Production of Antimicrobial Membranes Loaded with Potassium Sorbate using a Supercritical Phase Separation Process

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Abstract

The production of antimicrobial active packaging is one of the most interesting challenges in the food industry; its scope is to prolong the shelf-life of a food maintaining its safety and freshness. The major limitation of traditional techniques used to produce antimicrobial packaging, is the difficulty in controlling the release of the active agent from the device to the food surface.

In this work, a supercritical phase inversion process has been tested to produce Potassium Sorbate (PS) loaded Cellulose Acetate (CA) membranes, to be inserted in food packaging. The antimicrobial membranes have been obtained at different process conditions (pressures 150-250 bar, temperatures 35-55 °C) and at different polymer concentrations (10, 15 and 20% w/w). PS to CA weight ratio has been maintained constant at 5% w/w for all the formulations. The produced membranes have been characterized from a chemico-physical point of view and PS release tests have been performed. The best process parameters combination to obtain the longest PS release time (about 325 min) was 250 bar and 35 °C for 20% w/w CA membranes.

Keywords: Cellulose acetate, Potassium sorbate, Antimicrobial membranes, Phase separation, Supercritical CO_2.

1 Introduction

The production of antimicrobial active packaging is one of the most interesting challenges in the food industry. Its scope is to prolong the shelf-life of a food and to maintain its safety and freshness. Traditionally, antimicrobial agents are directly mixed with the food formulation; but, it is difficult to control the agent activity and selectivity, due to the reactions with the food components (Appendini & Hotchkiss, 2002; Min & Krochta, 2005). An alternative to this process, can be the use of antimicrobial devices and antimicrobial films, inserted in the food packaging.

Some characteristics are required for the effectiveness of an active packaging; in particular, a suitable concentration and a proper release rate of the active agent to avoid burst effects or a too slow release (Appendini & Hotchkiss, 2002; Vermeiren et al., 1999).

Scientific literature reports several techniques to produce antimicrobial packaging (Appendini & Hotchkiss, 2002; Cha & Chinnan, 2004; Vermeiren et al., 1999): 1) sachets containing volatile antimicrobial agents; 2) incorporation of volatile and non-volatile antimicrobial agents directly into polymers; 3) coating or adsorption of antimicrobials onto polymer surfaces; 4) immobilization of antimicrobials on polymers by ion or covalent linkages; 5) use of antimicrobial polymers.

The major limitation of all the proposed methods, is the difficulty in controlling the release of the active agent from the device to the food surface. Some authors tried to overcome this problem using multilayer films containing an active agent (Buonocore et al., 2005; Mastromatteo et al., 2009; Mastromatteo et al., 2010; Uz & Altınkaya, 2011) or producing films loaded with the antimicrobial agent (Gemili et al., 2009; Tankhiwale & Bajpai, 2012). But, they had the problem of cross-linking the polymer to prolong the release of the active compound. In the case of membrane-like processes, an asymmetric porous structure was produced by evaporation of the organic solvent (Gemili et al., 2009). Using this process, it is difficult to control membrane morphology, since the operative parameters are temperature and humidity only.

Potassium Sorbate (PS) is a potassium salt of the sorbic acid, well-known for its potential antifungal activity (Mehyar et al., 2014; Sayanjali et al., 2011b; Valencia-Chamorro et al., 2008). It

has been used to inhibit the growth of molds and yeasts; but, it is active also against *Staphylococcus aureus*, *Clostridium botulinum*, salmonellae and pseudomonads (Zamora & Zaritzky, 1987). Its stability is not a problem; but, its release is problematic. Sayanjali et al. (2011) produced by solvent evaporation, carboxymethyl cellulose based-edible film containing PS as antimicrobial agent. They demonstrated the efficacy of the antimicrobial film against *A. parasiticus* and *A. flavus*. Moreover, they coated pistachios with this film, obtaining molds growth inhibition (Sayanjali et al., 2011a). Basch et al. (2013) studied the antimicrobial effectiveness of nisin and PS incorporated in films produced by solvent evaporation technique of tapioca starch and its mixtures with hydroxypropyl methylcellulose. They verified that the combination of antimicrobials was more effective against *Listeria innocua* and *Zygosaccharomyces bailii,* than their individual incorporation (Basch et al., 2013).

However, in these studies, the authors produced dense films. Therefore, the active agent release and its action were reduced by the large mass transfer resistance in the polymer matrix. Moreover, solvent evaporation technique can produce the deposition of the active agent on the bottom surface of the film due to the slowness of the process, changing film performance in dependence of which surface is in contact with food.

To overcome conventional processes limitation, it could be possible to use supercritical assisted processes, taking advantage of the properties of supercritical fluids, such as negligible surface tension, high diffusivity and low viscosity. These characteristics have been already successful used to produce micro- and nano-particles, in pharmaceutical, food and biomedical field (Campardelli et al., 2015; Della Porta et al., 2013; Garofalo et al., 2014; Liparoti et al., 2015; Prosapio et al., 2014; Reverchon & Adami, 2013; Espirito Santo et al., 2014), in the separation and/or the extraction of natural compounds (De Melo et al., 2014).

A supercritical phase separation process has been proposed to produce loaded polymeric membranes; the literature shows that this process is more versatile with respect to the traditional ones, since changing operative conditions (i.e., pressure and temperature) it is possible to influence

kinetics and thermodynamics of the process (Reverchon & Cardea, 2004). It has been previously adopted to produce membranes loaded with a catalyst (Cardea & Reverchon, 2011), a drug (Cardea et al., 2014a; Cardea et al., 2014b; Cardea et al., 2010) and biological compounds for food applications (Baldino et al., 2014).

Therefore, the aim of this work is to use the supercritical phase inversion process to produce PS loaded membranes to be inserted in food packaging. Cellulose acetate (CA) has been selected as the polymer, since it is allowed by the Food and Drug Administration (FDA) for food contact. The antimicrobial membranes will be characterized from a chemico-physical point of view and PS release tests will be performed to evidence the effect of the membranes morphology on the kinetics of the active agent release.

2 Materials and Methods

Cellulose acetate, CA, (average M_n ca. 50000 with acetyl content of 39.7%), Acetone (purity 99.5%) and Potassium sorbate (the raw material is shown in Figure 1), PS, (purity 99.0%) were bought from Sigma-Aldrich (Milan, Italy); CO_2 (purity 99%) was purchased from Eurossigeno (Napoli, Italy). Distilled water was produced in our laboratory using an ISCO mod. AUTOSTILL DST/5. All materials were processed as received.

2.1 Antimicrobial membranes preparation

Polymer solutions were prepared dissolving CA in Acetone at 40 °C for 24 h. At the same time, a solution containing PS and water was prepared in an Eppendorf container under Vortex shaking at room temperature for 5 min; then, the two solutions (CA-Acetone and PS-water) were mixed for 1 h. CA amount varied between 10 and 20% w/w in Acetone and the PS amount ranged between 10 and 20% w/w in water; the ratio between CA-Acetone and PS-water solutions was maintained constant at 95:5. These weight percentages are summarized in Table 1. The solutions were distributed on stainless steel caps, having a diameter of 2 cm and a height of about 1 mm, and,

then, processed by Supercritical phase separation at different process conditions: temperatures ranging between 35 and 55 °C and pressures ranging between 150 and 250 bar.

Antimicrobial membranes were produced in a home-made laboratory apparatus previously described (Reverchon et al., 2004). The caps were rapidly put inside the high pressure vessel (a 316 stainless steel vessel with an internal volume of 80 mL) to minimize evaporation of the solvent. The vessel was closed and filled with SC-CO_2 up to the desired pressure, using a high pressure pump (mod. LDB1, Lewa, Germany). Pressure in the vessel was measured by a test gauge (mod. MP1, OMET, Italy) and regulated using a micrometering valve (mod. 1335G4Y, Hoke, USA). Temperature was regulated using PID controllers (mod. 305, Watlow, USA). At the exit of the vessel, a rotameter (mod. D6, ASA, Italy) was used to measure CO_2 flow rate, that was maintained constant at 1.5 Kg/h.

2.2 Antimicrobial membranes characterization

2.2.1 Field Emission Scanning Electron Microscopy (FESEM)

Antimicrobial membranes were cryofractured using liquid Nitrogen (SOL, Milan, Italy); then, the samples were sputter coated with gold (Agar Auto Sputter Coater mod. 108 A, Stansted, UK) at 30 mA for 150 s and analyzed by a FESEM (mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany), used to study membrane morphology.

2.2.2 Membrane pore size analysis

Sigma Scan Pro 5.0 (Jandel Scientific, San Rafael, Canada) and Origin 8.5 (Microcal, Northampton, USA) softwares were used to determine the average diameter of membrane pores. Images taken at various locations in the membrane were used for each calculation. We measured about 300 pores for each sample analyzed. Using Origin software, we first represented an histogram with the percentage of the pores having a given diameter; then, we performed a curve fitting to obtain the pore size distribution curve.

130

131 *2.2.3 Membranes porosity*

132 The porosity (ε) represents the "void space" of the membrane and was calculated from the
133 density of the membrane and the density of untreated CA:

134 $$\varepsilon = 1 - \frac{\rho_{memb}}{\rho_{CA}}$$

135 The membrane density was determined by measuring its volume and weight:

136 $$\rho_{memb} = \frac{Membrane\ weight}{Membrane\ volume}$$

137 The membrane volume was obtained using the Archimede's principle: the membrane was
138 waterproofed and subsequently immersed in pure water. Calculating the weight of the displaced
139 water, we measured the volume of the sample. The presence of PS was neglected in this calculation.
140 Five specimens were tested for each sample.

141

142 *2.2.4 Differential scanning calorimetry (DSC)*

143 DSC (DSC 30 Mettler, Toledo) was carried out to analyze and identify any changes in the
144 thermograms of pure substances compared to polymer/preservative formulations.

145 Calorimetric analysis was performed in the temperature range between -60 and 300 °C, with a
146 heating rate of 10 °C/min; the inert gas was Nitrogen, with a flow rate of 50 L/min.

147

148 *2.2.5 Energy dispersive X-ray spectroscopy (EDX)*

149 Antimicrobial membranes were cryofractured using liquid Nitrogen and sputter coated with
150 Chromium (EMITECH K575X peltier cooled). They were analyzed by EDX (INCA Energy 350,
151 Oxford Instruments) to observe the components distribution in the sample; in particular, Potassium
152 atoms were selected for PS and Carbon atoms for CA.

153

154 *2.2.6 X-Ray analysis*

X-Ray diffractograms were recorded using an X-ray powder diffractometer (mod. D8 Discover, Bruker AXS, Inc., USA) with a Cu sealed tube source. The measuring conditions were: Ni-filtered CuKα radiation, λ = 1.54 Å, 2θ angle ranging from 5° to 60° with a scan rate of 1 s/step and a step size of 0.037°.

2.2.7 Potassium sorbate release test

PS release kinetics were determined measuring the increase of active agent concentration in distilled water at room temperature. The antimicrobial membrane was placed in a bottle containing 50 mL of water, that simulate the food environment, and stirred at 50 rpm. To determine the PS release rate from the membrane and its concentration, analysis was carried out in continuous using a Varian (mod. Cary 50) UV/Vis spectrophotometer and reading the absorbance of the sample at 254 nm (that is the wavelength at which PS shows maximum absorption).

3 Results and Discussion

3.1 Effect of process parameters on antimicrobial membranes morphology

In the first part of the work, we studied the effect of PS incorporation in CA membranes to explore the possible interferences of solubilized PS on membranes morphology. Therefore, CA membranes at 10, 15 and 20% w/w in Acetone, loaded with PS at 5% w/w with respect the polymer, were prepared. We also analyzed the effect of SC-CO_2 solvent power, performing experiments at low, medium and high SC-CO_2 density, corresponding to 150 bar 55 °C (ρ_{CO_2} = 0.66 g/cm^3), 200 bar 45 °C (ρ_{CO_2} = 0.81 g/cm^3) and 250 bar 35 °C (ρ_{CO_2} = 0.90 g/cm^3), respectively. Indeed, the solvent power of SC-CO_2 is a function of its density, that increases with pressure and decreases with temperature.

Summarizing the results, at all the operative conditions tested, CA membranes maintained the shape and volume of the starting solutions. A macroscopic example of the final membrane is depicted in Figure 2.

181 The observation at macroscopic level confirms that shrinkage phenomena were avoided
182 during the supercritical process. When traditional phase separation processes are used, instead, the
183 final membrane structure can collapse due to the surface tension of the liquid system on the polymer
184 matrix, leading to a membrane with a reduced volume.

185 In Figure 3, FESEM images, taken at different enlargements, of the section of the
186 antimicrobial membranes at 10, 15 and 20% w/w CA, processed at 200 bar and 45 °C (medium SC-
187 CO_2 solvent power) are reported.

188 The morphology of the membranes is similar to the ones obtained in a previous work on CA
189 membranes (Reverchon et al., 2004), where CA-Acetone solutions were processed at pressures and
190 temperatures ranging between 100 and 200 bar and 45 - 65 °C, respectively. This comparison is
191 relevant, since it means that the presence of water, used to dissolve PS, does not interfere with the
192 SC-CO_2 induced phase separation of the CA-Acetone system. This result was not obvious, since it
193 is well know that, differently from Acetone, water at the usual phase separation conditions, shows a
194 very reduced affinity with SC-CO_2 and, therefore, its presence could interfere or modify the phase
195 separation process and the produced morphologies.

196 Summarizing the information in Figure 3, membranes structure varied from finger-like to
197 cellular, increasing CA concentration from 10 to 20% w/w. CA membranes at 15 and 20% w/w
198 were characterized by an uniform cellular morphology, in which the mean pore size decreased from
199 about 8 to 5 µm, increasing CA amount from 15 to 20% w/w, respectively. The same results were
200 observed at all tested process conditions; i.e., also when different pressures and temperatures were
201 used. The mean pore size measured for each membrane is reported in Table 2.

202 To explain these results, it is possible to refer to the classical phase separation theory (Van de
203 Witte et al., 1996) and previous works on membranes formation by supercritical phase separation
204 (Reverchon et al., 2004). At 10% w/w CA, a spinodal decomposition occurred and a fingers-like
205 morphology was obtained (Stropnik & Kaiser, 2002); at 15 and 20% w/w CA, the nucleation and

206 growth of the polymer poor-phase within the polymer rich-phase occurred, producing a cellular
207 structure.

208 Comparing the pore size distribution curves showed in Figure 4 and the related data in Table
209 3, we observed that, maintaining a constant CA concentration (e.g., 15% w/w), and varying process
210 conditions (T/p, °C/bar: 55/150, 45/200, 35/250), the mean pore size decreased and pore size
211 distribution sharpened. This is a consequence of SC-CO_2 density increase from about 0.66 to 0.90
212 g/cm^3, and, as previously indicated, of the increase of solvent power that influences the phase
213 separation kinetics. When SC-CO_2 solvent power increases, phase separation process becomes
214 faster and the lean polymer phase has not time enough to grow in the polymer rich phase. As
215 consequence, membrane structure is characterized by smaller pore size and pore size distribution is
216 sharper (Reverchon et al., 2004).

217 This series of experiments demonstrated that the presence of the preservative (i.e., PS) and
218 water, did not influence the CA membrane formation process from a kinetic and a thermodynamic
219 point of view.

220 When we observed the CA matrix at higher FESEM enlargements, we noted that PS is clearly
221 detectable as precipitated and encapsulated microparticles (Figure 5); whereas, before the process,
222 PS was a powder with an irregular shape of about 1 mm (see Materials and Methods section). Small
223 quantities of PS microparticles were distributed on the CA membrane surface; this phenomenon is
224 particularly evident for the 10% w/w CA membranes.

225 This is the first time that microparticles formation has been obtained during SC-CO_2 phase
226 inversion; i.e., simultaneous polymer phase separation and micronization of the active principle
227 during the same experiment. This is a relevant further information on the composite membrane
228 formation mechanism: solubilized PS has been released from the Acetone+water+CA solution by a
229 sort of supercritical antisolvent precipitation mechanism, largely used in the so-called SAS process
230 (Supercritical AntiSolvent micronization) (De Marco et al., 2015; Reverchon & De Marco, 2011).
231 This process is for some aspects similar to SC-phase separation; but, in the case of PS, it produced

microparticles, instead of PS participation during the formation of the continuous structure of the polymeric membrane. The fact that the particles are only partly incorporated in the polymer, indicates that they precipitated from the liquid phase, when the polymeric structure was already formed.

To explain this phenomenon, we have first to consider the preparation step of the samples and the kind of system formed by PS and CA. In particular, PS was dissolved in water and, then, added to the CA-Acetone solution, since PS is not soluble in this organic solvent. During the supercritical assisted process, SC-CO_2 can form a supercritical mixture with Acetone (Reverchon et al., 2004); whereas, SC-CO_2 and water show a very limited affinity (Sabirzyanov et al., 2002). Therefore, Acetone is rapidly eliminated during phase separation and it is very probable that part of water (in which PS tends to concentrate) is eliminated near the end of the process: only at this point, PS precipitates in form of microparticles that can fall in the empty spaces formed by membrane pores. This hypothesis could also justify why this phenomenon was mainly observed for 10% w/w CA membranes. Indeed, in that case, due to the minor amount of polymer, the entrapment capacity of the CA matrix was more limited.

3.2 DSC and EDX analyses

DSC analysis was performed to compare CA and PS thermograms with those of the polymer/PS formulations to identify eventual modifications of the processed materials. CA thermogram, in Figure 6, shows that the polymer glass transition temperature is located at about 78 °C and its melting temperature is about 237 °C. PS thermogram has a minimum at about 160 °C, which corresponds to its melting temperature.

Looking at thermograms of the antimicrobial membranes, the characteristic of CA were preserved, also at larger polymer concentration; whereas, PS fusion peak was no more detectable. It probably means that due to the fast supercritical processing, PS precipitated in an amorphous form.

257 This behavior was already observed in the case of Amoxicillin loaded in Poly(vinylidenefluoride)
258 aerogels for pharmaceutical applications (Cardea et al., 2011).

259 This result confirms the encapsulation and the micronization of PS in CA, since the polymer
260 matrix blocks PS reorganization in a more ordinate form (i.e., the crystalline state).

261 We also analyzed the antimicrobial dispersion inside the polymer matrix by EDX. In Figure 7,
262 element maps identifying CA and PS in a membrane at 15% w/w CA processed at 200 bar and 45
263 °C are reported. In particular, we selected Carbon atoms for CA and Potassium atoms for PS. As
264 shown in the pictures, PS (in red) was uniformly distributed in CA membrane (in green), since the
265 area in which Potassium was detected totally overlaps the Carbon area.

266 Moreover, we also verified by EDX that detected microparticles were formed by PS, as
267 reported in Figure 8. In particular, this figure shows the EDX signal of a single microparticle
268 selected from those observed in the previous FESEM image (see Figure 5).

269 Therefore, these analyses confirm that we produced antimicrobial membranes in which the
270 active principle was dispersed in the polymer matrix and, at 10% w/w CA, a small part of it also
271 precipitated on the membrane external surface.

272

273 **3.3 X-Ray analysis**

274 X-Ray analyses were performed to confirm PS solid state after the supercritical phase
275 separation process, as hypothesized in the results of DSC analysis. In Figure 9, we compared the X-
276 ray diffraction patterns of the raw materials with the CA+PS physical mixture and the CA+PS
277 membrane. As expected, PS is crystalline, CA is semi-crystalline and their physical mixture
278 maintained some peaks of the two compounds. The X-ray diffraction pattern of CA+PS membrane
279 shows instead that the sample is substantially amorphous.

280

281 **3.4 PS release tests from CA membranes**

Differently from the case of antimicrobial principles based on bioactive compounds (for example, an enzyme), PS stability is not a concern; therefore, activity tests were not performed. In this case, the most relevant characteristic to be measured, is the release rate of PS from CA membranes in water. Water is generally used as the medium to simulate a food (Buonocore et al., 2005; Uz & Altınkaya, 2011). Using the procedure described in Section 2.2.7, we tested the membranes at 10, 15 and 20% w/w CA processed at different SC-CO_2 solvent power. PS concentration (C_t) was normalized to the maximum detected PS concentration (C_{inf}) to allow the comparison among the curves, reported in Figure 10a-c. The aim of this analysis was to identify the parameters influencing the performance of the antimicrobial membrane with respect to PS release and the time required to achieve the maximum PS concentration in the medium.

The release curves showed an exponential trend for all samples tested; therefore, the release mechanism was the same and was reproducible for the different membranes, even changing CA concentration and the process operative conditions. A diffusion controlled mechanism can be hypothesized (Uz & Altınkaya, 2011) when these trends are observed.

PS release rate decreased with CA concentration at all operative condition tested; this result was expected considering that, increasing the polymer concentration, the membranes morphology changed (from macrovoids to cellular one) and the pore size decreased (Figure 2). Moreover, a slight burst effect can be observed in 10% w/w CA membranes; this phenomenon can be related to the presence of the precipitated PS microparticles, as previously discussed, on the CA membrane external surfaces, that make them easily available for a fast dissolution.

In Table 4, we reported the time required to achieve the maximum PS concentration for each sample. As expected, PS release rate decreased for the membranes produced at higher SC-CO_2 solvent power; e.g., for the membranes processed at 250 bar/35 °C. In Figure 10c, the maximum PS concentration was obtained after 75, 200 and 325 min for 10, 15 and 20% w/w CA, respectively. This result is consistent with the measured porosity values (Table 3) and with the decrease of the mean pores size (Figure 3), observed increasing the SC-CO_2 solvent power: smaller pores

308 determined a decrease of mass transfer coefficient of water and of PS+water solution inside the
309 membranes; i.e., more compact membranes lead to a slower PS release.

310 The maximum concentration of PS in water is not equivalent to a data about the duration of
311 the antimicrobial control exerted by the membrane. Indeed, the length of the preservative action will
312 depend on the quantity of microorganisms that is present in water and by the lithic action required.
313 Therefore, the measured release times have to be interpreted as the readiness of the membrane to
314 exert its antimicrobial action.

315 We also measured the maximum PS concentration released from CA membranes. In Table 5,
316 it is possible to observe as increasing the amount of CA in the polymer solutions (i.e., increasing the
317 amount of PS), the maximum concentration of PS increases, too. Moreover, for all the membranes
318 generated, the Minimal Inhibitory Concentration (MIC) for bacteria, that ranges from 15.7 - 250
319 mg/L (Gunes et al., 2013), was overcome. This aspect is very relevant for the application in active
320 packaging.

321 The process conditions did not affect the maximum PS concentration, that only depends on
322 the amount of PS in the starting solutions; on the other hand, the SC-CO_2 solvent power affects the
323 rate of PS release up to the maximum concentration, as previously observed in Table 4.

324

325 **4 Conclusions**

326 Different CA membranes loaded with PS were successfully produced by supercritical phase
327 separation and micronization of the active principle was simultaneously obtained. This result allows
328 to use these membranes in two ways: if a strong and fast microorganisms inhibition is required, the
329 membrane at lower polymer content is preferable, since PS release is faster; the other possibility is
330 to use membranes at higher polymer content, prolonging the inhibition effect and the safety of the
331 food.

Interpreting the results, the best combination to obtain the longest PS release time is the maximum SC-CO_2 solvent power and the maximum CA concentration, that in this work corresponds to the CA membrane at 20% w/w, obtained at 250 bar and 35 °C.

References

Appendini, P., & Hotchkiss, J. H. (2002). Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies, 3*(2), 113-126.

Baldino, L., Cardea, S., & Reverchon, E. (2014). Supercritical assisted enzymatic membranes preparation, for active packaging applications. *Journal of membrane science, 453*, 409-418.

Basch, C. Y., Jagus, R. J., & Flores, S. K. (2013). Physical and antimicrobial properties of tapioca starch-HPMC edible films incorporated with nisin and/or potassium sorbate. *Food and Bioprocess Technology, 6*(9), 2419-2428.

Buonocore, G., Conte, A., Corbo, M., Sinigaglia, M., & Del Nobile, M. (2005). Mono-and multilayer active films containing lysozyme as antimicrobial agent. *Innovative Food Science & Emerging Technologies, 6*(4), 459-464.

Campardelli, R., Baldino, L., & Reverchon, E. (2015). Supercritical fluids applications in nanomedicine. *The Journal of Supercritical Fluids, 101*, 193-214.

Cardea, S., Baldino, L., Pisanti, P., & Reverchon, E. (2014a). 3-D PLLA scaffolds formation by a supercritical freeze extraction assisted process. *Journal of Materials Science: Materials in Medicine, 25*(2), 355-362.

Cardea, S., Baldino, L., Scognamiglio, M., & Reverchon, E. (2014b). 3D PLLA/Ibuprofen composite scaffolds obtained by a supercritical fluids assisted process. *Journal of Materials Science: Materials in Medicine, 25*(4), 989-998.

Cardea, S., & Reverchon, E. (2011). Nanostructured PVDF-HFP membranes loaded with catalyst obtained by supercritical CO 2 assisted techniques. *Chemical Engineering and Processing: Process Intensification, 50*(7), 630-636.

Cardea, S., Sessa, M., & Reverchon, E. (2010). Supercritical phase inversion to form drug-loaded poly (vinylidene fluoride-co-hexafluoropropylene) membranes. *Industrial & Engineering Chemistry Research, 49*(6), 2783-2789.

361 Cardea, S., Sessa, M., & Reverchon, E. (2011). Supercritical CO 2 assisted formation of poly
362 (vinylidenefluoride) aerogels containing amoxicillin, used as controlled release device. *The*
363 *Journal of Supercritical Fluids, 59*, 149-156.
364 Cha, D. S., & Chinnan, M. S. (2004). Biopolymer-based antimicrobial packaging: a review. *Critical*
365 *Reviews in Food Science and Nutrition, 44*(4), 223-237.
366 De Marco, I., Rossmann, M., Prosapio, V., Reverchon, E., & Braeuer, A. (2015). Control of particle
367 size, at micrometric and nanometric range, using supercritical antisolvent precipitation from
368 solvent mixtures: Application to PVP. *Chemical Engineering Journal, 273*(0), 344-352.
369 De Melo, M., Silvestre, A., & Silva, C. (2014). Supercritical fluid extraction of vegetable matrices:
370 applications, trends and future perspectives of a convincing green technology. *The Journal*
371 *of Supercritical Fluids, 92*, 115-176.
372 Della Porta, G., Falco, N., Giordano, E., & Reverchon, E. (2013). PLGA microspheres by
373 Supercritical Emulsion Extraction: a study on insulin release in myoblast culture. *Journal of*
374 *Biomaterials Science, Polymer Edition, 24*(16), 1831-1847.
375 Garofalo, C., Capuano, G., Sottile, R., Tallerico, R., Adami, R., Reverchon, E., Carbone, E., Izzo,
376 L., & Pappalardo, D. (2014). Different insight into amphiphilic PEG-PLA copolymers:
377 Influence of macromolecular architecture on the micelle formation and cellular uptake.
378 *Biomacromolecules, 15*(1), 403-415.
379 Gemili, S., Yemenicioğlu, A., & Altınkaya, S. A. (2009). Development of cellulose acetate based
380 antimicrobial food packaging materials for controlled release of lysozyme. *Journal of Food*
381 *Engineering, 90*(4), 453-462.
382 Gunes, H., Gulen, D., Mutlu, R., Gumus, A., Tas, T., & Topkaya, A. E. (2013). Antibacterial effects
383 of curcumin: an in vitro minimum inhibitory concentration study. *Toxicology and industrial*
384 *health*, 0748233713498458.

Liparoti, S., Adami, R., & Reverchon, E. (2015). Supercritical Assisted Atomization: effect of operative conditions on PVP microparticle size and morphology. *The Journal of Supercritical Fluids, 97*, 31-35.

Mastromatteo, M., Barbuzzi, G., Conte, A., & Del Nobile, M. (2009). Controlled release of thymol from zein based film. *Innovative Food Science & Emerging Technologies, 10*(2), 222-227.

Mastromatteo, M., Mastromatteo, M., Conte, A., & Del Nobile, M. A. (2010). Advances in controlled release devices for food packaging applications. *Trends in food science & technology, 21*(12), 591-598.

Mehyar, G. F., Al-Qadiri, H. M., & Swanson, B. G. (2014). Edible coatings and retention of potassium sorbate on apples, tomatoes and cucumbers to improve antifungal activity during refrigerated storage. *Journal of Food Processing and Preservation, 38*(1), 175-182.

Min, S., & Krochta, J. (2005). Antimicrobial films and coatings for fresh fruit and vegetables. *Improving the safety of fresh fruit and vegetables*, 454-492.

Prosapio, V., Reverchon, E., & De Marco, I. (2014). Antisolvent micronization of BSA using supercritical mixtures carbon dioxide+ organic solvent. *The Journal of Supercritical Fluids, 94*, 189-197.

Reverchon, E., & Adami, R. (2013). Supercritical assisted atomization to produce nanostructured chitosan-hydroxyapatite microparticles for biomedical application. *Powder Technology, 246*, 441-447.

Reverchon, E., & Cardea, S. (2004). Formation of cellulose acetate membranes using a supercritical fluid assisted process. *Journal of membrane science, 240*(1), 187-195.

Reverchon, E., & De Marco, I. (2011). Mechanisms controlling supercritical antisolvent precipitate morphology. *Chemical Engineering Journal, 169*(1–3), 358-370.

Sabirzyanov, A. N., Il'in, A. P., Akhunov, A. R., & Gumerov, F. M. (2002). Solubility of Water in Supercritical Carbon Dioxide. *High Temperature, 40*(2), 203-206.

410 Santo, I. E., Campardelli, R., Albuquerque, E. C., de Melo, S. V., Della Porta, G., & Reverchon, E.
411 (2014). Liposomes preparation using a supercritical fluid assisted continuous process.
412 *Chemical Engineering Journal, 249*, 153-159.
413 Sayanjali, S., Ghanbarzadeh, B., & Ghiassifar, S. (2011a). Evaluation of antimicrobial and physical
414 properties of edible film based on carboxymethyl cellulose containing potassium sorbate on
415 some mycotoxigenic Aspergillus species in fresh pistachios. *LWT - Food Science and*
416 *Technology, 44*(4), 1133-1138.
417 Sayanjali, S., Ghanbarzadeh, B., & Ghiassifar, S. (2011b). Evaluation of antimicrobial and physical
418 properties of edible film based on carboxymethyl cellulose containing potassium sorbate on
419 some mycotoxigenic Aspergillus species in fresh pistachios. *LWT-Food Science and*
420 *Technology, 44*(4), 1133-1138.
421 Stropnik, Č., & Kaiser, V. (2002). Polymeric membranes preparation by wet phase separation:
422 mechanisms and elementary processes. *Desalination, 145*(1), 1-10.
423 Tankhiwale, R., & Bajpai, S. (2012). Preparation, characterization and antibacterial applications of
424 ZnO-nanoparticles coated polyethylene films for food packaging. *Colloids and Surfaces B:*
425 *Biointerfaces, 90*, 16-20.
426 Uz, M., & Altınkaya, S. A. (2011). Development of mono and multilayer antimicrobial food
427 packaging materials for controlled release of potassium sorbate. *LWT-Food Science and*
428 *Technology, 44*(10), 2302-2309.
429 Valencia-Chamorro, S. A., Palou, L., Del Rio, M. A., & Pérez-Gago, M. a. B. (2008). Inhibition of
430 Penicillium digitatum and Penicillium italicum by hydroxypropyl methylcellulose− lipid
431 edible composite films containing food additives with antifungal properties. *Journal of*
432 *agricultural and food chemistry, 56*(23), 11270-11278.
433 Van de Witte, P., Dijkstra, P., Van den Berg, J., & Feijen, J. (1996). Phase separation processes in
434 polymer solutions in relation to membrane formation. *Journal of membrane science, 117*(1),
435 1-31.

436 Vermeiren, L., Devlieghere, F., Van Beest, M., De Kruijf, N., & Debevere, J. (1999). Developments
437 in the active packaging of foods. *Trends in food science & technology, 10*(3), 77-86.
438 Zamora, M. C., & Zaritzky, N. E. (1987). Potassium Sorbate Inhibition of Microorganisms Growing
439 on Refrigerated Packaged Beef. *Journal of Food Science, 52*(2), 257-262.

440

Figure Captions

Figure 1. Untreated potassium sorbate powder.

Figure 2. Pictures of an antimicrobial CA membrane processed by Supercritical phase separation at 200 bar and 45 °C.

Figure 3. FESEM images of the section of antimicrobial CA membranes produced at 200 bar and 45 °C and at different CA concentrations: (a) 10% w/w, (b) 15% w/w, (c) 20% w/w, and at a constant ratio PS/CA=5% w/w.

Figure 4. Pore size distribution of antimicrobial membranes at 15% w/w CA, processed at different SC-CO_2 solvent power.

Figure 5. FESEM image showing PS microparticles partly encapsulated in the CA 10% w/w matrix.

Figure 6. Thermograms of CA, PS and CA plus PS membranes processed at 200 bar, 45 °C.

Figure 7. EDX analysis of the antimicrobial membrane at 15% w/w CA processed at 200 bar, 45 °C: a) Potassium atoms; b) Carbon atoms.

Figure 8. EDX analysis of a microparticle formed inside the 10% CA membrane: a) FESEM picture; b) Potassium atoms; c) Carbon atoms.

Figure 9. X-Ray analysis of PS, CA, CA+PS membrane and CA+PS physical mixture.

Figure 10. PS release curves from CA membranes at 10, 15 and 20% w/w in water, processed at: a) 150 bar, 55 °C; b) 200 bar, 45 °C; c) 250 bar, 35 °C.

461 **Table Captions**

462 **Table 1.** Weight percentages of each component used for the samples preparation.

463 **Table 2.** Mean pore size of antimicrobial membranes section.

464 **Table 3.** Porosity of the antimicrobial membranes phase separated at 150/55, 200/45, 250/35 p/T.

465 **Table 4.** Time to reach the maximum PS concentration changing SC-CO_2 solvent power.

466 **Table 5.** Maximum PS concentration released from CA membranes obtained at different SC-CO_2

467 solvent power.