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Smart Membranes Production by Supercritical Phase Inversion to Increase Food Shelf-life

Lucia Baldino, Stefano Cardea*, Ernesto Reverchon

Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II, 132, 84084, Fisciano (SA), Italy
scardea@unisa.it

The freshness of minimally processed foods is also determined by their appearance; browning due to oxidative phenomena is one of the main reasons of discarded by consumers. To overcame this problem, the development of antioxidant active packaging has gained increasing interest in the food industry.
In this work, a supercritical phase inversion process was tested to produce Curcumin (Cu) loaded Cellulose Acetate (CA) membranes, to be inserted in food packaging as antioxidant devices. Active membranes were produced at different process conditions (pressures ranging between 150-250 bar and temperatures ranging between 35-55 °C), polymer amount (12, 18, 24% w/w) and at constant ratio Cu/CA (10% w/w). Cu-loaded membranes showed an homogeneous cellular morphology with a mean pore size lower than 12 μm. Cu release was measured and a maximum release time of about 2 days was obtained; moreover, antioxidant tests were also performed on the loaded membranes, showing an activity up to 90%.

1. Introduction

The freshness of minimally processed foods is also determined by their appearance; browning due to oxidative phenomena is one of the main reasons of discarded by consumers. To overcame this problem, the development of antioxidant active packaging has gained increasing interest in the food industry.
Curcumin is a polyphenolic compound and is the main phytochemical compound contained in *Curcuma longa L*. It has been demonstrated that it exerts antioxidant and antimicrobial activities (Zorofchian et al., 2014). Several studies have been also performed about its use as antibiotic (Wang et al., 2009), antiviral (Chen et al., 2010) and antifungal (Sharma et al., 2009) drug. However, in several cases, the curcumin release was obtained after few hours from the carrier (Vimal et al., 2011) and its maximum antioxidant activity was lower than 80% (Sonkaew et al., 2012).
Processes assisted by supercritical CO_2 (SC-CO_2) have been proposed as alternative to the traditional ones to improve product morphology and safety, in terms of solvent residues (Baldino et al., 2015a). Among them, the formation of membranes by SC-CO_2 assisted phase inversion has been particularly successful (Huang et al., 2007; Shi et al., 2013; Cardea et al., 2014; De Marco et al., 2014; Baldino et al., 2015b; Zaherzadeh et al., 2015; Baldino et al., 2017). In this process, SC-CO_2 acts as the non-solvent for the polymer and as solvent for the liquid used to dissolve the polymer. The result is a phase inversion process with the formation of a continuous polymer phase and a supercritical (liquid+CO_2) discontinuous phase, that is released during the process, producing porous interconnected structures. The advantages of this technique over the liquid-liquid based process are the flexibility of SC-CO_2 action with pressure and temperature, that allows to produce membranes tailored in morphology and pore size, and the complete elimination of the organic solvent (De Marco et al., 2015; Baldino et al., 2016a).
The aim of this work is to produce smart membranes to be added in food packaging for the controlled release of curcumin, used as antioxidant agent. Membranes of cellulose acetate (CA) containing curcumin (Cu) were produced by SC-CO_2 phase inversion. Release studies performed on these membranes, were useful to verify the preservation of the antioxidant activity of curcumin and the length of the release of the active principle in an appropriate liquid medium.

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2. Materials and methods

Cellulose acetate, CA, (average Mn ca. 50000 with acetyl content of 39.7%), Acetone (purity 99.5%), Curcumin, Cu, (purity >65%), Ethanol (purity >99.8%) and DPPH• (2,2-diphenyl-1-picryl-hydrazyl) were bought from Sigma-Aldrich (Milan, Italy); CO_2 (purity 99%) was purchased from Morlando Group S.R.L. (Sant'Antimo, NA - Italy). All materials were processed as received.

Solutions were prepared dissolving CA at 12, 18 and 24% w/w in acetone at 35 °C, 100 rpm for 12 h. Then, Cu, at 10% w/w with respect to CA, was added to the solutions, that were mixed for other 4 h at 35 °C and 100 rpm. The obtained solutions, were distributed on stainless steel caps, having a diameter of 2 cm and an height of about 1 mm. The caps were rapidly put inside the high pressure vessel (a 316 stainless steel vessel with an internal volume of 200 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). Then, they were processed by SC-CO_2 phase inversion at different process conditions: temperatures ranging between 35 and 55 °C, pressures ranging between 150 and 250 bar, for 3 h. 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.

Membranes were cryo-fractured 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) to study membrane morphology.

Sigma Scan Pro 5.0 (Jandel Scientific, San Rafael, Canada) and Origin 8.5 (Microcal, Northampton, USA) were used to determine the average diameter of membrane pores. Images taken at various locations in the membrane were used for each calculation. About 300 pores for each sample were measured.

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

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

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

$$\rho_{\mathrm{memb}} = \frac{\text{Membrane weight}}{\text{Membrane volume}}$$

The membrane volume was obtained using the Archimede's principle: the membrane was waterproofed and subsequently immersed in pure water. Calculating the weight of the displaced water, we measured the volume of the sample. Five specimens were tested for each sample.

Cu release kinetics were determined measuring the increase of drug concentration in 100 mL of an aqueous solution at 80% v/v distilled water and 20% v/v Ethanol (Simulant C, according to the Regulation (UE) N. 10/2011), at room temperature. Cu loaded membrane was placed in a bottle containing the medium and stirred at 50 rpm. To determine the Cu release rate from the membrane, analysis was carried out in continuous using a Varian (mod. Cary 50, Palo Alto, CA) UV/Vis spectrophotometer and reading the absorbance of the sample at 430 nm, that is the wavelength at which Cu shows maximum absorption.

The radical scavenging capacity of the CA+Cu membranes was determined by DPPH• method. A 0.1 mM solution of DPPH• was prepared in ethanol and 1 mL of this solution was added to 3 mL of the solution Cu+water+ethanol, obtained from the release test previously performed. This final solution was mixed by Vortex for 2 min and incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm. The capability to scavenge the DPPH• radical was calculated using the following equation:

$$I\,(\%) = (1 - As/Ac) \times 100$$

where Ac is the absorbance of the control (1 mL, DPPH• solution) and As is the absorbance of the solution in the presence of Cu (Baldino et al., 2016b).

3. Results and discussion

We performed a first series of experiments at 200 bar, 45 °C and 3 h contacting time. Acetone was used as the organic solvent, since CA and Cu are largely soluble in it. CA membranes at 12, 18 and 24% w/w were produced, containing a percentage of 10% w/w of Cu with respect the CA content.

In Figure 1, an example of the morphology shown by CA+Cu membranes at different CA concentrations is reported. The observed morphology is cellular with open and regular pores along the membrane section.

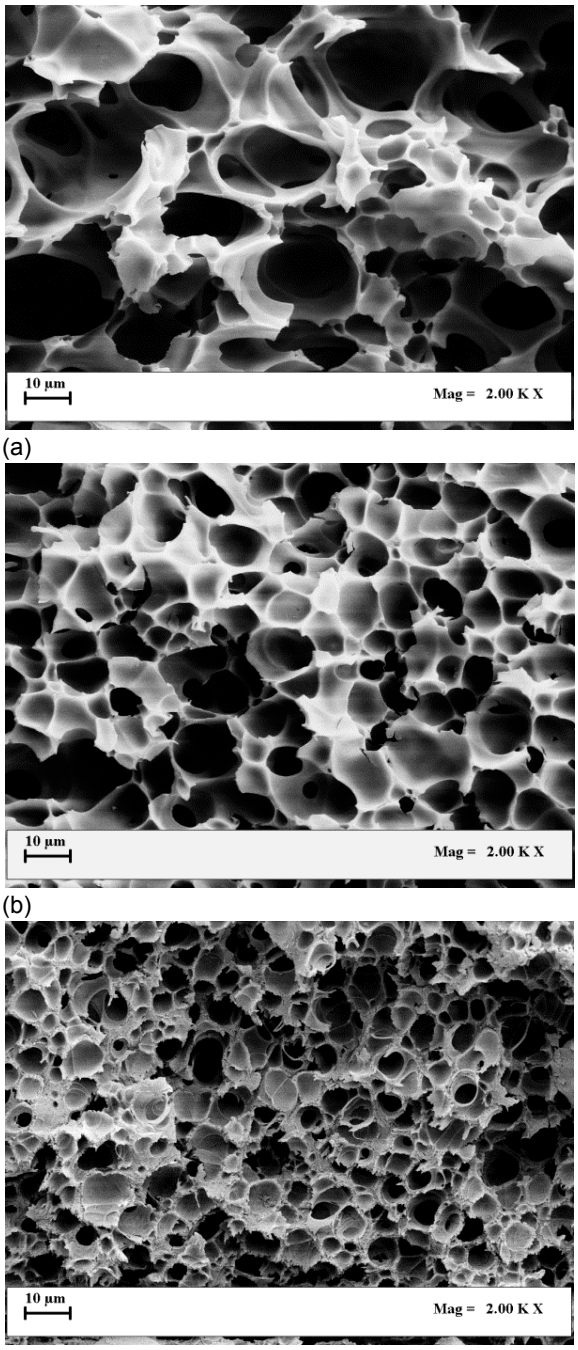

Figure 1: FESEM pictures of CA+Cu membranes section obtained at 200 bar, 45 °C, 3 h. (a) 12% w/w CA, (b) 18% w/w CA, (c) 24% w/w CA.

This result can be explained considering that the demixing mechanism occurred in all cases above the critical point between the binodal and spinodal curves of the miscibility gap in the ternary diagram, reported as example in Figure 2. In particular, the nucleation and growth of the polymer lean phase (i.e., acetone) in the polymer rich phase (i.e., CA) was obtained, producing a porous morphology. The membranes mean pore size decreases with CA content from about 10 µm to 9 µm to 7 µm for CA % of 12, 18 and 24% w/w, respectively. These results are consistent with the literature (Baldino et al., 2017), since when the polymer concentration

increases, the membrane structure generally becomes denser, namely, the pore mean diameter decreases. Moreover, the mean pore size increases and its distribution enlarges when SC-CO_2 density is decreased (for p/T values 250/35, 200/45, 150/55). The data collected for all the produced membranes are reported in Table 1. In this case, the explanation is related to the kinetics of the phase inversion process; indeed, the CO_2 density influences its solvent power. Therefore, reducing the SC-CO_2 solvent power, changing the operative conditions, the process becomes slower and, as a consequence, the polymer lean phase has more time to expand in the polymer rich phase, producing larger pores. The overall porosity decreased from 82% to 74% to 68% for the membranes at 12, 18 and 24% w/w CA, respectively.

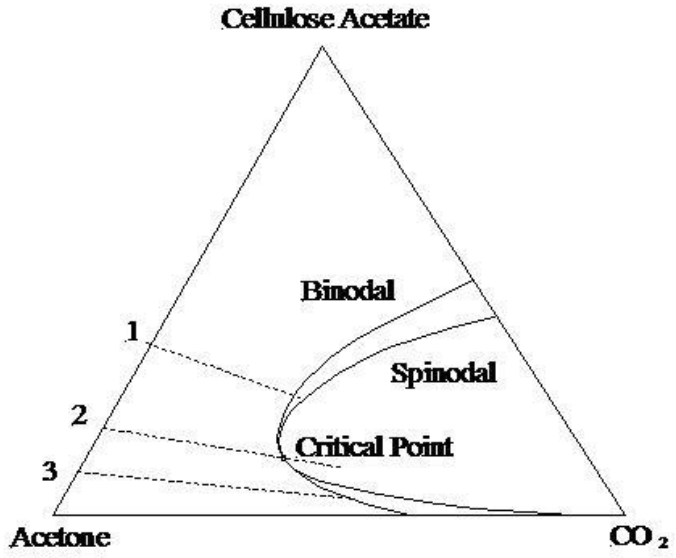

Figure 2: Qualitative ternary diagram of the system CA/acetone/CO2.

Table 1: Mean pore diameter ± standard deviation (μm) of the CA membranes loaded at 10% w/w Cu and at all different process conditions used in this work.

Cu 10% w/w	CA 12% w/w	CA 18% w/w	CA 24% w/w
150 bar – 55 °C	11.50±3.80	9.13±2.80	7.40±2.65
200 bar – 45 °C	10.03±2.90	9.28±2.72	6.75±2.53
250 bar – 35 °C	8.37±2.12	8.30±1.77	5.14±1.32

Extensive series of UV/Vis release tests of curcumin from the CA membranes were performed using the technique described in Materials and Methods. The Cu release curves were largely dependent on CA percentage and, correspondingly, on the membranes pore diameter, as indicated, for example, for the case 200 bar, 45 °C, reported in Figure 3. In this diagram, the Cu concentration measured during the time (C_t), normalized to the maximum Cu concentration detected (C_{inf}) versus time, is represented. Only 1.5 h were necessary to obtain the maximum concentration of curcumin in the dissolution medium in the case of the membrane at 12% w/w CA; after 3.3 h curcumin maximum concentration was obtained for 18% w/w CA and the longest time to reach the maximum curcumin concentration was 50 h for 24% w/w CA membranes. In particular, for membranes at 24% w/w CA, the more dense structure, due to the smaller pores, showed a very relevant influence on the release rate of Cu and, thus, on the resistance at the Cu mass transfer mechanism. The flexibility of the supercritical phase inversion process in dependence of the operative conditions and polymer concentration, permitted to obtain membranes characterized by a porous morphology, but with different pore diameter, that influenced the active principle release time. In this way, the optimum combination between process parameters/polymer parameters can be selected in dependence of the specific membrane application.

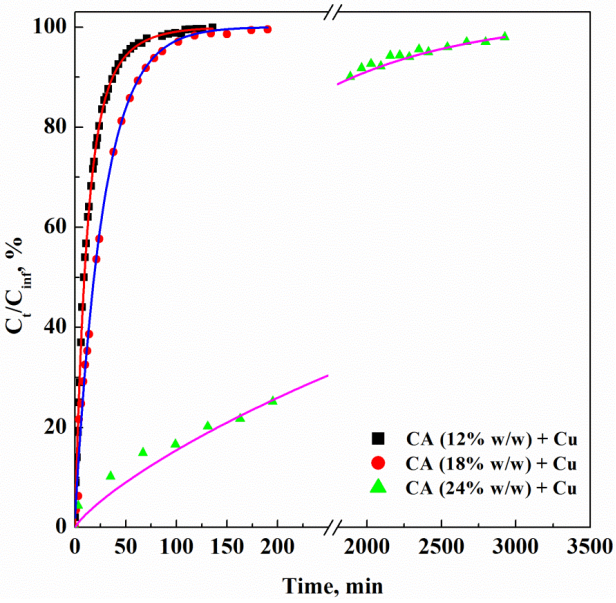

Figure 3: Cu release curves from CA membranes at 12, 18 and 24% w/w, obtained at 200 bar, 45 °C, 3 h.

We also measured the antioxidant activity of Cu released from the CA membranes, using the procedure described in Materials and Methods section.

Table 2: Antioxidant activity of 10% w/w Cu loaded CA membranes obtained at 200 bar, 45 °C, 3 h.

Sample	I (%)
CA (12% w/w) + Cu (10% w/w)	78
CA (18% w/w) + Cu (10% w/w)	84
CA (24% w/w) + Cu (10% w/w)	90

A curcumin antioxidant activity ranging from about 80% to 90% was observed, demonstrating that Cu maintained its properties after the supercritical phase inversion process. This result indicates that the mild supercritical processing avoids delicates molecules and drugs degradation/denaturation, as already ascertained in other previous works (Baldino et al., 2016b; Baldino et al., 2017), overcoming the limitations of the traditional phase inversion processes, such as solvent induced phase separation and thermally induced phase separation, in which the use of toxic organic solvents or high temperature, reduced their application to treat this kind of substances loaded in the membrane matrix.

4. Conclusions and perspectives

Smart CA+Cu membranes have been successful produced in this study by supercritical phase inversion process. It was possible to modulate membrane pore size, by changing operative conditions or polymer concentration, obtaining a very long release of curcumin that maintained up to 90% of its native antioxidant activity. These membranes can be modulated in terms of kinetics of drug release to the specific target required in food preservation and shelf life increase, used as small and active antioxidant devices.
In perspective, other natural active principles can be loaded in the membranes; for instance, combining the antioxidant activity with the antimicrobial activity, to extend the application of these devices also to more perishable food products or to food imported/exported.

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