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Encapsulation of volatiles in nanofibrous polysaccharide membranes for humidity-triggered release

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ABSTRACT

A single-step electrospinning process will be applied to a blend of edible carbohydrate polymers (pullulan and β-cyclodextrin) to encapsulate bioactive aroma compounds and allow a humidity-triggered release. The encapsulation is rapid and efficient and the final product is an active nanofibrous membrane that can be directly used for food or active packaging applications. The membrane hosts small and homogeneously dispersed crystals of cyclodextrin–aroma complexes which are formed during the electrospinning. With this type of structure, the release of aroma compound is negligible at ambient conditions (23 °C and 55% UR) even at high temperature (up to 230 °C), and it occurs beyond a given relative humidity threshold (90%), useful for food packaging applications. The mass fraction of free aroma released is directly related to the water activity of the system, namely, $\varphi = a_W^n/(a_W^n + K_{app})$ explaining the observed key role played by the relative humidity on the release of the aroma compounds.

1. Introduction

The growing concern for the promotion of health and prevention of disease through improved nutrition has led to numerous attempts to develop food-grade delivery systems, including active packaging, to encapsulate, protect and deliver bioactive components (Lesmes & McClements, 2009; López-Rubio, Sanchez, Wilkanowicz, Sanz, & Lagaron, 2012; Sozer & Kokini, 2008). Volatile substances with antimicrobial features, such as natural essential oils, absolutes, essences, extracts, resins, infusions, etc. are of great interest for the active packaging industry and their efficient encapsulation and release represent a major challenge, considering their high fugacity and the fact that they are very sensitive to heat, oxygen and light.

Most of the active packaging studies reported in the literature concern the dispersion of the active agent in carriers with limited surface areas, such as polymer films and layers, sometimes with not negligible losses of volatile compounds during production and storage (Appendini & Hotchkiss, 2002; Guillard, Issoupov, Redl, & Gontard, 2009). The controlled release of active substances from these structures is mainly governed by concentration-dependent passive diffusion (Vega Lugo & Lim, 2009).

Recently, electrospinning has received great attention in functional food and active food packaging systems (Bhardwaj & Kundu, 2010; Chen, Remondetto, & Subirade, 2006; Kayaci & Uyar, 2012a, 2012b; Kriegel et al., 2008; Lagaron & Lopez-Rubio, 2011; Sanchez-Garcia, Lopez-Rubio, & Lagaron, 2010). This simple technique allows the production of nanofibrous polymeric unwoven membranes formed by polymer fibers with diameters ranging from tens to hundreds of nanometers (Frenot & Chronakis, 2003; Yua, Yanga, Quiana, & Lia, 2012; Zheng-Ming Huang, Zhang, & Kotaki, 2003). By virtue of their submicron to nano-scale diameter and very large surface area, electrospun fibers may offer a number of additional advantages compared to film and sheet carriers, as they are more responsive to changes in the surrounding atmosphere (e.g., relative humidity and temperature changes), which enables a tunable release of the entrapped compounds (Vega Lugo & Lim, 2009). Moreover, since the electrospinning process takes place at ambient conditions, electrospun fibers are more suitable to encapsulate thermally labile substances than fibers prepared by conventional melt spinning or films produced by extrusion process, or other encapsulation methods, such as spray drying and fluid bed coating (Qi, Hu, Xu, & Wang, 2006; Xu et al., 2006; Zuidam &

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Shimoni, 2007, chap. 4). Furthermore, electrospinning seems suitable to trap aroma compound inclusion complexes (AC-IC) within the meshes of the membrane. This is the case of cyclodextrins inclusion complexes with hydrophobic substances (Koontz, Marcy, O'Keefe, & Duncan, 2009). Different literature is present on the electrospinning of cyclodextrins with polymer (Celebioglu Uyar, 2011; Chen et al., 2013; Kayaci & Uyar, 2012a, 2012b; Uyar, Hacaloglu, & Besenbacher, 2009, 2011), but few works are focalized on inclusion of active cyclodextrin in biopolymer, even less on active system bases on complete edible polysaccharides, for food and food packaging applications. Moreover, in these systems the AC-IC is formed before electrospinning in a multi-steps process. Electrospun soy proteins and zein have been used for controlled release of allyl isothiocyanate (Vega Lugo & Lim, 2009) and as carriers for catechins (Li & Kakuda, 2009), respectively. However, edible proteic systems, such as the above mentioned, or gelatin and collagen, cannot be electrospun from aqueous solutions due to extensive hydrogen bonding resulting in gel formation, and therefore toxic or aggressive solvents are required to produce these nanofibers. Casein or wheat proteins have poor electrospinnability and need to be mixed with other polymers such as poly ethylene oxide (PEO), poly vinyl alcohol (PVA) or other synthetic biopolymers like polylactic acid or ε-caprolactone with the use of toxic solvents (Aceituno-Medina, Lopez-Rubio, Mendoza, & Lagaron, 2013; Karim et al., 2009; Kriegel et al., 2008), thus limiting the edibility of the resulting systems. Moreover some proteins like gluten, soy proteins or milk proteins could be related to allergenicity problems.

Edible polysaccharides are commonly used in food applications as coating agents, thickening agents, or additives for technological aims; they are not allergenic and do not need toxic solvents to be electrospun (Karim et al., 2009; Stijnman, Bodnar, & Tromp, 2011). Pullulan is a natural, water-soluble linear polysaccharide industrially produced by fermentation of starch syrup with a selected strain of *Aureobasidium pullulans*. It consists of maltotriose units (α-1,4 linked glucose molecules) polymerized by α-1,6-glucosidic bonds forming a stair-step-type structure and has good electrospinnable properties. This polymer however alone is not suitable to encapsulate hydrophobic compounds because of its hydrophilic nature and a linear structure.

In this work, we report an edible polysaccharide system that can be used as active device or active coating material on traditional packaging, produced by a single-step electrospinning process. In this system β-cyclodextrin crystals encapsulate aroma compounds and are simultaneously fixed to the meshes of pullulan nanofibers. Considering its capacity of encapsulating and releasing antimicrobial aroma compounds, the system demonstrates a potential applicability for enhancing microbial safety, in particular of fresh foods. The morphology of the nanofibrous membranes and the retentive capacity of the edible nanofibrous system were evaluated, and the release of aroma compounds was investigated at various relative humidities (RH) and described phenomenologically.

One of the features that make the electrospinning attractive is the simplicity, versatility and low cost of the equipment. Operating simplicity of the equipment clashes, however, with the complexity of the set-up of experimental variables to produce nanofibers with controlled morphology. The production on an industrial scale starting in the 90s in various fields including the Life Science to the development of drug delivery carrier and today there are several patents. The industrial application is related to niche areas because of the difficulty of monitoring large-scale processes and the active packaging sector could be one of these niche areas (Huang, Zhang, & Kotaki, 2003; Kotaki, 2005).

2. Experimental

2.1. Materials

Pullulan was a food grade preparation (PF-20 Grade, 200 kD) of Hayashibara Biochemical Laboratories Inc. (Okayama, Japan) and was kindly supplied by Giusto Faravelli (Milan, Italy). β-Cyclodextrin was purchased from Sigma–Aldrich (Milan, Italy). Aroma compound (AC), i.e., perillaldehyde, was used as a model of bioactive aroma compound, it was the 90% of the essential oil of perilla extracted and purified in our laboratories from leaves of the plant *Perilla frutescens.* Doubly distilled water was used as solvent to prepare the emulsions. Sodium chloride, potassium chloride and potassium nitrate were purchased from Sigma–Aldrich (USA). Methanol and ethanol were supplied by Fluka analytical (Spain).

2.2. Preparation of electrospinning solutions

A polymer solution was prepared by dissolving pullulan dry powder in water (20 wt%) at room temperature under 4 h stirring. After dissolution, two different methods were used:

(i) The pullulan solution was mixed with a preformed AC-IC in a ratio of 25% of AC-IC with respect to the dry pullulan. The mixture was homogenized in 10 mL glass vials using an Ultra Turrax T25 IKA blender (IKA Works, Guangzhou, China) running at 10,000 rpm for 5 min. The AC-IC preparation was previously performed with the precipitation method at 16:84 wt% (AC:β-cyclodextrin) (Partanen, Ahro, Hakala, Kallio, & Pirkko, 2002) in water solution. The product was then filtrated and dried.

(ii) The pullulan solution was mixed with an amount of dry free β-cyclodextrins (25 wt% with respect to dry pullulan) and with 10% wt% (AC/β CD), containing more than 90 wt% of the active compound perillaldehyde. The solution was emulsified using the Ultra Turrax in the same conditions of (i) (10,000 rpm × 5 min). It must be noticed that on adding cyclodextrins the system turns to a water-in-water emulsion because of the thermodynamic uncompatibility of the two polymers (Grinberg & Tolstoguzov, 1997): cyclodextrin rich aqueous droplets (few micron size) are dispersed within an aqueous pullulan rich phase.

2.3. Electrospinning

Plastic syringes (10 mL) fitted with a metallic needle (Hamilton) were filled with the polymeric emulsions and placed in a KDS100 syringe pump (KD-Scientific, New Hope, PA) at flow rates of 0.5 mL/h. The needle of the syringe was linked to a Spellman SL150 high voltage power supply by an alligator clip, while a foil-covered copper tray, positioned at 12 cm in front of the needle, was used as collector and grounded. For the electrospinning of the emulsions, the electrical potential was set at values of 15 kV. The production time of the single membrane was stopped at 15 min, the membranes were removed from the collector and dried.

2.4. Field-Emission Scanning Electron Microscopy (FE-SEM)

Scanning electron microscopy images were obtained from a Sigma Field Emission microscope (Carl Zeiss Microscopy, LLC) at accelerating 5 kV voltage and 6 mm working distance, with a 30 μm width slit. The samples were first gold sputtered (Sputtering Polaron E 5100) for 30 s (rate 1 nm s^{-1}) with argon and 18 mA current intensity.

2.5. Thermogravimetric analysis (TGA)

The amount and rate of mass loss of the membranes containing hydrophobic volatile compounds with β-cyclodextrin were measured as a function of temperature and time. For comparison, a mechanical mixture of dry cyclodextrin and pure aroma compound perillaldehyde and AC-IC produced by precipitation method were also analyzed. Thermogravimetric analysis (TGA) was performed under nitrogen atmosphere with a Perkin Elmer TGA 4000 instrument. The instrument output is the TGA trace, namely mass loss-vs-*T*. The selected temperature range, from 30 to 450 °C, was scanned at a constant 20 °C/min heating rate. Each run was repeated at least twice. The raw data were converted into the corresponding time derivative trace, DTG, and expressed in mg/K units. The software PeakFit v. 4.0 (Jandel Scientific, San Rafael, CA, USA) was used to mathematically deconvolute the DTG traces in Gaussian components, each related to the contribution of a single compound (water, perillaldehyde, low mass compounds from the thermal degradation of cyclodextrin and pullulan) to the overall mass release.

2.6. Extraction and quantification of aroma in the nanofibrous membranes

Perillaldehyde was extracted from nanofibrous membranes by immersing the membrane in 5 mL of methanol under continuous stirring for 24 h at 500 rpm and by an ultrasonic treatment for 10 min. The alcoholic phase containing the aroma compound was separated by using glass wood, dried over anhydrous sodium sulphate and analyzed via total vaporization technique (TVT) by head space gas chromatography (HSGC) (Mod HS 40, Perkin Elmer) combined with a Clarius 600 GC-FID (Perkin Elmer) equipped with a TRB-WAX column (30 m × 0.53 mm, film thickness of 1 μm) and a flame ionization detector (FID). Helium was used as carrier gas with a 2 mL/min flow rate. After a 30 min rest time at 80 °C, the oven temperature was programmed to rise from 50 to 230 °C at 15 °C/min rate, then held at 250 °C for 5 min. Injector and detector temperatures were 230 and 260 °C, respectively. Injections were carried out in a split mode with a 1:20 ratio. The calibration curve was obtained with different concentrations of perillaldehyde in methanol and the residual quantity was quantified with the External Standard Method. The estimated extraction efficiency was about 93%, for every kind of membrane considered. Analyses were performed in triplicate. Results were expressed as residual amount of aroma compounds per gram of dry membrane.

2.7. Evaluation of aroma losses during storage

In order to quantify the losses during storage, membranes were stored at 55% RH and 23 °C for 45 days, and then analyzed with HSGC. These results were analyzed by means of a one-way and multifactor analysis of variance, using the LSD test with a 95% confidence interval for the comparison of the test mean.

To simulate a sensorial analysis, so to evaluate the perceptible odor of the membranes, the electronic nose was also used. Electronic nose analyses were performed with a Portable Electronic Nose (PEN2) (Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany)) operating with the Enrichment and Desorbtion Unit (EDU). A 35 mg membrane sample was placed in a 22.5 mL airtight Pyrex® glass vial with a Silicon/Teflon disk in the cap for 1 h equilibration at 35 ± 2 °C. The disk was eventually pierced before the analysis with the electronic nose and EDU. The relevant operating procedure was reported in a previous work (Benedetti, Buratti, Spinardi, Mannino, & Mignani, 2008). All samples were analyzed three times and the average of the sensor responses was used for the statistical analysis. Principal Component Analysis (PCA) was applied, as an exploratory tool, to study the changes of the aroma compounds in the head-space of the vial containing the membrane at the various RH conditions tested. Calculations were performed with MATLAB v. 6.5 program (Mathworks). The same type of samples were analyzed also with HSGC to quantify the aroma compound released from the membranes immediately after production and at 1, 2, 6 days after electrospun process. In this case the sample was let rest at 35 °C for 1 h as equilibration time, while the GC conditions were the same as above (Section 2.6). Both electronic nose and HSGC data were compared using PCA analysis (MATLAB software v. 6.5).

2.8. Aroma compound release from membranes as a function of relative humidity and time

Single membranes produced by electrospinning technique were weighed and put in four different chambers at 23 ± 2 °C and constant relative humidity (RH) of 55%, 75%, 85% and 92%. During each experiment the chamber humidity was controlled using an humidity probe. Membranes were removed from the chambers at given time intervals for analysis; the amount of aroma compound was immediately determined with the extraction method described in Section 2.7. The experimental data were treated with the software Table Curve 4.0 (Jandel Scientific, San Rafael, CA, USA) according to models presented in next sections of this paper.

3. Results and discussion

3.1. Production and morphology of the nanofibrous encapsulating system

Traditional encapsulation techniques (e.g., precipitation method, fluid bed coating) allow production of AC-IC as crystalline powders that can be used directly in the field of fragrances and food flavoring. On the contrary, for active food packaging applications, the AC-IC usually need to be inserted in polymeric matrixes. Such systems, that are typically films and layer coatings, must enable the interaction of the encapsulated substance with the surrounding medium. This means that the matrix should be characterized by a large surface area and to be constituted by a water-reactive polymer. Pullulan is a natural, water-soluble linear polysaccharide industrially produced by fermentation of starch syrup with a selected strain of *A. pullulans*. It consists of maltotriose units (α-1,4 linked glucose molecules) polymerized by α-1,6-glucosidic bonds forming a stair-step-type structure. This carbohydrate can be electrospun to prepare unwoven membranes of edible polysaccharide matrix able to incapsulate aroma compound through β-cyclodextrine.

Pure pullulan nanofibers were obtained by electrospinning of aqueous solutions by using the following optimal process parameters: 20 wt% pullulan solution, 0.5 mL/h flow rate, 15 kV applied voltage and 12 cm tip-to-collector distance. Macroscopically, the membranes appeared white, homogeneous and smooth (Fig. 1a). SEM images showed in these conditions thin (250 ± 50 nm diameter) and uniform pullulan fibers and a morphology characterized by randomly oriented fibers, creating a pseudo-porous structure (Fig. 1b). The same process conditions were found suitable also for the electrospinning of solutions containing pullulan, β-cyclodextrin (25 wt% with respect to pullulan) and the aroma compound (perillaldehyde 10 wt% with respect to β-cyclodextrin), which allowed the formation of AC-IC complexes. Membranes containing the β-cyclodextrin AC-IC and membranes prepared with pullulan alone showed comparable morphologies: the only difference was the presence of AC-IC crystals along the entangled fibers of the formers (Fig. 1c).

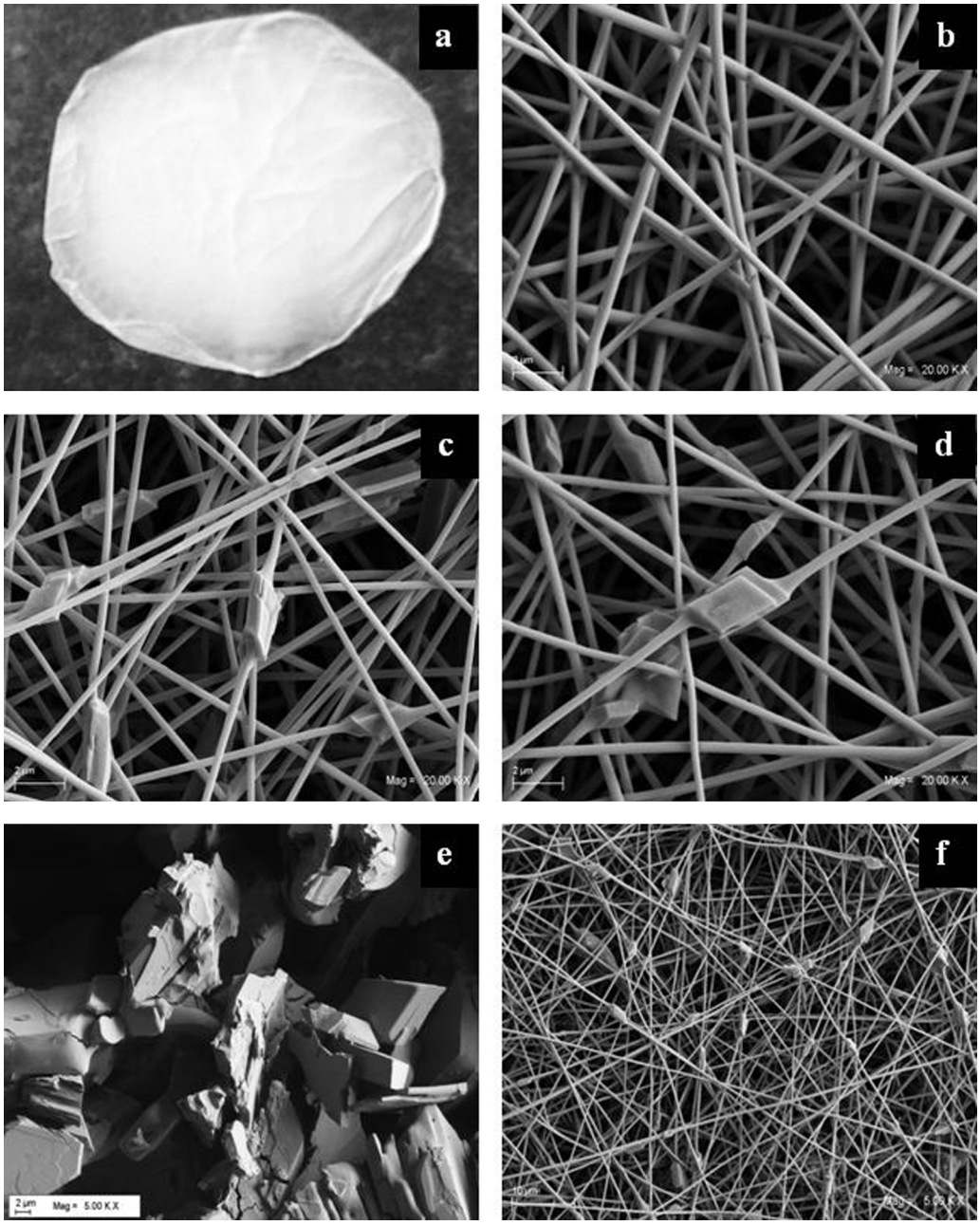

Fig. 1. Images of macroscopical appearance electrospun pullulan membrane (a); scanning electron micrograph of nanofibers prepared by using: pullulan solution (b), pullulan, β-cyclodextrin and perillaldehyde solution (c) and pullulan solution with preformed AC-IC (magnification 20k×). Morphological structures of AC-IC (e) and AC-IC in the nanofibrous membranes (f) (magnification 5k×).

These results show that it is possible to encapsulate aroma compounds directly in a single step electrospinning treatment applied to a dispersed aqueous system containing β-cyclodextrin, aroma compound and pullulan, without preforming the AC-IC crystals. No differences were evidenced in the morphology of the membranes produced by electrospinning with or without the AC-IC preformation step (Fig. 1d). This is probably due to the fact that electrospinning implies the instantaneous evaporation of the solvent, transforming the starting emulsion in a dry nanofibrous matrix embedding crystalline structures. The inclusion complex is probably preformed in emulsion step and very rapidly stabilized during the electrospinning process.

One of the advantages of the formation of the AC-IC within the nanofibrous membrane in a single step is that these membranes can directly be used as active macroscopically homogeneous devices (Fig. 1a) to be glued as labels onto a packaging wrap. Moreover, the encapsulation achieved with the classic precipitation method implies first a decrease of temperature for precipitation and then a temperature rise that is required to slowly evaporate the solvent: the final result is a mixture of amorphous and polycrystalline material where crystal growth can progress (Fig. 1e) and a powder that should be incorporated in a packaging materials. In the case of encapsulation within a nanofibrous pullulan matrix, the situation is totally different: the crystals formed are smaller and regularly dispersed within the pullulan nanofibrous matrix (Fig. 1f), probably as a consequence of the rapid solvent evaporation. Two structures (crystals of AC-IC and pullulan nanofibers) are evident and, apparently, the crystals "envelope" the fibers: this can be explained reminding that, in spite of the chemical similarity (saccharide nature),

β-cyclodextrin and pullulan are thermodynamically uncompatible, which means that they tend to form separate aqueous phases in the presence of excess solvent, because of different exclusion volumes (Beebe, Pell, & Seasholtzt, 1998). These phases form a dispersed system, namely droplets of aqueous β-cyclodextrin are dispersed in the aqueous pullulan rich solution. The thermodynamic incompatibility that takes the β-cyclodextrin droplets apart from the surrounding pullulan-rich dispersion medium is equivalent to a surface tension effect between the β-cyclodextrin aqueous droplets and solvated bunches of pullulan molecules (Schiraldi, Fessas, & Signorelli, 2012). Once the solvent is quickly sucked out in the electrospinning process, the β-cyclodextrin rich droplets generate small crystals (with a 50–100 nm size) around the pullulan fibers that come from the starting solvated polymer.

3.2. Aroma encapsulating capacity

In order to evaluate the aroma encapsulating capacity of the system, HS-GC was used. These analyses allowed measuring the amount of aroma in the pullulan/β-cyclodextrin electrospun membranes, immediately after their preparation via electrospinning. This percentage is 1.85 ± 0.1 wt% perillaldehyde/dry membrane, which corresponded to 8.2 ± 0.5 wt% perillaldehyde/β-cyclodextrin with a ratio β-cyclodextrin/pullulan of 1:4. The conical cavity of the oligosaccharide β-cyclodextrin is hydrophobic and able to bind non-polar molecules in water solutions. Taking into account that the molecule of β-cyclodextrin (molecular weight 1134.98 g/mol) can bind one molecule of perillaldehyde (molecular weight 150.22 g/mol), the maximum amount of aroma that can be encapsulated by β-cyclodextrin is less than 9 wt%. The experiments with different mass ratios confirmed that these values actually correspond to the maximum retention capacity of the system and that any excess of free perillaldehyde is lost during either the electrospinning process or the earlier stage of storage, as discussed in Section 3.4). The retention capacity of aroma compound of pure pullulan nanofibrous membranes (i.e., in absence of β-cyclodextrin) resulted almost negligible (<0.1 wt% over total dry matter in the membranes). The two ways of fixing the AC-IC complexes to the pulluan membranes (namely, by mixing preformed complexes and by a one-step electrospinning) did not show significant differences as for the amount of encapsulated perillaldehyde.

3.3. Thermal characteristics

Thermo-gravimetric Analysis (TGA) was used to assess the thermal stability of: pure aroma compound (AC) with dry β-cyclodextrins, AC-IC complexes, and AC-IC complexes fixed within the pullulan membranes. The record of a TGA run encompasses a wide temperature range (see insert in Fig. 2a) where mass loss occurs because of various events such as release of water, release of the aroma compound, degradation of perillaldehyde and pullulan, which take place in different temperature spans with partial overlaps. It is expedient to use the corresponding time derivative (DTG) trace where the different contributions to the overall mass loss appear as peaks or shouldered peaks. In the present case, the DTG data were referred to the fraction, α, of the overall mass released at the end of the run namely, $d\alpha/dt$, and, since the experiments were carried out at a given and constant heating rate, were expressed in K^{-1} units (Fig. 2).

The relevant DTG traces were, if necessary, de-convoluted in a sum of Gaussian components to split the shouldered peaks of the original record. Fig. 2b indicates that the release of perillaldehyde started at 129 °C and reached a maximum rate at 180 °C. Fig. 2a (dotted line) shows the DTG trace collected from a AC-IC humid sample: the mass lost in the 30–150 °C and 210–315 °C temperature ranges was related to the release of moisture and perillaldehyde, respectively, while the mass loss at higher temperature was related to the volatiles formed in the thermal degradation of β-cyclodextrin. The enhancement of thermal stability of AC-IC respect to pure aroma compound is due to the inclusion complexation. Fig. 2c reports the DTG trace of the AC-IC complex embedded in a pullulan matrix: the comparison with the trace in Fig. 2a (dotted line) of the AC-IC indicates a further enhanced stability of the complex in presence of pullulan. The deconvolution in Gaussian components of the trace allowed the split of the water release in a couple of contributions and suggests that water can be present in at least two different environments (e.g., imbibing water and water bound to CD and/or pullulan). The signal related to the perillaldehyde starts at 230 °C (i.e., at higher temperature with respect to the behavior of the AC-IC powder) and is weaker and spanned in a smaller temperature range. This can be a consequence of the morphological characteristics described above.

3.4. Stability of the encapsulation system during storage

The first goal of encapsulating volatile compounds is protecting them against environmental factors that cause losses. From a design point of view, novel controlled release systems should be primarily stable at the storage conditions over a long time span (Peppas & Brannon-Peppas, 1996).

In order to assess the aroma retentive capacity of the nanofibrous membranes during time, a storage life study was carried out at ambient conditions (55 ± 5% RH and 23 °C) using the nanofibrous membranes containing perillaldehyde. Results showed that the loss of perillaldehyde from the pullulan/β-cyclodextrin membranes after 7 days was 15 ± 2%, whereas from 7 to 45 days there were no further significant losses ($p < 0.05$), thus proving the stability of this encapsulation system. In absence of β-cyclodextrin, the small amount of aroma retained after the preparation continued to decline and finally, after 21 days, the volatile was no longer detectable.

There were no statistically significant differences ($p < 0.05$) in the retention capacity of the system when it was fabricated with or without the preformation the AC-IC. Thus, the single-step-fabricated pullulan/AC-IC membranes were preferred and used to determine the losses of aroma compound during the first 6 days of storage, through HSGC coupled to electronic nose to identify the aromatic profile. A PCA plot is shown in Fig. 3 and includes the data regarding the perillaldehyde released to the headspace and e-nose sensors signals, after 0 (freshly made), 1, 2 and 6 days. There is a clear differentiation of the aromatic profiles of samples according to their storage time. The response of two of the e-nose sensors (W5S and W2S in Fig. 3) and the free volatile was strongly correlated (bottom right of the PCA plot), as confirmed by a separate data analysis (linear correlations with $R^2 > 0.99$). Freshly made samples are located in the same quadrant (bottom right) of the PCA plot, indicating that they are characterized by high values of these three variables. Therefore, differences between fresh and 1-day-membranes suggest that there is an important loss of aroma during the early stage of storage. Considering only the data provided by the W5S sensor and the amount of free volatile quantified with HSGC, it was possible to estimate that around 90% of the aroma loss occurs within the first day.

The fast release of aroma during the early stage of storage is due to the excess of volatile that cannot be effectively encapsulated inside the β-cyclodextrin. Once this excess is quickly lost, the system remains stable without losses during months if kept at a relatively low humidity. Therefore, the nanofibrous device can be considered suitable to preserve the volatile compound

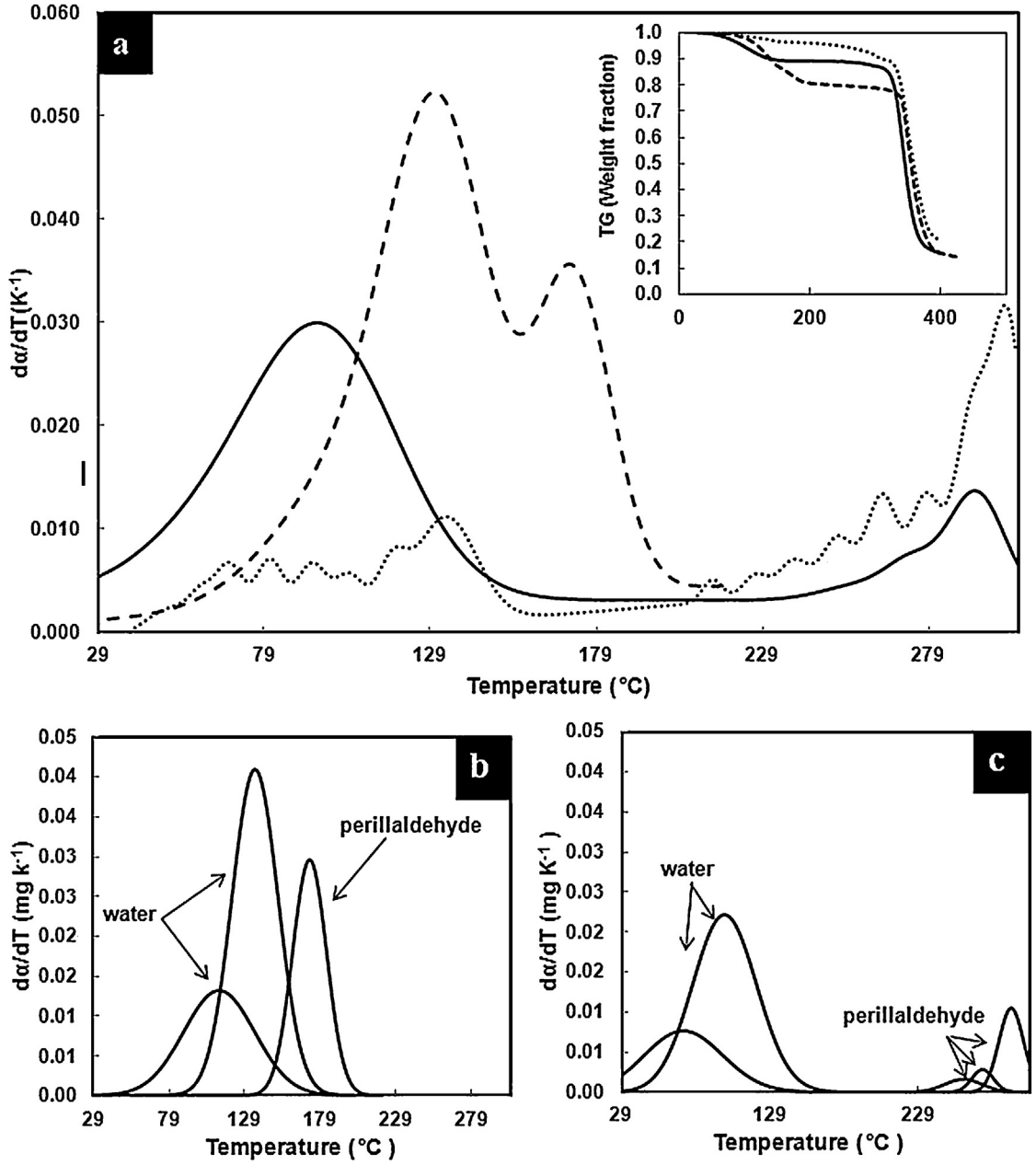

Fig. 2. (a) Raw TGA traces referred to the mass fraction (in the box) and the respective DTG traces of a mixture of perillaldehyde and β-cyclodextrin (dashed line), aroma compound inclusion complex (AC-IC) (dotted line), and nanofibrous membranes with AC-IC (continuous line); (b) deconvolution of DTG trace of a mixture of perillaldehyde and β-cyclodextrins; and (c) deconvolution of DTG trace of nanofibrous membranes with AC-IC.

and masking its characteristic odor until use at high relative humidity.

3.5. RH-dependent release of aroma and release kinetics

Various mathematical models are present in literature to describe controlled delivery processes that can be of interest for food and pharmaceutical applications (Pothakamury & Barbosa-Canovas, 1995; Siepmann & Siepmann, 2008). These models mainly differ for the role the carrier plays in controlling core release (Ayala-Zavala, Del Toro Sanches, Parrilla, & Gonzalez-Aguilar, 2008). The discussion on this subject should start from the fundamental assumption that, no matter the kinetic model chosen, the behavior of the system is mainly governed by the "distance" from the thermodynamic equilibrium relevant to encapsulated and released species. The state of the system considered in the present work can be described with a couple of co-existing thermodynamic equilibrium, each governed by equilibrium constant:

$$\mathrm{CD} + n_1(\mathrm{H_2O}) \leftrightarrow \mathrm{CD(aq)}$$

$$K_h = \frac{c(\mathrm{CD, aq})}{c(\mathrm{CD}) \times a_{\mathrm{W}}^{n_1}} \quad (1)$$

$$c(\mathrm{CD, aq}) + \mathrm{AC} \leftrightarrow \mathrm{CD\text{-}AC(aq)} + n_2\mathrm{H_2O}$$

$$K = \frac{c(\mathrm{CD\text{-}AC, aq}) \times a_{\mathrm{W}}^{n_2}}{c(\mathrm{CD, aq}) \times c(\mathrm{AC})} \quad (2)$$

where CD, AC and CD-AC stand for β-cyclodextrin, free aroma compound (perillaldehyde) and inclusion complex, respectively, c is the

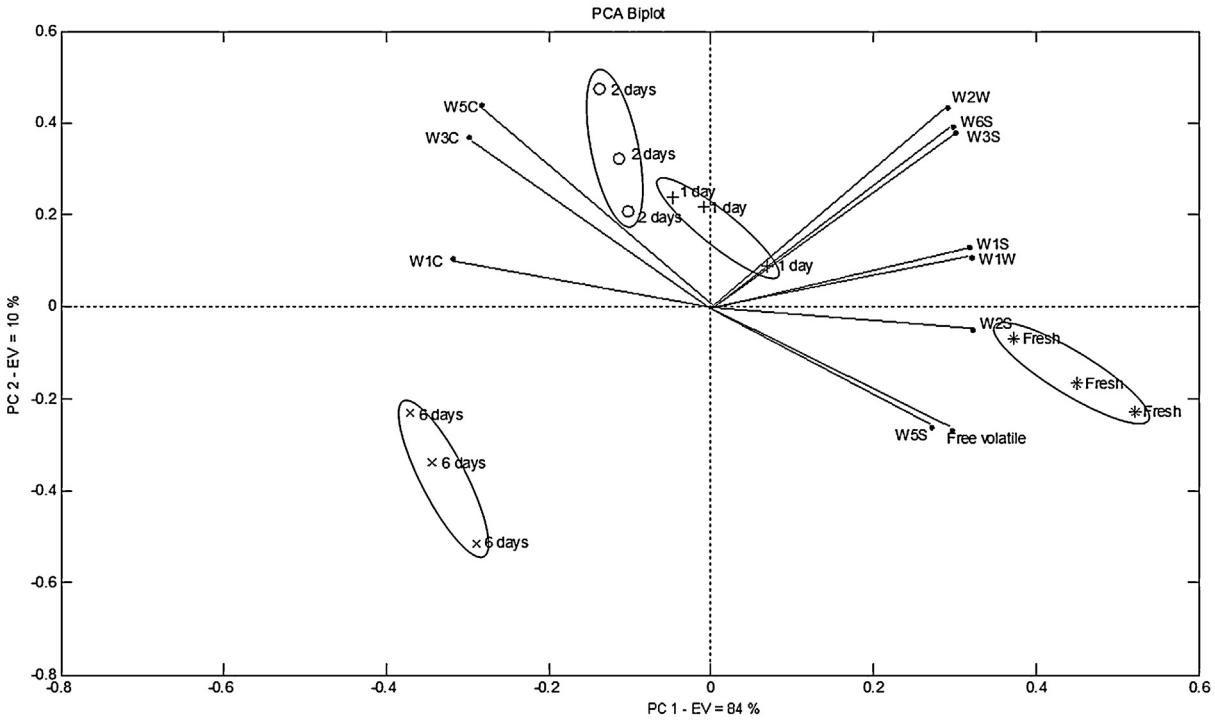

Fig. 3. Score plot and loading plot of the aroma profile of nanofibrous membranes during storage.

symbol for any suitable kind of concentration, and a_W is the activity of water (a_W = RH/100).

Both equations explicitly indicate the major role played by the relative humidity, RH on the release of process. Taking into account that the overall mass of perillaldehyde is split in the free and bound species, namely, $M(AC) = [m(AC) + m(AC \text{ in CD-AC})]$, the relevant concentration ratio, $c(AC)/c(CD\text{-}AC)$, in Eq. (2) can be replaced by the corresponding mass fractions of AC, namely $\varphi/(1-\varphi)$. Combining Eqs. (1) and (2), the following expression for φ can be obtained:

$$\varphi = \frac{a_W^n}{a_W^n + K_{app}} \tag{3}$$

where $n = (n_2 - n_1)$ and $K_{app} = K \times K_h \times c(CD)$.

Eq. (3) was used to fit the trend of the experimental data that are reported in Fig. 4. The figure shows that the release of the volatile may take place for $a_W \geq 0.9$, while it is much smaller for lower a_W. This suggests that the hydrophobicity of perillaldehyde may play a key role in the releasing process. The crystals are immobilized at the nanofibers web; the excess of water molecules could weaken the interaction between host and guest of the complexes CD-AC, e.g., because of conformational changes, thus favoring the expulsion the hydrophobic perillaldehyde toward the pullulan nanofibers that also swell in presence of water molecule and permit the release of perillaldehyde in the outer environment. The macroscopic effect of this change is the color variation of the membranes that turn from white to translucent on increasing RH.

At RH > 92%, that are the condition of the environment of a packed fresh food, the release of the perillaldehyde reaches the equilibrium in about 1 day and the quantity released is about 90% of

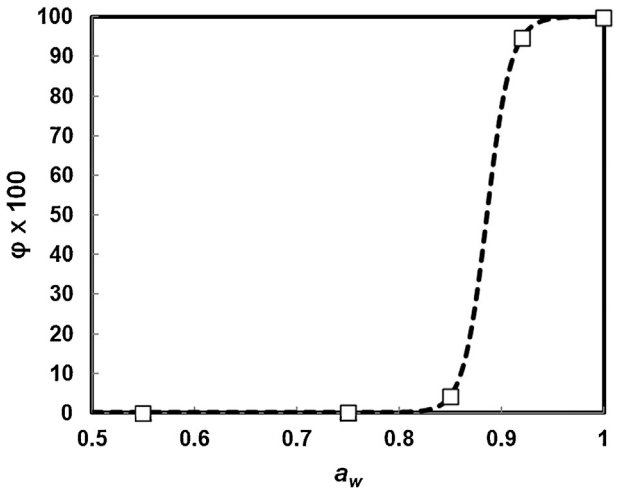

Fig. 4. Fraction of the aroma compound release at equilibrium (φ) as a function of activity of water at 23 °C: experimental data (□) and related fit (dotted line) according to Eq. (3).

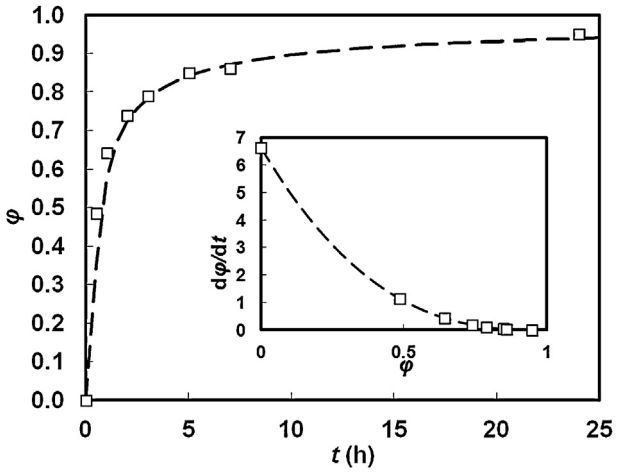

Fig. 5. Kinetic parameterization and fitting of the perillaldehyde release at 92% RH at room temperature. φ is the mass fraction of released aroma.

the total, this is suitable for food applications and can be described (at room temperature) with a classical kinetic expression:

$$\frac{d\phi}{dt} = k(1-\phi)^{\nu} \quad (4)$$

Fig. 5 shows the fit of the experimental data with $\nu = 2.64$ and $k = 6.63\ h^{-1}$. Although Eq. (4) has a phenomenological meaning, the large value of the kinetic order, ν, suggests a multi-step mechanism that may not be assessed on the basis of the evidence collected for the present work.

4. Conclusions

Volatile substances with antimicrobial features, such as natural essential oils, essences, extracts, are of great interest for active food and packaging systems, and their efficient encapsulation and release represent a major challenge to preserve them from evaporation, heat, oxygen and light from production until use.

We demonstrate that it is possible to immobilize aroma compounds directly in an edible polysaccharide nanofibrous matrix via electrospinning, in a single step process, just applying the treatment to a dispersed aqueous solution containing β-cyclodextrin, pullulan and the aroma compound, without preforming the AC-IC. The behavior of the cyclodextrin-aroma-compound complex is mainly governed by the "distance" from the thermodynamic equilibrium relevant to stoichiometric balance between encapsulated and released species. At equilibrium, the ratio of concentrations of aroma compound between the polymer matrix and the surrounding environment is defined by the partition coefficient. The complex is, in fact, bound to the pullulan nanofibers of the membrane and remains stable for months without losses of volatiles if stored at ambient relative humilities, and at different temperatures, as usually happens for food packaging materials. The release of the volatile takes place for $a_W \geq 0.9$ and the equilibrium is reached in about 24 h, with a release of 90% at 92% RH and a release much smaller for lower a_W. These systems could be used to release aroma compounds in fresh foods, thanks to the antimicrobial properties of perillaldehyde, in the package of high a_W food products that can experience microbial degradation and therefore require a special protection. The pullulan non-woven membranes obtained, in fact, can be directly used as active macroscopically homogeneous devices, as they can be can be glued as labels onto packaging wraps for food and food packaging applications, taking advantage of the antimicrobial capacity of certain aroma compounds, such as perillaldehyde. The load limit capacity of the aroma compound is closely related to the solubility of the β-cyclodextrins and to their possibility to be electrospun. Studies at high temperature and with high cyclodextrins concentrations are in progress. Therefore, as mentioned above, the system demonstrates promising features for the food packaging industry. In the case of study, the material is edible and so water soluble, but the work in progress concerns also the development of non-edible cyclodextrin structures allowing the efficient mass exchange in the gas phase, due to the interactions occurring between the system of encapsulation (pullulan-CD-flavor) and the water vapor with, at the same time, conferring a protection from the direct contact with the aqueous phase.

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