in 88 mL of distilled water, at 80 °C for 1 h under constant magnetic stirring (PVA*). In the case of the sample containing RE (PVA/Ros), 20 mL of water were replaced by 20 mL of the extract, which was added dropwise under constant stirring and then mixed for 30 min. PVA solution became turbid after RE addition, suggesting the formation of particles as a consequence of solvent displacement phenomenon. In

Table 1
Composition and properties of electrospun solutions. Citric acid and rosemary content are added over PVA solution (PVA + water + ethanol). % Ros (w/w) corresponds to rosemary solids.

Solution	Water	Ethanol:water (70:30 v/v)	% PVA (w/w)	% CA (w/w)	% Ros (w/w)	Conductivity (μS/cm)	Viscosity (cP)
PVA/Ros	68	20	12	0.6	1.2	690 ± 1	434 ± 5
PVA	68	20	12	0.6	0	495 ± 1	400 ± 5
PVA*	88	0	12	0.6	0	810 ± 1	297 ± 5

order to study the effect of ethanol in the aqueous PVA electrospinning solution, a sample was prepared by replacing 20 mL of water with 20 mL of ethanol:water (70:30) mixture (PVA). Compositions and nomenclature of each sample are presented in Table 1.

Solution viscosity and conductivity were measured by a Brookfield viscometer (model LV DV-E) and by an Orion Versastar conductimeter (Thermo scientific), respectively. The obtained results are presented in Table 1.

Size of the nanoparticles formed after RE addition to distilled water (López-Córdoba et al., 2017) was determined using a Laser Diffraction Particle Size Analyzer SALD-3101 (LS, Shimadzu, Japan). Additionally, a drop of the same dispersion was air dried at 50 °C, sputtered with a thin layer of platinum and examined by scanning electron microscopy (SEM) using a field emission gun Zeiss SUPRA 40. SEM images were used to determine rosemary particle diameter distribution by measuring diameter of 100 particles with ImageJ free software.

Solutions were placed in a 10 mL plastic syringe connected to a multi-nozzle injector with 6 needles of 0.8 mm inner diameter. A rotating drum collector of 6 cm diameter covered by an aluminum foil was located 20 cm from the needles. During electrospinning, the syringe was pushed employing a syringe pump (APEMA PC11U, Argentina) with a controlled feed rate of 2.2 mL/h, and a voltage of 30 kV was applied between the needles and the collector. An experimental arrangement scheme is shown in Fig. 1. Electrospun mats were treated at 190 °C for 10 min to induce crosslinking between PVA and CA in order to improve water stability of mats (López-Córdoba et al., 2016).

2.4. Mats characterizations

Morphology of the resulting fibers was examined by SEM with the same microscope described above. Electrospun mats were sputtered with a thin layer of platinum just before observation to improve sample conductivity. SEM images were used to determine fiber diameters with the help of ImageJ free software. The diameter distribution (histogram) and average fiber diameter were obtained.

Chemical characterization was performed by Fourier Transform Infrared Spectroscopy (FTIR) using a Jasco FT-IR 4100 spectrometer in ATR mode. Each spectrum was recorded as the average of 64 scans with a resolution of 2 cm^{-1}. In order to compare PVA and PVA/Ros mats, spectrums were normalized with respect to the C–H bending band (1328 cm^{-1}), which was not observed in the rosemary powder spectrum and corresponds to a functional group that is not expected to interact with any of the components.

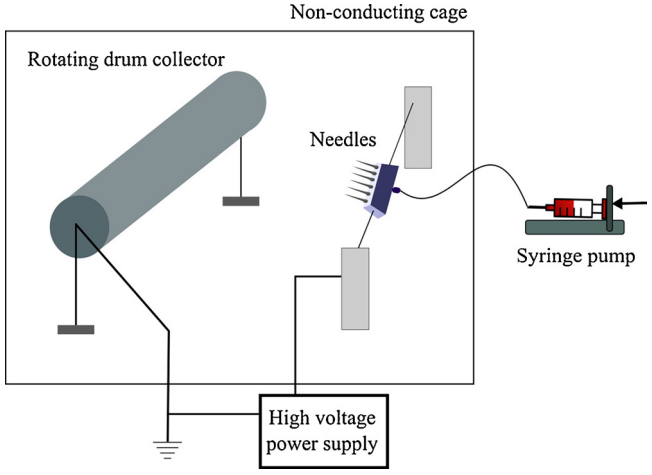

Fig. 1. Electrospinning arrangement scheme.

Thermogravimetric analyses (TGA) were performed for the rosemary dried extract, PVA and PVA/Ros mats. For this purpose, samples of about 2 mg were placed in aluminum pans in the TGA balance. Tests were performed using a Shimadzu DTG-60 (Japan) under a dry nitrogen atmosphere in the range of 25–550 °C at a heat rate of 10 °C/min.

Polyphenol extraction from PVA/Ros mats was carried out to measure total polyphenols retained, rosemary component distribution, and antioxidant activity of mats. A complete mat of 366 mg was submerged in 100 mL of ethanol:water solution (70:30 v/v) at 50 °C under reflux. This procedure was chosen to be similar to the RE preparation from dried rosemary leaves. Aliquots were extracted from the solution at 1, 2, and 3 h and the polyphenols content and antioxidant activity were measured by Folin-Ciocalteu and DPPH· assays, respectively. Antioxidant activity was expressed as Trolox equivalent (μmol TE/g mats).

High Performance Liquid Chromatography (HPLC) analyses were carried out using a Dionex UltiMate 3000 (Thermo Scientific) coupled to a LTQ XL mass spectrometer (Thermo Scientific) via an electrospray interface. The employed method was adapted from Herrero et al. (2010): the column used was a Hypersil Gold column (50 mm × 2.1 mm, d.p. 1.9 m) (Thermo Scientific) and the mobile phases consisted of ACN (0.1 formic acid, A) and water (0.1% formic acid, B) eluted according to the following gradient: 0 min, 95% B; 0.52 min, 95% B; 5.25 min, 40% B; 9.30 min, 5% B; 9.75 min; 5% B; 10.5 min, 95% B; 13.5 min, 95% B. Flow rate was 0.3 mL/min and injection volume was 5 μL.

Release studies were performed using three different food simulants: hydrophilic food simulant (ethanol 10% v/v), acid food simulant (3% acetic acid w/v) and hydrophobic (ethanol 50% v/v) (European Commission, 2011). PVA/Ros mats samples (~200 mg) were immersed in 20 mL of food simulant and placed in a shaker at 125 rpm. Samples were taken at different times and polyphenols released were measured using the Folin-Ciocalteu method described previously. Initial polyphenols released were measured at 20 s. Assays were performed in triplicate, and results were fitted with Fick's diffusion law, Power Law and Weibull model using SciPy (Oliphant, 2007).

3. Results and discussion

3.1. Rosemary extract characterization

Total phenolic content of RE was 10.6 mg GAE/mL. With respect to the antioxidant activity, extracts exhibited an efficient concentration EC_{50} = 0.37 μL/mL (5.45 μg/mL) and an antioxidant activity of (80 ± 4) μmol TE/mL. Rosemary extracts exhibit excellent antioxidant activity, as reported by many authors: for example Moreno et al. (2006) obtained EC_{50} = 33 μg/mL for a rosemary extract in methanol obtained by a Soxhlet apparatus; Herrero et al. (2010) reported EC_{50} = 11.4 μg/mL when working with pressurized liquid extraction in ethanol; Rodríguez-Rojo, Visentin, Maestri, and Cocero (2012) reported

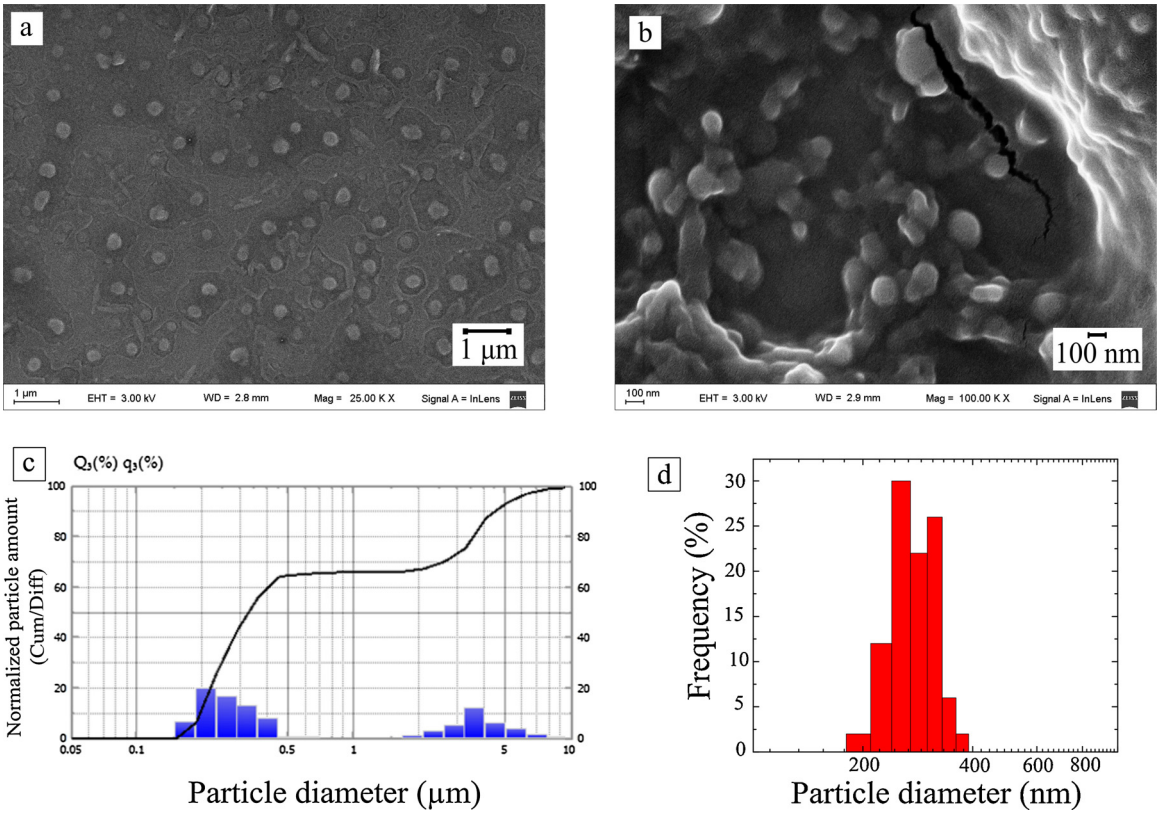

Fig. 2. SEM images of rosemary nanoparticles (a, b) and particles size distributions measured by LS (c) and by SEM micrographs (d).

EC_{50} = 35.4 µg/mL when working with de-oiled leaves by ethanol extraction; and López-Córdoba et al. (2017) reported EC_{50} = 90.01 µg/mL for an ethanol extract. A high concentration extract was obtained in this work by following the recently developed method by Oliveira et al. (2016), who optimized extraction of rosemary main antioxidants in different ethanol:water solutions.

Recently, López-Córdoba et al. (2017) reported the precipitation of nanoparticles from a rosemary extract when it was added to a starch filmogenic solution. A similar process must be expected when adding RE to PVA electrospinning, as a consequence of the precipitation of non-water soluble components by a solvent displacement phenomenon. Fig. 2 shows SEM images (Fig. 2a, b) and a comparison between the size distribution of rosemary extract nanospheres, obtained by LS (Fig. 2c) with respect to those obtained by SEM (Fig. 2d). LS measurement showed two particle distributions, centered at around 300 nm and 3 µm. An exhaustive study by SEM did not show micrometers particles and revealed the presence of agglomerated nanoparticles with a similar size to those observed by LS (Fig. 2b). Dismissing agglomerates, the average diameter resulted (307 ± 33) nm for LS measurements, and a similar value was measured by SEM (282 ± 39 nm).

3.2. Mats characterization

Representative SEM images, fiber diameter distribution, and average fiber diameter of the different electrospun mats are shown in Fig. 3. Uniform fibers were obtained for the PVA* solution, with an average diameter of (120 ± 21) nm. When adding ethanol to the solution, average diameter of the fibers increased to (231 ± 52) nm and a strong widening was observed in its distribution. As explained by Zhang, Yuan, Wu, Han, and Sheng (2005), ethanol addition decreased the surface tension of PVA water solution and simultaneously increased solvent evaporation rate during electrospinning. These effects and the lower conductivity, as reported in Table 1, could be responsible for the fibers widening and the broader distribution. For the PVA/Ros fibers, a similar distribution with respect to PVA was obtained, with an average diameter of (223 ± 80) nm, indicating that ethanol addition and not the rosemary component is responsible for fibers morphology changes and increased diameter.

Fig. 4a presents the FTIR spectrum of mats and rosemary dried extract. Characteristic bands of PVA are assigned to O—H stretching in hydroxyl group (3000–3600 cm^{-1}), C–H symmetric and asymmetric stretching (2854, 2921 cm^{-1}), C–O stretching in alcohol group (1096 cm^{-1}), and C–H bending (1328 and 1424 cm^{-1}). The band centered at 1144 cm^{-1} is associated with C–O bending in crystalline fraction of PVA (Krimm, 1960). Crosslinking of PVA electrospun mats with CA induced by heat treatment was previously studied by López-Córdoba et al. (2016). In this work, the same heat treatment was used and the successfully crosslinking reaction was evidenced by the band centered at 1745 cm^{-1}, which corresponds to C=O stretching of ester group. Rosemary extract includes a wide variety of organic molecules that presents aromatic and phenolic groups. FTIR spectrum showed stronger bands corresponding to O—H stretching of phenol group (3000–3600 cm^{-1}), C–H stretching (2929 cm^{-1}), C=C ring stretching (1597 cm^{-1}), and C–OH stretching of phenolic groups (1264 cm^{-1} and 1029 cm^{-1}). Electrospun mats containing rosemary extract present the 1597 cm^{-1} band corresponding to C=C stretching, confirming the presence of rosemary compounds. PVA/Ros also presents a broadening in the 1096 cm^{-1} band toward lower wave numbers, probably due to an overlap with C–OH band from rosemary phenolic compounds (Fig. 4c). The band corresponding to C–H stretching (2921 cm^{-1}) showed a double peak in PVA/Ros spectrum due to the presence of vibration mode from $C–H_3$ belonging to some rosemary components, such as carnosol or rosmanol, among others. The band corresponding to O—H stretching in PVA/Ros (centered at 3296 cm^{-1}) presented a 14 cm^{-1} shift to lower values with respect to PVA band (3310 cm^{-1}), suggesting hydrogen bond interactions between PVA and polyphenolic compounds from the extract (Fig. 4b). Interaction between PVA and the rosemary components could help retain it inside fibers, protect them

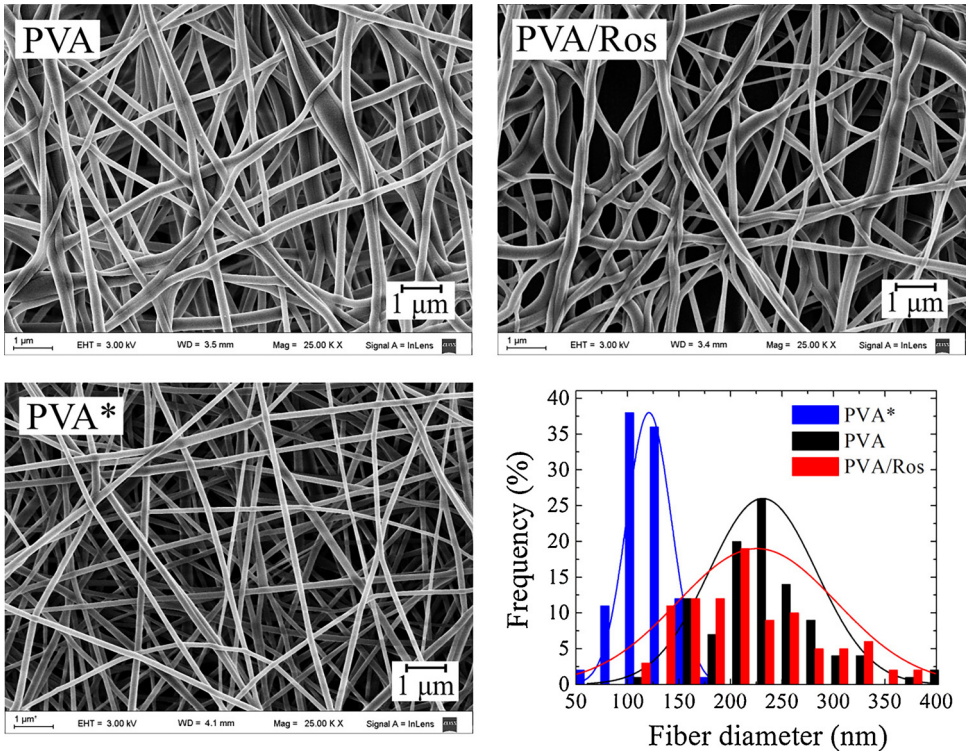

Fig. 3. SEM micrographs of the obtained mats and diameter size distribution.

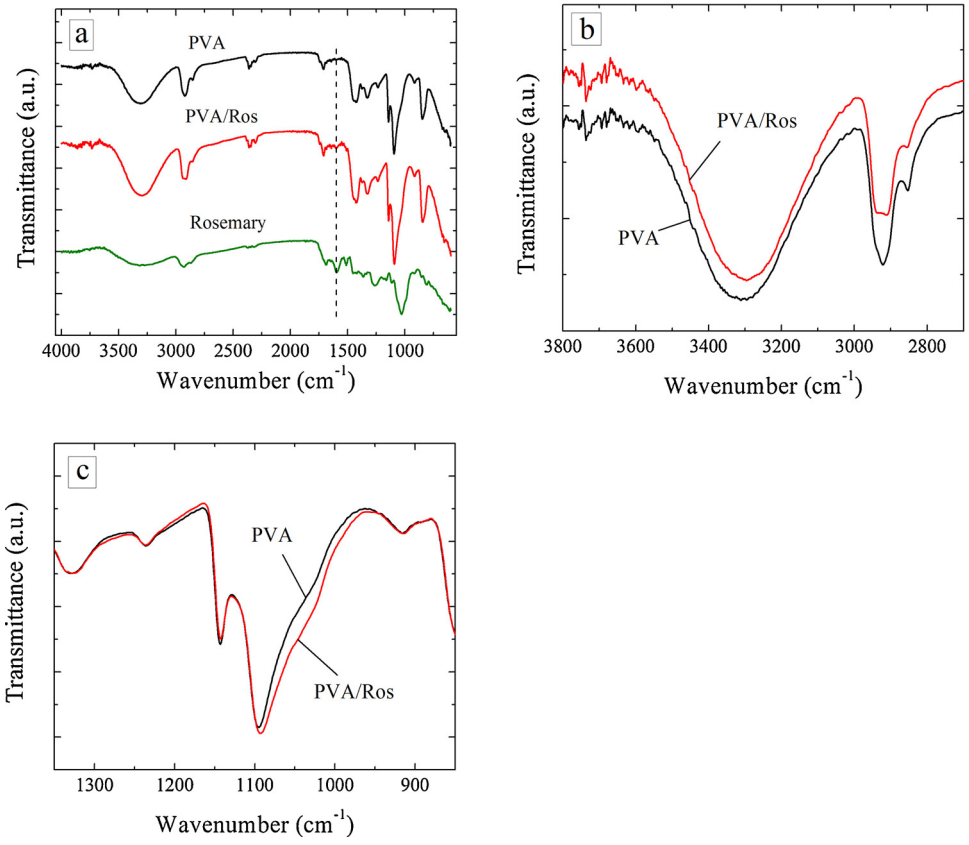

Fig. 4. FTIR spectra of PVA mats and rosemary powder extract (a), and detailed differences between PVA and PVA/Ros mats (b, c).

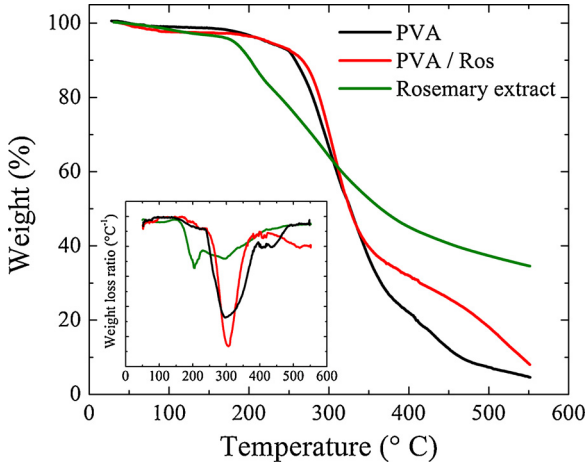

Fig. 5. TGA curves of PVA mats and rosemary powdered extract.

during heat treatment and possibly decrease their release kinetics.

Thermogravimetric analysis of PVA, PVA/Ros mats and rosemary powder extract are shown in Fig. 5. PVA thermal degradation occurs in two steps, as evidenced by the derivative curve. The first degradation (200–250 °C) corresponds to the elimination of side groups, which form water and other volatile compounds such as ketones and acetaldehyde, while the second degradation process (350–450 °C) is associated with decomposition of the main polymer chain (Holland & Hay, 2001; López de Dicastillo et al., 2017). Rosemary powder extract presented a slow thermal degradation at a wide range of temperatures, beginning at about 170 °C and leaving 37% of residual mass at 550 °C. In the derivative curve two thermal degradation processes were distinguished, one centered at 205 °C and other at 300 °C. The first one was associated to the loss of water, ethanol and volatile phenolic compounds, while the second was attributed to the decomposition of bioactive constituents (Cordeiro et al., 2013). The overall 7% of mass losses till 190 °C suggest that the heat treatment used for PVA crosslinking in PVA/Ros could degrade a portion of entrapped rosemary components. This 7% represents an overestimation of rosemary component losses during heat treatment, since degradation may be slowed by PVA molecular interaction discussed in FTIR section. PVA/Ros mat also degraded in two steps: the first step was similar to the one observed for PVA mat, while the second showed a lower slope. In the derivative curve this effect is evidenced by the reduction of second peak's area. This difference between PVA and PVA/Ros curves (~14%) was greater than the rosemary mass incorporated. This result indicates that rosemary addition delayed thermal degradation of PVA, suggesting chemical interaction between rosemary components and PVA side chains, in agreement with FTIR results. Additionally, rosemary extract degraded about 38% between 170 and 310 °C, but PVA/Ros did not show any extra weight loss in that range, supporting the theory that PVA encapsulated and thermally stabilized rosemary components. Residual mass at 550 °C showed a 3% difference between PVA and PVA/Ros corresponding to rosemary substances presented in that material.

3.3. Polyphenols encapsulation, antioxidant activity and release studies

Polyphenol contents did not show significant differences between extraction at 1, 2 and 3 h, and the average value obtained was (15.4 ± 0.5) mg GAE/g of PVA/Ros mats. During mats fabrication, 20 mL of ethanolic extract were mixed with a solution containing 12 g of PVA (Table 1); therefore, the theoretical concentration of polyphenols encapsulated in PVA/Ros was 17.7 mg GAE per material gram. Comparing this value with the polyphenols recovered in the extraction, a (87 ± 3) % of polyphenols retention was achieved. As discussed earlier, a fraction of rosemary components could be lost during the heat treatment performed at 190 °C. However, retention percentage measured by the Folin-Ciocalteu method indicates that most of the rosemary polyphenols were retained by PVA fibers during the electrospinning and heat treatment processes.

DPPH· assay was performed with the same ethanol mixture containing the extracted polyphenols from PVA mats, resulting in an antioxidant capacity of (120 ± 8) μmoles TE/g. As discussed in the preceding paragraph, during mats fabrication 20 mL of RE were incorporated to 12 g of PVA. Comparing the antioxidant activity of the extract (Section 3.1) with that measured for PVA/Ros mats, (90 ± 10) % of the antioxidant activity of RE incorporated was retained. This result lines up well with the percentage of polyphenols retention, indicating that RE polyphenols react in the same manner with both reagents. Thus, the antioxidant activity of PVA/Ros mats will be proportional to the released polyphenols. Many recent articles in active food packaging reported values for DPPH scavenging activity of polymeric films with different antioxidants. Reports in which antioxidant activity was expressed as Trolox equivalents (Araújo et al., 2015; De Freitas et al., 2017; Genskowsky et al., 2015; Rodsamran & Sothornvit, 2017) were compared directly with PVA/Ros results (Table S1). However, several papers only reported the percentage of inhibition (Busolo & Lagaron, 2015; Ghadetaj, Almasi, & Mehryar, 2018; Kadam, Pankaj, Tiwari, Cullen, & O'Donnell, 2015; Piñeros-Hernandez et al., 2017), and values could not be directly compared since the mass of material, the volume used in the extractions and DPPH concentration used are different in each paper. Antioxidant activity was normalized as reduced DPPH moles per mass of material and compared with PVA/Ros results (Table S2). The proposed PVA/Ros electrospun mats presented higher activity with respect to other materials recently presented in the literature (details are presented as Supplementary material).

In order to compare composition of RE with components effectively incorporated into PVA/Ros mats, HPLC assays were carried out, and results are presented in Fig. 6. RE chromatogram showed a wide variety of substances in which two main zones were identified: polar molecules determined at shorter times (about 5 min), and non-polar substances determined at longer times (about 8 min). Chromatogram of the recovered components from PVA/Ros mats (also obtained in ethanol:-water solution 70:30 w/w at 50 °C) showed a similar distribution as the RE chromatogram, indicating that most of the molecules were retained

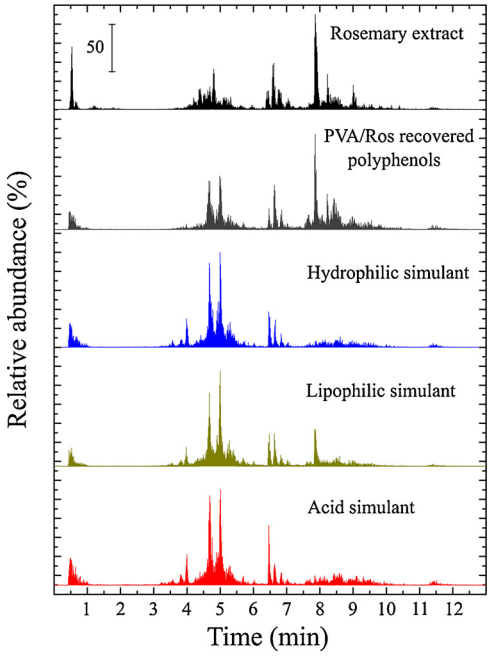

Fig. 6. HPLC chromatograms of rosemary extract and food simulants after release of polyphenols from PVA/Ros mats.

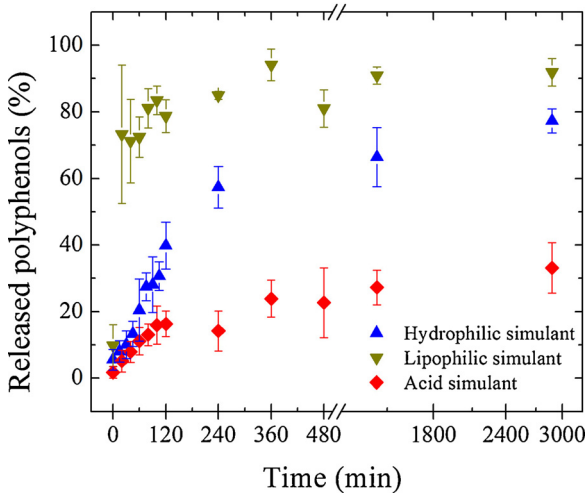

Fig. 7. Rosemary polyphenols released to different aqueous food simulants. Scale of X axis becomes logarithmic starting from 500 min.

during electrospinning, and then recovered by the chosen extraction method.

The nature of rosemary components released from electrospun fibers in different food simulants was also investigated by HPLC. PVA/Ros mats were immersed in hydrophilic, lipophilic and acid food simulants for 2 days. The obtained solutions were analyzed by HPLC. Results are presented in Fig. 6, where it can be seen that polar molecule peaks obtained at short times are released in all food simulants, but non-polar molecules could not be recovered in hydrophilic and acidic simulant, and only a small fraction was released in lipophilic simulant. This is associated with hydrophobicity of some components: for example, carnosic acid and carnosol are hydrophobic, and it is not expected that they could be released in a polar solvent.

Results discussed previously showed that a suitable extraction allows the recovering of most of the components from PVA/Ros fibers, but hydrophilic and acidic food simulants extraction failed in non-polar molecule recovery. A successful application of PVA/Ros mats for food packaging will depend on antioxidants release. Mats capability to release rosemary polyphenols in time was studied. Fig. 7 presents the polyphenols release at different times for hydrophilic, lipophilic and acidic food simulants, where *Released polyphenols (%)* is calculated with respect to the maximum concentrationof polyphenols recovered:

$$\textit{Released Polyphenols}\,(\%) = 100\frac{M_t}{M_0} \tag{2}$$

where M_t is the polyphenols amount released at t time and M_0 is the total amount of encapsulated polyphenols (M_0 = 15.4 mg GAE/g).

Mats submerged in all simulants showed an initial release of about 5–10% which could be associated to rosemary particles adsorbed on fibers surface. Mats submerged in lipophilic simulant showed a burst release, reaching (72 ± 6)% polyphenol release in just 60 min, and (92 ± 4)% after 2 days. This behavior is associated with higher solubility of rosemary extract components in ethanolic medium and agrees with HPLC results, which indicated that lipophilic food simulant was the only medium able to recover hydrophobic substances. On the other hand, mats submerged in aqueous and acidic simulants showed a slower and more controlled release, associated with the non-solubility of rosemary nanoparticles. Maximum release in these mediums was reached after 2 days, achieving (77 ± 4) and (33 ± 8)% for aqueous and acidic simulant, respectively. López-Córdoba et al. (2017) reported the opposite behavior when submerging starch films containing rosemary extract in hydrophilic and lipophilic food simulants. This difference may be a consequence of the high solubility of starch in water in comparison with ethanol. Very likely, polyphenol release may be enhanced by the dissolution of starch in water. In the present research, citric acid crosslinked PVA is not soluble in water or ethanol, so the only mechanisms that could be considered are diffusion or polymer relaxation. In a recent paper, Talón, Trifkovic, Vargas, Chiralt, and González-Martínez (2017) demonstrated that release behavior of polyphenols from starch-chitosan films is largely affected by the polarity and pH of the solvent, and also found that cross-linking starch-chitosan with tannic acid inhibits polyphenols release to acid medium. In this work, a smaller amount of polyphenols were release in acid food simulant, which could also be attributed to citric acid crosslinking of PVA. Additionally, release rate was slower in an acidic medium compared to hydrophilic and lipophilic mediums. Colín-Orozco et al. (2015) studied rosemary release from poly(ethylene oxide)/whey protein electrospun fibers to buffer solutions with different pH, and also observed that release rate was slower in acidic mediums.

If rosemary polyphenols keep their antioxidant activity in each food simulant, and considering that polyphenols are the main antioxidant, it is possible to estimate the antioxidant activity in lipophilic, hydrophilic and acidic food simulants, respectively. Estimated antioxidant activity in each medium, expressed as μmol TE/g and as DPPH• percentage inhibition, was compared with the total antioxidant activity of other recently developed packaging material (see Supplementary material). Independently of the food simulant, PVA/Ros mats showed higher antioxidant activity than other materials reported in the literature.

Release from polymeric systems may be a consequence of different phenomena, like diffusion, swelling, and polymer chain relaxation, among others (Dash, Murthy, Nath, & Chowdhury, 2010). Diffusion can be described by Fick's second law. For an electrospun mat, a cylindrical geometry shall be considered, and the solution to Fick's equation results (Crank, 1975):

$$\frac{M_t}{M_\infty} = 1 - \sum_1^\infty \frac{4}{r^2\alpha_n{}^2}\exp[-D\alpha_n^2 t], \tag{3}$$

where M_t/M_∞ is the released polyphenols at time t divided by the polyphenols amount at equilibrium (infinite time), r is the fiber average radius, D is the diffusion coefficient and r. α_n are the positive roots of Bessel function of first kind of zero order. Nevertheless, many controlled release systems do not follow a pure diffusional process, so several other approaches were developed to consider other mechanisms. A useful equation to describe Fickian and non-Fickian release is the well-known Power Law (Ritger & Peppas, 1987), which only considers the first portion of the release curve ($M_t/M_\infty < 60\%$):

$$\frac{M_t}{M_\infty} = kt^n, \tag{4}$$

where k is a kinetic constant, and n is a diffusional exponent characteristic of the release system mechanism. A value of n close to 0.45 indicates that Fick diffusion is the ruling mechanism, a value close to 0.89 indicates Case II transport (where polymer chain relaxation rules the release), and a value between 0.45 and 0.89 indicates an anomalous release. Another alternative to describe release behavior is the empirical Weibull function, valid for the entire release curve:

$$\frac{M_t}{M_\infty} = 1 - exp(a.\ t^b), \tag{5}$$

where a and b are constants. The b parameter indicates the release mechanism: for values smaller than 0.75 release occurs according to Fick's diffusion Law, intermediate values (0.75–1) indicate Case II transport, and values of $b > 1$ indicate complex release mechanisms.

Polyphenols release curves (Fig. 7) were normalized with respect to the polyphenols amount at equilibrium in each medium, and were then fitted according to Eqs. (3)–(5). Resulting parameters are reported in Table 2. The first value of each curve was dismissed as it corresponds to initial polyphenols released by desorption. Release to lipophilic food simulant overcomes the 60% in only 15 min; thus the Power Law model could not be applied. Moreover, adjustment with the other models was

Table 2
Resulting parameters from adjustment of polyphenols release with Fick, Power Law and Weibull Model.

	Fick		Power law			Weibull		
	D (cm^2/s)	R^2	k	n	R^2	a	b	R^2
Hydrophilic	6.83×10^{-16}	0.92	0.77	0.85	0.96	0.00445	1.03	0.97
Lipophilic	1.37×10^{-14}	0.42	–	–	–	0.568	0.279	0.75
Acid simulant	6.70×10^{-16}	0.92	2.19	0.66	0.98	0.0419	0.548	0.93

not satisfactory (according to correlation coefficient). Although release mechanisms could not be determined, a high amount of polyphenols were released in a few minutes to lipophilic medium, so PVA/Ros electrospun mats may be used as active packaging in contact to lipophilic food that require a high amount of polyphenols in a short time period. Release to hydrophilic and acidic food simulants could be fitted with all the models employed. Diffusion coefficients showed similar results for hydrophilic and acidic mediums, indicating that diffusion of polyphenols in PVA mats occurred at a similar rate. It should be considered that PVA mats must be described as a swelling-controlled release device. According to this model, food simulants penetrate PVA nanofibers at a rate that controls the release of polyphenols, so *D* values correspond to effective diffusion coefficients of swollen fibers. In agreement with this idea, the obtained *n* values of Power Law were 0.85 and 0.66 for hydrophilic and acid simulants, respectively. These results indicate that swelling and chain relaxation are the leading mechanisms in polyphenols release to hydrophilic simulant, while an anomalous release occurs in acidic simulant.

Results obtained by fitting with Weibull model give similar information as Power Law: *b* values resulted 1.03 and 0.55 for hydrophilic and acid simulants, respectively. The obtained values of *b* indicate that chain relaxation rules the release to hydrophilic simulant, while anomalous release occurs in acid simulant. Both Power Law model (applied to the first 60% of release) and Weibull model (applied to the entire release curve) results suggest a difference in the chain relaxation of PVA when it is submerged in hydrophilic or acid mediums, affecting polyphenols release kinetics. This difference could be attributed to the dependence of swelling with pH. Jin and Hsieh (2005) studied the equilibrium swelling of casting films and electrospun mats of PVA/PAA, and reported that swelling increased with increasing pH, which is in accordance with our findings. Despite the difference in the release mechanisms, the main difference between hydrophilic and acid food simulants is the rosemary polyphenols concentration at equilibrium: a relatively high amount of polyphenols were released to hydrophilic medium after 2 days, while a low amount of polyphenols were released to acid medium.

Application of PVA/Ros mats to hydrophilic and acid foods seems to be suitable since release is sustained over prolonged time periods, however in lipophilic foods higher amounts of polyphenols will be released in a short time period. More research must be done to determine the relationship between the amount of polyphenols released and the extension of shelf life of particular foods.

4. Conclusions

PVA active mats retained (88 ± 3) % of rosemary polyphenols initially included in the electrospinning solution, showing an excellent antioxidant activity. Rosemary polyphenols were released in all food simulants studied, however the release rate and percentage depended strongly on the medium. For lipophilic simulant (92 ± 4) % of polyphenols release was achieved, while (77 ± 4) % were released in hydrophilic simulant and only (33 ± 8) % in acid simulant. The release mechanisms were also different: a burst release was observed in lipophilic medium, polymer chain relaxation was the main mechanism in hydrophilic simulant, while in acid simulant both polymer chain relaxation and Fick's diffusion were involved.

PVA electrospinning process was successful in incorporating most of bio-active components observed in the initial rosemary extract. Most of the rosemary components could be released in lipophilic simulant, while only polar components could be released in hydrophilic or acid food simulant.

This work evinces PVA electrospun mats to be an excellent material to entrap and deliver rosemary components for food packaging applications, with particular differences in the release kinetics according to the food characteristics.

Funding sources

This work was supported by the following organizations: CONICET (PIP 2014–2016, 11220120100508CO), University of Buenos Aires (UBACYT2014–2017, 20020130100495BA), ANPCyT (PICT-2012-1093, PICT 2016-2940).

Acknowledgment

We thank Dr. Elsa López Loveira (3ia – UNSAM) for assistance with HPLC-MS measurements.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fpsl.2018.08.006.

References

Araújo, G. K. P., Souza, S. J., Silva, M. V., Yamashita, F., Gonçalves, O. H., Leimann, F. V., ... Shirai, M. A. (2015). Physical, antimicrobial and antioxidant properties of starch-based film containing ethanolic propolis extract. *International Journal of Food Science & Technology, 50*(9), 2080–2087.

Bonilla, J., Sobral, P. J., & do, A. (2017). Antioxidant and antimicrobial properties of ethanolic extracts of guarana, boldo, rosemary and cinnamon. *Brazilian Journal of Food Technology, 20*.

Brand-Williams, W., Cuvelier, M.-E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*(1), 25–30.

Busolo, M. A., & Lagaron, J. M. (2015). Antioxidant polyethylene films based on a resveratrol containing clay of interest in food packaging applications. *Food Packaging and Shelf Life, 6*, 30–41.

Camerlo, A., Vebert-Nardin, C., Rossi, R. M., & Popa, A.-M. (2013). Fragrance encapsulation in polymeric matrices by emulsion electrospinning. *European Polymer Journal, 49*(12), 3806–3813.

Carvalho, R. N., Jr, Moura, L. S., Rosa, P. T. V., & Meireles, M. A. A. (2005). Supercritical fluid extraction from rosemary (*Rosmarinus officinalis*): Kinetic data, extract's global yield, composition, and antioxidant activity. *The Journal of Supercritical Fluids, 35*(3), 197–204.

Celebioglu, A., & Uyar, T. (2017). Antioxidant vitamin E/cyclodextrin inclusion complex electrospun nanofibers: Enhanced water solubility, prolonged shelf life, and photostability of vitamin E. *Journal of Agricultural and Food Chemistry, 65*(26), 5404–5412.

Celebioglu, A., Yildiz, Z. I., & Uyar, T. (2018). Fabrication of electrospun eugenol/cyclodextrin inclusion complex nanofibrous webs for enhanced antioxidant property, water solubility, and high temperature stability. *Journal of Agricultural and Food Chemistry, 66*(2), 457–466.

Colín-Orozco, J., Zapata-Torres, M., Rodríguez-Gattorno, G., & Pedroza-Islas, R. (2015). Properties of poly(ethylene oxide)/whey protein isolate nanofibers prepared by electrospinning. *Food Biophysics, 10*(2), 134–144.

Cordeiro, A., Medeiros, M. L., Santos, N. A., Soledade, L. E. B., Pontes, L., Souza, A. L., ... Souza, A. G. (2013). Rosemary (Rosmarinus officinalis L.) extract. *Journal of Thermal Analysis and Calorimetry, 113*(2), 889–895.

Crank, J. (1975). *The mathematics of diffusion* (2nd ed.). Clarendon Press.

Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica, 67*(3),

217–223.
De Freitas, T. B., Santos, C. H. K., da Silva, M. V., Shirai, M. A., Dias, M. I., Barros, L., ... Leimann, F. V. (2017). Antioxidants extraction from Pinhão (Araucaria angustifolia (Bertol.) Kuntze) coats and application to zein films. *Food Packaging and Shelf Life, 15*, 28–34.
Delgado, M. C. O., Galleano, M., Añón, M. C., & Tironi, V. A. (2015). Amaranth peptides from simulated gastrointestinal digestion: Antioxidant activity against reactive species. *Plant Foods for Human Nutrition, 70*(1), 27–34.
DeMerlis, C. C., & Schoneker, D. R. (2003). Review of the oral toxicity of polyvinyl alcohol (PVA). *Food and Chemical Toxicology, 41*(3), 319–326.
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.
European Commission (2011). Commission regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. *Offical Journal of the European Union, 12*(1), 1–89.
Farghal, H. H., Karabagias, I., El Sayed, M., & Kontominas, M. G. (2017). Determination of antioxidant activity of surface-treated PET films coated with rosemary and clove extracts. *Packaging Technology and Science, 30*(12), 799–808.
Genskowsky, E., Puente, L. A., Pérez-Álvarez, J. A., Fernandez-Lopez, J., Muñoz, L. A., & Viuda-Martos, M. (2015). Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (Aristotelia chilensis). *LWT-Food Science and Technology, 64*(2), 1057–1062.
Ghadetaj, A., Almasi, H., & Mehryar, L. (2018). Development and characterization of whey protein isolate active films containing nanoemulsions of Grammosciadium ptrocarpum Bioss. essential oil. *Food Packaging and Shelf Life, 16*, 31–40.
Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M. C., Alemán, A., & Montero, P. (2009). Antioxidant properties of tuna-skin and bovine-hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chemistry, 112*(1), 18–25.
Herrero, M., Plaza, M., Cifuentes, A., & Ibáñez, E. (2010). Green processes for the extraction of bioactives from Rosemary: Chemical and functional characterization via ultra-performance liquid chromatography–tandem mass spectrometry and in-vitro assays. *Journal of Chromatography A, 1217*(16), 2512–2520.
Ho, C.-T., Chen, J., Lu, G., Huang, M.-T., Shao, Y., & Chin, C.-K. (1998). *Antioxidative and antitumorigenic properties of rosemary*. ACS Publications.
Holland, B. J., & Hay, J. N. (2001). The thermal degradation of poly (vinyl alcohol). *Polymer, 42*(16), 6775–6783.
Ibanez, E., Kubátová, A., Señoráns, F. J., Cavero, S., Reglero, G., & Hawthorne, S. B. (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry, 51*(2), 375–382.
Jacobsen, C., Garcia-Moreno, P. J., Mendes, A. C., Mateiu, R. V., & Chronakis, I. S. (2018). Use of electrospinning for encapsulation of sensitive bioactive compounds and applications in food. *Annual Review of Food Science and Technology, 9*(1).
Jin, X., & Hsieh, Y.-L. (2005). pH-responsive swelling behavior of poly (vinyl alcohol)/poly(acrylic acid) bi-component fibrous hydrogel membranes. *Polymer, 46*(14), 5149–5160.
Joye, I. J., & McClements, D. J. (2013). Production of nanoparticles by anti-solvent precipitation for use in food systems. *Trends in Food Science & Technology, 34*(2), 109–123.
Kadam, S. U., Pankaj, S. K., Tiwari, B. K., Cullen, P. J., & O'Donnell, C. P. (2015). Development of biopolymer-based gelatin and casein films incorporating brown seaweed Ascophyllum nodosum extract. *Food Packaging and Shelf Life, 6*, 68–74.
Kayaci, F., & Uyar, T. (2012). Encapsulation of vanillin/cyclodextrin inclusion complex in electrospun polyvinyl alcohol (PVA) nanowebs: prolonged shelf-life and high temperature stability of vanillin. *Food Chemistry, 133*(3), 641–649.
Kayaci, F., Ertas, Y., & Uyar, T. (2013). Enhanced thermal stability of eugenol by cyclodextrin inclusion complex encapsulated in electrospun polymeric nanofibers. *Journal of Agricultural and Food Chemistry, 61*(34), 8156–8165.
Kayaci, F., Sen, H. S., Durgun, E., & Uyar, T. (2014). Functional electrospun polymeric nanofibers incorporating geraniol–cyclodextrin inclusion complexes: High thermal stability and enhanced durability of geraniol. *Food Research International, 62*, 424–431.
Krimm, S. (1960). *Infrared spectra of high polymers. Fortschritte der Hochpolymeren-Forschung*. Springer51–172.
Lemma, S. M., Scampicchio, M., Mahon, P. J., Sbarski, I., Wang, J., & Kingshott, P. (2015). Controlled release of retinyl acetate from β-cyclodextrin functionalized poly(vinyl alcohol) electrospun nanofibers. *Journal of Agricultural and Food Chemistry, 63*(13), 3481–3488.
López de Dicastillo, C., Garrido, L., Alvarado, N., Romero, J., Palma, J. L., & Galotto, M. J. (2017). Improvement of polylactide properties through cellulose nanocrystals embedded in poly(vinyl alcohol) electrospun nanofibers. *Nanomaterials, 7*(5), 106.
López-Córdoba, A., Castro, G. R., & Goyanes, S. (2016). A simple green route to obtain poly(vinyl alcohol) electrospun mats with improved water stability for use as potential carriers of drugs. *Materials Science and Engineering: C, 69*, 726–732.
López-Córdoba, A., Medina-Jaramillo, C., Piñeros-Hernandez, D., & Goyanes, S. (2017). Cassava starch films containing rosemary nanoparticles produced by solvent displacement method. *Food Hydrocolloids, 71*, 26–34.
Moreno, S., Scheyer, T., Romano, C. S., & Vojnov, A. A. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research, 40*(2), 223–231.
Mujica-Garcia, A., Sonseca, A., Arrieta, M. P., Yusef, M., López, D., Gimenez, E., ... Peponi, L. (2016). Electrospun fibers based on biopolymers. *Advanced Surface Engineering Materials,* 385–438.
Oliphant, T. E. (2007). SciPy: Open source scientific tools for Python. *Computing in Science & Engineering, 9*, 10–20. https://doi.org/10.1109/MCSE.2007.58.
Oliveira, G. A. R., Oliveira, A. E., Conceição, E. C., & Leles, M. I. G. (2016). Multiresponse optimization of an extraction procedure of carnosol and rosmarinic and carnosic acids from rosemary. *Food Chemistry, 211*, 465–473.
Piñeros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A., & Goyanes, S. (2017). Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. *Food Hydrocolloids, 63*, 488–495.
Ribba, L., Parisi, M., D'accorso, N. B., & Goyanes, S. (2014). Electrospun nanofibrous mats: From vascular repair to osteointegration. *Journal of Biomedical Nanotechnology, 10*(12), 3508–3535.
Ritger, P. L., & Peppas, N. A. (1987). A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *Journal of Controlled Release, 5*(1), 23–36.
Rodríguez-Rojo, S., Visentin, A., Maestri, D., & Cocero, M. J. (2012). Assisted extraction of rosemary antioxidants with green solvents. *Journal of Food Engineering, 109*(1), 98–103.
Rodsamran, P., & Sothornvit, R. (2017). Bioactive coconut protein concentrate films incorporated with antioxidant extract of mature coconut water. *Food Hydrocolloids, 79*, 243–252.
Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). *14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in enzymology*, *vol. 299*, Elsevier152–178.
Talón, E., Trifkovic, K. T., Vargas, M., Chiralt, A., & González-Martínez, C. (2017). Release of polyphenols from starch-chitosan based films containing thyme extract. *Carbohydrate Polymers, 175*, 122–130.
Torres-Giner, S., Wilkanowicz, S., Melendez-Rodriguez, B., & Lagaron, J. M. (2017). Nanoencapsulation of aloe vera in synthetic and naturally occurring polymers by electrohydrodynamic processing of interest in food technology and bioactive packaging. *Journal of Agricultural and Food Chemistry, 65*(22), 4439–4448.
Tu, Z., Moss-Pierce, T., Ford, P., & Jiang, T. A. (2013). Rosemary (Rosmarinus officinalis L.) extract regulates glucose and lipid metabolism by activating AMPK and PPAR pathways in HepG2 cells. *Journal of Agricultural and Food Chemistry, 61*(11), 2803–2810.
Urbančič, S., Kolar, M. H., Dimitrijević, D., Demšar, L., & Vidrih, R. (2014). Stabilisation of sunflower oil and reduction of acrylamide formation of potato with rosemary extract during deep-fat frying. *LWT-Food Science and Technology, 57*(2), 671–678.
US Food and Drug Administration, 2017. FDA cite: 21CFR176.170.
US Food and Drug Administration, 2003. FDA cite: 21CFR176.
US Food and Drug Administration, 2003. FDA GRN no. 141.
Wen, P., Zhu, D.-H., Wu, H., Zong, M.-H., Jing, Y.-R., & Han, S.-Y. (2016). Encapsulation of cinnamon essential oil in electrospun nanofibrous film for active food packaging. *Food Control, 59*, 366–376.
Wen, P., Zong, M.-H., Linhardt, R. J., Feng, K., & Wu, H. (2017). Electrospinning: A novel nano-encapsulation approach for bioactive compounds. *Trends in Food Science & Technology, 70*, 56–68.
Yang, Y., Song, X., Sui, X., Qi, B., Wang, Z., Li, Y., ... Jiang, L. (2016). Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. *Industrial Crops and Products, 80*, 141–147.
Zhang, C., Yuan, X., Wu, L., Han, Y., & Sheng, J. (2005). Study on morphology of electrospun poly(vinyl alcohol) mats. *European Polymer Journal, 41*(3), 423–432.